AMELIORATIVE EFFECTS OF L-GLUTAMINE ON RECTAL TEMPERATURE, HAEMATOLOGICAL AND OXIDATIVE STRESS CHANGES IN RED SOKOTO GOATS DURING THE HOT-DRY SEASON

BY

OHIEMI BENJAMIN OCHEJA

DEPARTMENT OF PHYSIOLOGY
FACULTY OF VETERINARY MEDICINE
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA

NOVEMBER, 2015

AMELIORATIVE EFFECTS OF L-GLUTAMINE ON RECTAL TEMPERATURE, HAEMATOLOGICAL AND OXIDATIVE STRESS CHANGES IN RED SOKOTO GOATS DURING THE HOT-DRY SEASON

BY

Ohiemi Benjamin OCHEJA, DVM (ABU) 2011
MSc/VET-MED/47117/2012-2013

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN VETERINARY PHYSIOLGY

DEPARTMENT OF VETERINARY PHYSIOLOGY
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA

NOVEMBER, 2015

DECLARATION

I declare that the work reported in this dissertation entitled **'Ameliorative Effects of L-glutamine on Rectal Temperature, Haematological and Oxidative Stress Changes in Red Sokoto Goats During the Hot-dry Season'** was carried out by me in the Department of Veterinary Physiology, Ahmadu Bello University, Zaria-Nigeria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Ohiemi Benjamin OCHEJA

_____	_____	_____
Name of Student	Signature	Date

CERTIFICATION

This dissertation entitled **'AMELIORATIVE EFFECTS OF L-GLUTAMINE ON RECTAL TEMPERATURE, HAEMATOLOGICAL AND OXIDATIVE STRESS CHANGES IN RED SOKOTO GOATS DURING THE HOT-DRY SEASON'** carried out by Ohiemi Benjamin OCHEJA 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.

Professor J. O. Ayo

_____ _____ Date_____

Chairman, Supervisory Committee

Dr T. Aluwong

_____ _____ Date_____

Member, Supervisory Committee

Dr N. S. Minka

_____ _____ Date_____

Member, Supervisory Committee

Dr M. U. Kawu

_____ _____ Date_____

Head of Department

Professor K. Bala

_____ _____ Date_____

Dean, School of Postgraduate Studies

DEDICATION

This dissertation is dedicated to the Almighty God for seeing me through this programme, also to my aunt, Mrs Celina Ocheja, for her invaluable assistance and to all who believe in hardwork.

ACKNOWLEDGEMENTS

I thank Almighty God for His unfathomable love bestowed upon me throughout the period of this programme. Indeed, He has been faithful and all praise, honour and adoration be unto Him forever and ever.

My profound appreciation goes to the chairman of my supervisory committee, Professor J. O. Ayo, for his mentorship, generosity, scholarly and fatherly advice. I express my profound gratitude to the other members of my supervisory committee: Drs T. Aluwong and N. S. Minka, for their indispensable contributions to this work. I appreciate their selfless sacrifices and financial support. The timely and indispensable assistance of my colleagues, Drs G. O. Ijale, I. C. Egbuniwe, E. S. Idoga and F. O. Zakari, during the conduct of this research work is highly appreciated. Special thanks to Dr L. S. Yaqub for his encouragement and support throughout this work. Many thanks to Chief Emmanuel Nwosu and other technical staff of the Departments of Veterinary Physiology, and Veterinary Pharmacology and Toxicology for their technical assistance, especially during my research work. I also appreciate the Heads of Departments of Veterinary Physiology, Dr M. U. Kawu; Veterinary Pathology, Dr M. Bisalla; and Veterinary Anatomy, Professor J. O. Hambolu for granting me access to Research Laboratory, livestock pen and postgraduate class, respectively. A big thank you to my lecturers, Drs T. Dzenda, V.O. Sinkalu and M. Maidawa, for their constant encouragement.

I am eternally grateful to my aunt, Mrs Celina Ocheja, who has been my benefactor since the demise of my father. The kind of love and support I enjoyed from her is unprecedented that no word of gratitude will be enough. God bless her. I also appreciate my late father, Mr Sani Ocheja for his love and most especially the discipline he inculcated in me, which has modelled me to the person I am today. The memories of times spent with you remain evergreen in me. To my mother, Mrs Cecilia Ocheja, you remain the best mum in the world. Thank you for your prayers, love and support.

The love, support and encouragement I enjoyed from my entire family are unquantifiable. I thank my grandmother, uncles, aunts, in-laws and cousins for being a family I always want to be associated with. Special thanks to Dr Akoji Ocheja for giving me the car that was of great help; Mr Ugbede Ocheja for his financial assistance; and Barr. E.I. Ojotu, who assisted in the purchase of the L-glutamine. I also appreciate Ogolowa Marcel, Ejeh Monday, Drs Adamu Blessing, Adanu Williams, Ejeh Jerry, Okpanachi Jerome, Zirra Abraham, Anpe Nanshak, Mhon Frank Gerrard, Anyika Kingsley, Onwuama Kenechukwu, Ameh Odinya, Uchendu Chidebere, Peter Ofemile, Ehizibolo Peter, Olusola O. Oladipo, Abdulrazak I. Bello, Ogundele Abidemi, Ngbede Emmanuel, Ofemile Peter, Falalu Sakeena, Ayo Ake and Pharmacist James Fatika for their assistance in different capacities. I thank Mr Olu Ayegbusi, Mr Dennis and Mallam Yunusa of Chemical Pathology, Ahmadu Bello University Teaching Hospital, Veterinary Pharmacology and Veterinary Clinical Pathology Laboratories, respectively,

for their assistance. Finally I appreciate all members of ‘Chatham house’ (Veterinary Anatomy postgraduate class) for making my stay worthwhile.

The list of all those who contributed to this work is endless. Those whose names do not appear here are also highly appreciated, and may God reward you abundantly. Thank you.

ABSTRACT

The experiment was conducted with the aim of determining the ameliorative effects of L-glutamine administration on rectal temperature, haematological and oxidative stress changes in Red Sokoto goats during the hot-dry season. Twenty eight apparently healthy adult Red Sokoto goats of about 1-year-old, comprising 14 bucks (7 experimental and 7 control) and 14 non-pregnant does (7 experimental and 7 control) served as subject of the study. Goats in the experimental group were administered L-glutamine at 0.2 g/kg body weight *per os* once daily, while those in the control group were given distilled water for 21 days. Rectal temperature and, blood samples for haematological and biochemical analyses, were obtained 3 days before, during and on day 7 after L-glutamine administration. The thermal environmental variables of dry-bulb temperature, relative humidity, and temperature-humidity index during the study period had range values of 24.5 - 35.2 °C, 41.7 - 84.0 % and 74.1 - 84.0, respectively. The mean rectal temperature of 38.78 ± 0.06 °C recorded in experimental group was lower than the corresponding value of 38.93 ± 0.06 °C recorded in control group, during the period L-glutamine administration. The packed cell volume, haemoglobin concentration, erythrocyte count, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, and total leucocyte, neutrophil and lymphocyte counts, and neutrophil:lymphocyte ratio were higher ($P < 0.05$) in experimental group as compared to the control group. Erythrocyte osmotic fragility in experimental group was lower ($P < 0.05$) than in the control group only at 0.5 and 0.7 % during the period of L-glutamine administration. The MDA concentration in the experimental group (1.47 ± 0.1 nmol/L) was lower ($P < 0.05$) than the corresponding value of 1.75 ± 0.1 nmol/L recorded in the control group, during L-glutamine administration. In the experimental group, SOD activity (2.29 ± 0.04 IU/L)

was higher ($P < 0.05$) than that obtained in the control group (2.10 ± 0.04 IU/L) during L-glutamine administration. The glutathione peroxidase activities was higher ($P < 0.05$) in experimental than the control goats during L-glutamine administration. Catalase activity recorded in the experimental goats (48.96 ± 0.9 IU/L) was significantly ($P < 0.05$) higher than that obtained in control goats (44.04 ± 0.9 IU/L) during the period of L-glutamine administration. In conclusion, L-glutamine ameliorated the heat stress-induced changes in rectal temperature, haematological and oxidative stress parameters of Red Sokoto goats during the hot-dry season.

TABLE OF CONTENTS

Page

Title Page---------- i

Declaration---------- ii

Certification---------- iii

Dedication---------- iv

Acknowledgements---------- v

Abstract---------- vii

Table of Contents---------- ix

List of Tables---------- xiii

List of Figures---------- xiv

List of Appendices---------- xv

List of Abbreviations---------- xvii

CHAPTER ONE: INTRODUCTION---------- 1

1.1 Background of the Study---------- 1

1.2 Statement of Research Problem---------- 2

1.3 Justification of the Study---------- 3

1.4 Aim of the Study---------- 5

1.5 Objectives of the Study---------- 5

1.6 Statement of Research Hypotheses---------- 6

CHAPTER TWO: LITERATURE REVIEW---------- 7

2.1 Stress in Livestock---------- 7

2.1.1 Stress response in animals---------- 8

2.1.2 Thermal stress---------- 9

2.1.3 Reactive oxygen species and cellular redox system---------- 11

2.1.4 Reactive oxygen species and oxidative stress---------- 12
2.1.5 Antioxidants and their roles in amelioration of heat stress---------- 13
2.1.6 Biomarkers of stress---------- 15
2.2 L-glutamine ---------- 19
2.2.1 Physiological functions of L-glutamine---------- 19
2.2.2 Glutamine and glutathione---------- 22
2.3 Goats---------- 23
2.3.1 Domestication of goats---------- 24
2.3.2 Breeds of goats in Nigeria---------- 24
2.3.3 Thermoneutral zone in goats---------- 26
2.3.4 Effect of heat stress on goats---------- 26
2.3.5 Effect of heat stress on haematological parameters of goats---------- 28
CHAPTER THREE: MATERIALS AND METHODS---------- **30**
3.1 Experimental Site---------- **30**
3.2 Animals and Management---------- **30**
3.3 Experimental Measurements---------- **31**
3.3.1 Thermal environmental parameters ---------- 31
3.3.2 Determination of liveweight of goats---------- 31
3.3.3 Measurement of rectal temperature---------- 32
3.3.4 Collection of blood samples---------- 32
3.3.5 Evaluation of haematologic parameters---------- 32
3.3.6 Determination of serum total protein and albumin---------- 35
3.3.7 Determination of erythrocyte osmotic fragility---------- 36
3.3.8 Determination of malondialdehyde concentration---------- 36
3.3.9 Determination of serum antioxidant enzyme activities---------- 37

3.4 Data Analyses---------- 39

CHAPTER FOUR: RESULTS---------- 40

4.1 Thermal Environmental Parameters---------- 40

4.2 Effect of Treatment on Liveweight of the Goats during the Study Period---------- 42

4.3 Fluctuations in Rectal Temperature of Red Sokoto Goats administered with L-glutamine during the Hot-dry Season---------- 44

4.4 Effect of L-glutamine Administration on Haematological Parameters---------- 49

4.4.1 Effect of L-glutamine administration on packed cell volume---------- 49

4.4.2 Effect of L-glutamine administration on haemoglobin concentration------ 49

4.4.3 Effect of L-glutamine administration on red blood cell count---------- 50

4.4.4 Effect of L-glutamine administration on mean corpuscular volume-------- 50

4.4.5 Effect of L-glutamine administration on mean corpuscular haemoglobin- 50

4.4.6 Effect of L-glutamine administration on mean corpuscular haemoglobin concentration---------- 51

4.4.7 Effect of L-glutamine administration on total white blood cell count------ 51

4.4.8 Effect of L-glutamine administration on neutrophil count---------- 51

4.4.9 Effect of L-glutamine administration on lymphocyte count---------- 52

4.4.10 Effect of L-glutamine administration on neutrophil:lymphocyte ratio----- 53

4.5 Effect of L-glutamine on Biomarkers of Oxidative Stress in Red Sokoto Goats during the Hot-dry Season---------- 56

4.5.1 Erythrocyte osmotic fragility in Red Sokoto goats during the study period---------- 56

4.5.2 Variation in malondialdehyde concentration---------- 60

4.5.3 Variation in activities of antioxidant enzymes---------- 62

4.6 Sexual variation---------- 66

CHAPTER FIVE: DISCUSSION---------- 68

5.1 Thermal Environmental Parameters---------- 68

5.2 Fluctuations in Rectal Temperature of Red Sokoto Goats administered with L-glutamine during the Hot-dry Season---------- 69

5.3 Effect of L-glutamine Administration on Haematological Parameters---------- 70

5.4 Erythrocyte Osmotic Fragility in Red Sokoto Goats during the Study Period---------- 73

5.5 Variation in Malondialdehyde Concentration---------- 74

5.6 Variation in Antioxidant Enzyme Activities---------- 74

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS---------- 76

6.1 Conclusions---------- 76

6.2 Recommendations---------- 76

REFERENCES---------- 78

APPENDICES---------- 99

LIST OF TABLES

Table	Title	Page
Table 4.1:	Thermal environmental parameters in the pen during the study period----------	**41**
Table 4.2:	Fluctuations in Rectal temperature (°C) of Red Sokoto goats administered with L-glutamine during the hot-dry season-------	**45**
Table 4.3:	Erythrocytic parameters and serum protein of Red Sokoto goats administered with L-glutamine during the hot-dry season----	**54**
Table 4.4:	Leucocyte counts in Red Sokoto goats administered with L-glutamine during the hot-dry season----------	**55**

LIST OF FIGURES

Figure	Title	Page
Figure 4.1:	Effect of treatment with L-glutamine on liveweight of the goats during the study period----------	**43**
Figure 4.2:	Diurnal fluctuations in rectal temperature (°C) of Red Sokoto goats before L-glutamine administration in the hot-dry season----------	**46**
Figure 4.3:	Diurnal fluctuations in rectal temperature (°C) of Red Sokoto goats during three weeks of L-glutamine administration in the hot-dry season----------	**47**
Figure 4.4:	Diurnal fluctuations in rectal temperature (°C) of Red Sokoto goats day 7 after L-glutamine administration in the hot-dry season----------	**48**
Figure 4.5:	Dynamics of changes in erythrocyte osmotic fragility in Red Sokoto goats before L-glutamine administration during the hot-dry season---	**57**
Figure 4.6:	Dynamics of changes in erythrocyte osmotic fragility in Red Sokoto goats during 3-week L-glutamine administration in the hot-dry season----------	**58**
Figure 4.7:	Dynamics of changes in erythrocyte osmotic fragility in Red Sokoto goats day 7 after L-glutamine administration during the hot-dry season----------	**59**
Figure 4.8:	Effect of L-glutamine administration on serum malondialdehyde concentration in Red Sokoto goats during the hot-dry season----------	**61**
Figure 4.9:	Effect of L-glutamine administration on serum superoxide dismutase activity in Red Sokoto goats during the hot-dry season----------	**63**
Figure 4.10:	Effect of L-glutamine administration on serum glutathione peroxidase activity in Red Sokoto goats during the hot-dry season----------	**65**
Figure 4.11:	Effect of L-glutamine administration on serum glutathione peroxidase activity in Red Sokoto goats during the hot-dry season----------	**67**

LIST OF APPENDICES

Appendix	Title	Page
Appendix I:	Mean ± SEM erythrocyte osmotic fragility in both experimental and control goats the study period----------	100
Appendix II:	Dynamics of changes in oxidative stress parameters in Red Sokoto goatsadministered with L-glutamine during the hot-dry season-------	101
Appendix III:	Rectal temperature (°C) of goats before L-glutamine administration----------	102
Appendix IV:	Rectal temperature (°C) of goats week one of L-glutamine administration----------	103
Appendix V:	Rectal temperature (°C) of goats week two of L-glutamine administration----------	104
Appendix VI:	Rectal temperature (°C) of goats week three of L-glutamine administration----------	105
Appendix VII:	Rectal temperature (°C) of goats week after L-glutamine administration----------	106
Appendix VIII:	Packed cell volume (%) in Red Sokoto goats during the study period----------	107
Appendix IX:	Haemoglobin concentration (g/dL) in Red Sokoto goats during the study period----------	108
Appendix X:	Red blood cell count (x 10^6/µL) in Red Sokoto goats during the study period----------	109
Appendix XI:	Absolute white blood cell count (x 10^3/µL) in Red Sokoto goats during the study period----------	110
Appendix XII:	Absolute neutrophil count (x 10^3/µL) in Red Sokoto goats during the study period----------	111
Appendix XIII:	Absolute lymphocyte count (x 10^3/µL) in Red Sokoto goats during the study period----------	112
Appendix XIV:	Serum malondialdehyde concentration (nmol/L) in goats during the study period----------	113
Appendix XV:	Superoxide dismutase activity (IU/L) in Red Sokoto goats during the study period----------	114

Appendix XVI: Glutathione peroxidase activity (IU/L) in Red Sokoto goats during the study period---------- 115

Appendix XVII: Catalase activity (IU/L) in Red Sokoto goats during the study period---------- 116

LIST OF ABBREVIATIONS

ACTH	Adrenocorticotrophic hormone
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AVP	Arginine-vasopressin
CAT	Catalase
CRH	Corticotrophin-releasing hormone
Cu	Copper
DBT	Dry-bulb temperature
DNA	Deoxyribonucleic acid
EOF	Erythrocyte osmotic fragility
ERK	Extracellular signal-regulated kinase
GPx	Glutathione-peroxidase
GSH	Reduced glutathione
GSSG	Oxidised glutathione
H_2O_2	Hydrogen peroxide
Hb	Haemoglobin
H_o	Null hypothesis
$HO^{\cdot}$	Hydroxyl radical
HPA	Hypothalic-pituitary-adrenocortical axis
HR	Heart rate
JNK	cJun N-terminal kinase
MCHC	Mean corpuscular haemoglobin concentration
MDA	Malondialdehyde
Mn	Manganese
mTOR	Mammalian target of rapamycin
NaCl	Sodium chloride

NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Reduced NADP
$O_2^{\cdot -}$	Superoxide anion
PCV	Packed cell volume
PUFA	Polyunsaturated fatty acids
RBC	Red blood cell
RH	Relative humidity
RNA	Ribonucleic acid
$RO_2^{\cdot}$	Alkoxyl radical
ROS	Reactive oxygen species
RR	Respiratory rate
RT	Rectal temperature
SAM	Sympathetic adrenomedullary
Se	Selenium
SEM	Standard error of mean
SOD	Superoxide dismutase
TBARS	Thiobabituric acid reactive substances
THI	Temperature-humidity index
UK	United Kingdom
USA	United States of America
WBC	White blood cell count
Zn	Zinc

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Increasing world population and demand for animal protein have led to an increase in livestock production (Koknaroglu and Akunal, 2013). Small ruminants, including goats, occupy an important economic and ecological niche in agricultural systems throughout the developing countries (Devendra, 2001). In Nigeria and many other developing countries, goats are kept primarily for meat. They also represent a veritable resource for economic development, livelihood security and are of major cultural importance (Taneja, 1982; Terill, 1986). Livestock performance in developing countries is far behind that recorded in the developed world, although it has been predicted that the performance in developing countries (including Nigeria) would sustain the future growth of the world's meat production (Rendaudeau *et al*., 2012). The Red Sokoto goat is the predominant and the most widely distributed breed in the Northern Savannah zone of Nigeria. It is recognized for its high-quality skin, which is used in leather industry, locally and internationally (Akpa *et al*., 1998).

Goats are considered to be adapted to harsh environmental conditions better than other domestic species (Silanikove, 2000a), although their productivity is affected by extreme climatic conditions (Lu, 1989; Minka and Ayo, 2012). Goats are subjected to heat stress, when there is an imbalance between heat production within the body and heat loss. Under heat stress conditions, reactive oxygen species (ROS) are produced in excess of what the body can cope with. The increased ROS induce the lipoperoxidation of polyunsaturated fatty acids and, consequently, leading to the destruction of cell

membrane (Sunil Kumar *et al.,* 2011). The increased ROS production, induced by heat stress occurs in the skeletal muscular mitochondria through the elevation of membrane potential due to increase in oxygen consumption, especially at initial phase of heat exposure (Azad *et al*., 2010).

1.2 Statement of Research Problem

Goats are subjected to many stresses, including physical, nutritional, chemical, psychological and thermal stress. The productivity of goats in the tropics is adversely affected by the prevailing harsh environmental conditions, especially heat stress (Marai *et al*., 2007). During heat stress, there is a redistribution of the body resources, including protein and energy at the cost of increased growth, reproduction, production and health (Gupta *et al*., 2013a). Physiological and biochemical changes occur in the animal body, which directly or indirectly affect production (Ganaie *et al*., 2013). The hot-dry season in the Northern Guinea Savannah zone of Nigeria is thermally stressful to livestock, including goats (Minka and Ayo, 2012). In this zone, goats are mostly managed extensively, where they are left to graze on open field, with little or no provision for shelter. Browsing of goats on open fields during most of the hours of the day, make them susceptible to environmental stress (Gupta *et al*., 2013a). Shade provision can reduce the effect of direct sunlight (Minka and Ayo, 2007a), but this only has little effect on radiant heat. Ambient temperature is the most important factor negatively affecting farm animal performance and welfare (Koknaroglu, 2008). High ambient temperatue and high relative humidity induce heat stress in animals, especially during the hot-humid season. Heat stress affects all systems of the body, resulting in reduced libido in males (Perez-Crespo *et al*., 2008), reduced fertility in female (Sakatani *et al*., 2004) and increased susceptibility to diseases (Roth, 2008). Although goats are resistant

to thermal stress to a greater extent compared to other livestock (Silanikove, 2000a), they suffer from heat and cold stresses outside their comfort zone (Mishra, 2009). Environmental heat stress weakens efficient animal production, resulting in significant economic losses to farmers (Rhoads *et al*., 2013). Many investigations have been carried out in goats to demonstrate the beneficial role of antioxidant nutrients like vitamin C, A and E (Banerjee *et al*., 2003; Minka and Ayo, 2010; Sunil Kumar *et al*., 2011) when used against heat stress, but little attention has been paid to antioxidant amino acids, especially L-glutamine. Heat stress has been associated with reduced level of L-glutamine, the most abundant amino acid in plasma (Wu *et al*., 2011).

1.3 Justification of the Study

Goat is an important source of animal protein for humans in warm climates (Kirton, 1988), and the meat (chevon) from local breeds is sought after in many parts of the world (Devendra and Burns, 1983) because of its palatability. Rearing of animals, including goats under extreme environmental conditions reduces their productivity. The hot-dry season in the Northern Guinea Savanna zone, which extends from March to May (Igono *et al*., 1982), is thermally stressful to livestock, including goats (Ayo *et al*., 1988). The adverse effects of high ambient temperature and relative humidity on the health and productivity of goats have been documented (Jindal, 1980). Heat stress is one of the important climatological thermal conditions (Broucek *et al*., 2007) in the hot regions of the world (Altan *et al*., 2003). Its effects on livestock results in the generation of excessive ROS beyond the capacity of the tissue antioxidant defence system (Akinwade and Adebule, 2003), resulting in oxidative stress (Sies, 1991; Lykkesfeldt and Svendsen, 2007). In general, livestock with health problems and the most productive animals (for example, animals with the highest growth rate or milk

production) are at greatest risk of heat stress, thereby requiring the most attention (Nienaber and Hahn, 2007). Antioxidants, both enzymatic and non-enzymatic, provide necessary defence against oxidative stress as a result of thermal stress (Sunil Kumar *et al*., 2011). Antioxidant nutrient supplementation, especially vitamins C, A and E, and amino acids have been shown to attenuate the negative effects of environmental stress (Banerjee *et al*., 2003; Minka and Ayo, 2010; Sunil Kumar *et al*., 2011). L-glutamine is a conditionally-essential amino acid (Wu *et al*., 2011). Amongst many of the physiological functions of the amino acid, it exhibits antioxidant property, acting directly as a scavenger of ROS and indirectly as an important intermediate in the synthesis of glutathione. Glutathione is the most abundant small molecular-weight antioxidant, crucial for defending cells against oxidative stress (Wu *et al*., 2004; Morris *et al*., 2008; Stipanuk *et al*., 2009). L-glutamine is a major substrate for multiple metabolic pathways and has a plethora of key regulatory functions in animals (Wu *et al*., 2011). It is a major energy substrate for rapidly-dividing cells, including enterocytes and immunocompetent cells. Thus, L-glutamine plays a vital role in the maintenance of gut integrity and immunity functions (Curi *et al*., 2005; Li *et al*., 2007; Xi *et al*., 2012). Wu *et al*. (2013) reported that L-glutamine play important roles in regulating gene expression, cell signaling, antioxidative responses, neurotransmission and immunity in mammals.

The physiological parameters of rectal temperature (RT), heart rate (HR) and respiratory rate (RR) are the most relevant on-the-spot diagnostic parameters of the state of an animal's health before any laboratory analysis is carried out (Ayo *et al*., 1998). They are important in evaluating adaptability of domestic animals to various environmental stress factors (Bianca, 1976; Ayo *et al*., 1998). The fluctuations in the parameters, partly

caused by variations in the ambient temperature above or below the zone of comfort, provide accurate information on the effect of stress factors acting on the body (Piccione and Caola, 2002). Farm animals try to cope with stressors using behavioural and physiological stress responses, aiming to restore homeostasis. When the responses are not successful, or when they are overwhelmed, typical behavioural and physiological symptoms of stress occur. In this case, the welfare of the animal is clearly compromised (Etim *et al*., 2013). Behavioural responses are the first to be invoked in challenging thermal environments, and they reflect the preferences of the animals exposed to specific conditions. The resting pattern of the animals provides a readily observable indicator of the environment adequacy and, thus, the level of thermal balance. Changes in haematological values are important in evaluating the responses of animals to various conditions (Zakari *et al*., 2014), including heat stress. Haematologic profiles in animals provides means of assessing the internal environment and understanding the cause of impairment in homeostasis (Sattar and Mirza, 2009). Although, the beneficial roles of L-glutamine have been documented in the pig and poultry (Soltan, 2009; Wu *et al*., 2011), such information is lacking in goats.

1.4 Aim of the Study

The aim of the study was to determine the ameliorative effects of L-glutamie administration on some physiological responses of goats during the hot-dry season.

1.5 Objectives of the Study

They were to determine:

a. Rectal temperature, haematologic (packed cell volume, erythrocyte count, haemoglobin concentration, total and differential leucocyte counts, and

neutrophil/lymphocyte ratio), and oxidative stress parameters of Red Sokoto goats during the hot-dry season.

b. Ameliorative effect of L-glutamine on heat stress-induced changes in rectal temperature, haematologic and oxidative stress parameters of Red Sokoto goats during the hot-dry season.

1.6 Statement of Research Hypotheses (Ho)

a. Heat stress does not alter significantly the physiologic responses of goats during the hot-dry season.

b. Administration of L-glutamine exerts no significant effects on the physiologic responses of goats to heat stress during the hot-dry season.

CHAPTER TWO

LITERATURE REVIEW

2.1 Stress in Livestock

In animal husbandry, stress is an unavoidable (reflex) reaction that all animals experience in their lives; and is perceived as a mental or bodily tension, produced by external or internal stressors (Selye, 1977, von Borell, 2001). Stress, though very familiar, is difficult to define, and is the consequence of adverse effects of the environment or management systems that evoke adaptive changes in the physiology or behaviour of the animal to avoid malfunctioning (Terlouw *et al*., 1997; Fazio and Ferlazzo, 2003). Stress has adverse economic impact on growth and quality of animal products (Cuccuzza *et al*., 2014). It is a threat to which the body needs to adjust. Stress can be physical, psychological or interoceptive in nature, but usually contains components of all three classifications (von Borell, 2001). Depending on the duration, stress can either be acute or chronic. In general, chronic stress has greater potential impact on health and welfare of animals than acute stress because animals are exposed and react to stressor(s) for longer periods, thereby causing prolonged disruption to homeostasis and related biologic responses. Disruption in homeostasis adversely affects nervous coordination, reproduction, metabolism, cardiovascular activity, immune function, behavior, and under certain circumstances may produce pathologic lesions (Obernier and Baldwin, 2006; Earley *et al*., 2010). Whether a stressor can be classified as harmful depends on the way an animal is able to cope with a threatening situation as it gains a state of homeostasis. Thus, an individual's state of welfare can be measured and monitored in terms of behavioural and physiological alterations (Fazio and Ferlazzo, 2003; Kashinakunti *et al*., 2010).

2.1.1 Stress response in animals

The response to stressors requires a progression of events, beginning with sensing and signaling an animal's various biological mechanisms that a threat exists (Ewing *et al*., 1999). The response to stressors requires a progression of events beginning with sensing and signaling the animal's various biological mechanisms that a threat exists. These events are followed by activation of neurophysiologic mechanisms to resist and prevent major damage. The various sensory detectors not only receive information but transform that information into neural signals that are transmitted to either cognitive and/or non-cognitive centres of the nervous system to generate a co-ordinated response to the challenge. This response may be behavioural, autonomic, neuroendocrine and/or immunological (von Borell *et al*., 2007). The reaction of an animal to a stressor depends on the duration and intensity of the stressor, the animal's previous experience, its physiological status and the immediate environmental restraints (Fazio and Ferlazzo, 2003). It appears that an animal's perception of a stimulus as stressful is necessary to mount a stress response; and the activation of the stress response system does not occur, if an animal does not perceive an event to be fearful or stressful (Minton, 1994). Since stress occurs when an animal's homeostasis is disrupted, the stress response consists of a set of physiologic mechanisms, designed to return to homeostasis (Candiani *et al*., 2008). Two distinct systems link the initial perception of the stressors to this response; that is, the sympathetic adrenomedullary (SAM) axis, and the hypothalamic-pituitary-adrenocortical (HPA) axis. SAM axis involves the hypothalamus, pituitary and the sympathetic neural pathways to the adrenal medulla, and the release of adrenaline by the adrenal gland. It is responsible for the short-term (acute) behavioural and endocrine responses that prepare the animal for an immediate response to environmental adjustment, referred to as the fear-fight-flight syndrome (von Borell, 2001). The HPA

stress-response systems represent 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: viz, the alarm reaction; stage of resistance; and stage of exhaustion.

The alarm stage is characterized by mobilisation of all defence mechanisms in the body to combat adverse effects of stress. If the stress is so severe that continued exposure is incompatible with life, the animal will die within a few hours during this stage. Otherwise, a stage of adaptation or resistance will ensue, since no animal can be maintained continuously in a state of alarm. The adaptive stage is characterized by the cessation or diminishing of the initial symptoms. After a more prolonged exposure to the stressor, this acquired adaptation is lost and the third stage (stage of exhaustion) ensues, progressing to death, unless the animal receives emergency aid from some outside sources (Selye, 1946). The HPA axis responds to a variety of stressors by synthesizing and releasing four key hormones: corticotrophin-releasing factor/hormone (CRH), arginine-vasopressin (AVP), adrenocorticotrophic hormone (ACTH), and glucocorticoids. Glucocorticoids serve as the final effectors of HPA axis and are critically involved in modulating the response to any psychologic or physical stressor (Earley *et al*., 2010; Bellavance and Rivest, 2014).

2.1.2 Thermal stress

Livestock are exposed to numerous stresses, including physical, nutritional, chemical, psychological and thermal stresses. In the ever-changing climatic scenario, thermal stress poses a great threat to animal productivity (Gupta *et al*., 2013a). Thermal stress is the perceived discomfort and physiological strain, associated with an exposure to an

extreme hot or cold environment (Ganaie *et al*., 2013). In tropical and sub-tropical regions, high ambient temperature is the major constraint on animal production (Marai *et al*., 2007; Nardone *et al*., 2010); but the extreme low temperatures in temperate regions are also detrimental to livestock, as they challenge the animal's ability to maintain energy, thermal, water, hormonal and mineral balance (Silanikove, 1992). During thermal stress, physiological and biochemical changes occur in the body of the animal, which directly or indirectly affect the production. During thermal stress, the body resources, including protein and energy are redistributed, and the cost is decreased growth, reproduction, production and impaired health (Ganaie *et al*., 2013).

Ambient temperature determines metabolic rate, heart rate and other important body parameters of animals, so an extreme temperature can easily distress the animal body. The effect of high ambient temperature is further aggravated, when heat stress is accompanied by high relative humidity (Gupta *et al*., 2013a). A variety of livestock production systems and/or geographical locations are associated with exposure to environmental conditions outside their thermo-neutral zone (West, 2003). According to West (2003), heat stress may be characterized by intense radiation energy, for an extended period of time, with high relative humidity. Thus, there is often little relief from heat during the evening hours, and intense bursts of combined heat and relative humidity further depress performance of animals. Reduction in performance of animals during heat stress is due mainly to high ambient temperature. The debilitating effects may be further compounded when elevated ambient temperature is coupled with solar radiation (Al-Haidary, 2006; Marai *et al*., 2007). Heat stress is of great concern among livestock owners in tropical countries because it decreases the antioxidant level, various hormones and electrolyte concentration, but increases lipid peroxidation *in vivo*.

Animals show various responses to heat stress at physiological, cellular, biochemical and molecular levels. Changes in various genes expression help in stress amelioration at cellular level. At molecular level, increased expression of stress-related genes and, hence, their protein products may help to combat the stress (Gupta *et al*., 2013a).

2.1.3 Reactive oxygen species and cellular redox system

Reactive oxygen species (ROS) are used to broadly describe O_2-derived free radicals such as superoxide anion (O_2•−), hydroxyl (HO•), peroxyl (RO_2•), and alkoxyl (RO•) radicals, as well as O_2-derived nonradical species such as hydrogen peroxide (H_2O_2) (Halliwell and Cross, 1994). They are majorly produced in the mitochondria in complex processes, involving the electron transport chain (Ames *et al*., 1993). Mitochondria-derived O_2•− is dismutated to H_2O_2 by manganese superoxide dismutase, and, in the presence of metal ions, highly reactive HO• is generated via Fenton and/or Haber–Weiss reactions, inflicting significant damage on cellular proteins, lipids, and DNA. (Andreyev *et al*., 2005). Most ROS are unstable due to their unpaired electron. This condition is thermodynamically unfavourable. Thus, the molecules will attempt to reach a more stable state by reacting with another molecule, cleaving a hydrogen atom from a carbon-hydrogen bond, and donating or accepting electrons from neighboring compounds (Halliwell and Gutteridge, 2007). This set up a chain reaction that ultimately lead to cellular damage and/death. There is a constant balance between endogenous antioxidant systems and generation of ROS. This equilibrium is shifted in favour of ROS in many conditions, resulting in oxidative stress. The result of this is damage to cellular molecules such as lipids, proteins and DNA. ROS play physiologic role as signaling molecules at low intracellular concentrations (Halliwell, 2012). They are deleterious, when their concentration rises above physiologic concentrations. The

chain reactions set up by ROS are usually intercepted by endogenous antioxidant systems.

2.1.4 Reactive oxygen species and oxidative stress

Oxidative stress is an active field of research in veterinary medicine, implicated in numerous disease processes, including sepsis, mastitis, enteritis, pneumonia, respiratory and joint diseases (Lykkesfeldt and Svendsen, 2007). It has also been associated with pathogenetic mechanisms of several human diseases, including atherosclerosis, neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases, cancer, diabetes mellitus, inflammatory diseases, as well as psychological diseases or ageing processes (Durackova, 2010). Although ROS are constantly generated *in vivo* as an integral part of metabolism, they may cause oxidative stress when their level exceeds the threshold value (Rahal *et al*., 2014), triggering progressive destruction of polyunsaturated fatty acids (PUFA), and ultimately leading to membrane destruction and cell death (Kumar *et al.*, 2011). Various kinds of stress, including heat and cold stress, increase the production of ROS, generated during normal body functions (Boveris and Chance, 1973). Although, low levels of ROS are essential for many beneficial biological functions such as cell signaling, apoptosis, necrosis and phagocytosis (Noori, 2012), their accumulation due to over-production or a decreased antioxidant defence mechanism causes oxidative stress. Oxidative stress leads to damage of biomolecules, including DNA/RNA, proteins and lipid peroxidation of membranes and disruption of normal cell metabolism (Spurlock and Savage, 1993). Functional oxidative modifications of cellular proteins, both reversible and irreversible cause cellular dysfunctions (Ho *et al*., 2013).

Deficiency of ROS may occur due to different kinds of stress (McDowell *et al*., 2007). Free-radical oxidation is activated in animals under various types of stresses, and lipid peroxidation products accumulate in various organs (Yarovan, 2008). There are reports which establish that heat stress stimulate excessive production of ROS (Bernabucci *et al*., 2002; Sivakumar *et al*., 2010).

2.1.5 Antioxidants and their roles in amelioration of heat stress

The body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced *in situ* (endogenous) or externally supplied through foods and/or supplements (exogenous). Endogenous and exogenous antioxidants act as "scavengers" by preventing and repairing damages caused by ROS (Valko *et al*., 2006; Chatterjee *et al*., 2007). Animals including goats have developed antioxidant defences due to exposure to oxidants from a variety of sources (Kirschvink *et al*., 2008). Heat stress results in generation of excessive ROS, which can induce oxidative stress, if the antioxidant capacity of the body is overwhelmed. Antioxidants are substances that, when present at low concentrations compared to those of the oxidizable substrate, significantly delay or inhibit oxidation of that substrate (Halliwell, 1997).

Endogenous antioxidant compounds in cells are classified as enzymatic antioxidants and non-enzymatic (metabolic and nutrient) antioxidants (Gupta *et al*., 2013b). The major enzymatic antioxidants include superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and glutathione reductase (GRx) (Fridovich, 1995; Halliwel, 2007; Pacher *et al*., 2007). The catalytic activity of these enzymes allows the transformation of superoxide anion into hydrogen peroxide (H_2O_2) and water, thereby

mitigating the harmful effects of ROS. SOD catalyses the dismutation of superoxide to H_2O_2, and it is considered the first defence against ROS (Halliwell and Chirico, 1993). Catalase catalyses the break-down of H_2O_2 to water and oxygen. GPx in addition to catalysing H_2O_2 to water and oxygen, also contributes to oxidative defence of the animal tissue by catalysing reduction of hydrogen and lipid peroxides. This prevents oxidative damage in cell membranes. Increased activity of SOD results in increased production of H_2O_2; and, thus, a simultaneous increase in GPx and CAT activities is required for protection against heat stress-induced ROS (Dröge, 2002).

Trace elements such as selenium (Se), zinc (Zn), copper (Cu) and manganese (Mn) play important catalytic role in the enzymatic activity of GPx and SOD (Zn, Mn, Cu) (Mates, 2000). Glutathione reductase, a flavoprotein enzyme, regenerates glutathione from oxidized glutathione (GSSG), with nicotinamide adenine dinucleotide phosphate (NADPH) as a source of reducing power (Halliwell, 2007). The non-enzymatic antioxidants are also divided into metabolic antioxidants and nutrient antioxidants. Endogenous antioxidants such as lipoid acid, glutathione, L-arginine, L-glutamine, co-enzyme Q10, melatonin, uric acid, bilirubin, metal-chelating proteins and transferrin (Willcox *et al*., 2004). They are produced in the body as a result of metabolism, and are also termed metabolic antioxidants, while nutrient antioxidants, belonging to exogenous antioxidants, are compounds, which cannot be produced in the body and must be provided through foods or supplements. They include vitamin E, vitamin C, carotenoids, trace metals (Se, Mn, Zn), flavonoids, omega-3 and omega-6 fatty acids (Powers and Jackson, 2008).

Nutrient antioxidants are involved in ROS detoxification (Gupta and Singh, 2013), and they facilitate the neutralisation of oxidative stress by endogenous antioxidants (Donaldson, 2004). Results obtained from an increasing body of research have convincingly demonstrated that antioxidant supplementation attenuates adverse effects of heat stress in goats (Sivakumar *et al*., 2010), sheep (Sejian *et al*., 2014; Chauhan *et al*., 2014), cattle (Zhang *et al*., 2014) and other domestic animals (Mert *et al*., 2014; Zhang *et al*., 2015).

2.1.6 Biomarkers of stress

A biomarker, or biological marker, generally refers to a measurable indicator of some biological state or condition. Biomarkers are often measured and evaluated to examine normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (Strimbu and Tavel, 2010). Numerous and rapidly evolving methodologies for evaluating oxidative stress in livestock are available to clinicians and researchers, each with their own distinct advantages and disadvantages (Celi, 2011). Changes in rectal temperature, respiratory rate and heat rate can be used to assess heat stress in goats (Minka and Ayo, 2012; Sharma *et al*., 2013)

Haematological examination is a reflection of an animal's responses to its external and internal environments (Esonu *et al*., 2001). Thus, blood values are used for evaluating stress and the well-being of the animal. At present, neutrophil:lymphocyte ratio is a well-accepted indicator for stress assessment in animals (Stanger *et al*., 2005). Erythrocyte osmotic fragility may be used as an indirect measure of lipid peroxidation (Uchendu *et al*., 2011; Minka and Ayo, 2011). Malondialdehyde, a by-product of peroxidation of polyunsaturated fatty acids is also a useful indicator of stress following

their reaction with thiobabituric acid (Janero, 1990). At the molecular level, there is alteration in expression of stress genes to counter stress conditions (Collier *et al*., 2008). Heat stress induced transcriptional activation and accumulation of heat shock proteins (HSP) is well known (Kregel, 2002). HSP expression act as a potential indicator of adaptation of goats to harsh environmental stress (Dangi *et al*., 2014). Serum antioxidant enzymes have been used as indicators of oxidative stress in goats (Qwele *et al*., 2013)

Absence of chronic stress is one of the pre-requisites of animal welfare. During stress, various endocrine responses are involved in the improvement of the fitness of the individuals (Mostl and Palme, 2002). Thus the plasma levels of hormones may be used as indicator of stress. Many hormones, for example ACTH, glucocorticoids, catecholamines and prolactin are involved in the stress response (Matteri *et al*., 2000). The adrenal glands have a key-role in hormonal reactions to stress as they are involved in both HPA and SAM systems (Moberg, 2000). Adverse situations trigger the adrenals to increase secretion of glucocorticoids and catecholamines; the front-line hormones to overcome stressful situations (Mostl and Palme, 2002). The hormones are determined as a parameter of adrenal activity and, thus, of disturbance.

2.1.6.1 Erythrocyte osmotic fragility as a biomarker of oxidative stress

The erythrocyte osmotic fragility is an *in vitro* test used to determine the extent of haemolysis produced by osmotic stress (Minka and Ayo, 2010), and this is dependent on the degree of functional integrity of the cell membrane, when placed in varying concentrations of salt (Oyewale *et al*., 2011). Erythrocytes are frequently used to evaluate oxidative stress because their membranes are rich in polyunsaturated fatty

acids (PUFA), which is a primary target for ROS, thus making the cells susceptible to lipid peroxidation (Brzezinska-Slebodzinska, 2001; Altan *et al*., 2003). The normal erythrocyte is remarkable for its durability, but its tolerance is altered under certain pathologic conditions (Sharma *et al*., 2009). Apart from its main role in transporting oxygen and carbon dioxide, the erythrocyte plays also an important role in antioxidative defence of the body. Direct exposure to ROS results in shortening of the half-life of the erythrocyte, even by 50% (Olszewska *et al*., 2012).

Environmental factors, including extremes of ambient temperature and high relative humidity provoke excessive generation of ROS (Vani *et al*., 2010) as a result of increased metabolism (Ramnath *et al*., 2008). The ROS induce oxidative damage of macromolecules, including membrane lipids (Altan *et al*., 2010; Celi, 2011). Extensive peroxidation of membrane lipids causes changes in cell fluidity, a fall in membrane potential and an increase in permeability to different ions that finally lead to haemolysis and cell death (Padayatty *et al*., 2003; Williams *et al*., 2008; Ambali *et al*., 2010; Bitla *et al*., 2011). Continuous exposure of haemoglobin to high concentration of oxygen and iron (Hanzama and Watanabe, 2000; Kollanjiappan *et al*., 2002), the inability of the erythrocyte to repair its membrane due to lack of nucleus (Bernabucci *et al*., 2002; Dorđevic *et al*., 2008) and depletion of the antioxidant status (Gultekin *et al*., 2001; Mansour and Mossa, 2009; Nazifi *et al*., 2009; Ambali *et al*., 2010) are amongst factors responsible for increased vulnerability of erythrocytes to oxidative stress. Thus, erythrocytes constitute an appropriate model to study oxidative stress (Aliciguzel, 2001). Haemolysis has been shown to increase in different animals, including transported goats (Minka and Ayo, 2007b; 2010), pigs (Asala *et al*., 2011) and horses (Yaqub *et al*., 2014).

2.1.6.2 Malondialdehyde as a biomarker of oxidative stress

Lipids, in particular those that are polyunsaturated, are prone to oxidation. Lipids are one of the most susceptible substrates to ROS damage, and biomarkers of lipid peroxidation are considered the best indicators of oxidative stress (Georgieva, 2005). Malondialdehyde (MDA) is one of the several low molecular-weight end-products formed during the ROS-induced decomposition of polyunsaturated fatty acids (PUFA) (Janero, 1990). MDA readily reacts with thiobarbituric acid, producing a red pigment which can be easily measured by spectrophotometry in the form of thiobarbituric acid-reactive substances (TBARS) (Janero, 1990). It is worth noting that the MDA assays have been criticized for low specificity and artifact formation, since only a fraction of the MDA measured is generated *in vivo*.

2.1.6.3 Rectal temperature as a biomarker of stress

Rectal temperature (RT) is a parameter that illustrates the mechanism of adaptation. Several researchers have studied rectal temperature as a physiologic parameter of adaptation in ruminants (Otoikhian *et al*., 2009; Sharma *et al*., 2013). Body temperature is a good measure of heat tolerance in animals. Silanikove (2000b) and Keim *et al*. (2002) reported that the best physiological parameter to objectively monitor animal welfare in hot environment is the RT. The hypothalamus serves as the main integrative control centre for the homeostasis of body temperature. It receives information from peripheral and central nervous systems, and exerts appropriate hormonal changes to maintain the internal milieu of the animals (Salama *et al*., 2014). When the physiological mechanism of an animal fails to negate the excessive heat load, RT increases. Such exposure of animal to heat stress evokes a series of drastic changes in

the biological functions, which include a decrease in feed intake, disturbances in water, protein, energy and mineral balances, enzymatic reactions, hormonal secretions and blood metabolism (Gwatibaya *et al*. 2007; Marai *et al*. 2007). Gebremedhin *et al*. (2008) reported that RT is perhaps the most reliable indicator of thermal heat stress because it drives other heat stress alleviating mechanisms.

2.2 L-glutamine

L-glutamine is a neutral amino acid, considered as a conditionally-essential amino acid (Yu *et al*., 2013). It is the most abundant amino acid in physiological fluids (cytoplasm, plasma, milk and foetal fluid) (Kwon *et al*., 2003; Wu, 2009; Xi *et al*., 2012), and in both plant and animal proteins (Li *et al*., 2011). L-glutamine was first isolated from beet juice in 1883 and later from edestin (a protein) in 1933; and was first synthesized chemically in 1933 (Wu *et al*., 2011). Although L-glutamine is synthesized *de novo* in almost all cells in the animal's body, it is synthesized mainly in the skeletal muscle (Patrick, 2000; Wernaman, 2008) by the enzyme glutamine synthase from glutamate and ammonia. The high levels of L-glutamine in physiologic fluids (Wu *et al*., 2011) and the low levels in catabolic states such as occur in trauma, sepsis and oxidative stress (Boelens *et al*., 2001; Gleeson, 2008) suggest that L-glutamine is conditionally-essential in both health and disease.

2.2.1 Physiological functions of L-glutamine

Besides being a constituent of protein, L-glutamine performs several physiologic functions, which include participation in multiple metabolic pathways and regulatory mechanisms (Roth, 2008; Wu *et al*., 2011). L-glutamine is the major energy substrate

for rapidly-dividing cells, including enterocytes, immunologically-challenged lymphocytes and other cell types, providing adenosine triphosphate (ATP) for intracellular protein turn-over (Xi *et al*., 2012), nutrient transport through plasma membrane, cell growth and migration as well as maintaining the integrity of cells (Curi *et al*., 2005). L-glutamine participates in the maintenance of acid-base balance and cellular volume in animals (Roth, 2008). It is the main interorgan carrier of nitrogen, and provides carbon atoms for intermediary metabolism (Young and Ajami, 2001; Newsholme *et al*., 2003). It is also a precursor for the synthesis of purine and pyrimidine nucleotides, which are essential for the proliferation of cells. It is a major amino acid for the endogenous synthesis of citruline and arginine in most mammals, including pigs, sheep, goats and cattle (Hanczakowska and Niwińska, 2013). L-glutamine contributes to the synthesis of polyamines, amino sugars, and is essential for the synthesis of nicotinamide adenine dinucleotide phosphate (NADP) co-factors (Young and Ajami, 2001). Importantly, L-glutamine serves as a precursor of glutamate in the synthesis of glutathione; the most abundant small-molecular-weight antioxidant in cells, crucial for defending cells from oxidative stress (Wu *et al*., 2004; Morris *et al*., 2008; Stipanuk *et al*., 2009; Mates *et al*., 2013).

L-glutamine down-regulates the expression of genes responsible for oxidative stress and immune activation, and increases the expression of genes that are necessary for cell growth and removal of oxidants (Hanczakowska and Niwińska, 2013). L-glutamine has been shown to influence several cellular signaling pathways, including the extracellular signal-regulated kinase (ERK), cJun N-terminal kinase (JNK) (Ko *et al*., 2001) and the mammalian target of rapamycin (mTOR) pathways in diverse cell types, including skeletal muscle, the small intestine and placental cells (Kim *et al*., 2011a; Xi *et al*.,

2010). A significant body of evidence indicates that glutamine preserves the gut barrier function and prevents permeability to toxins and pathogens from the gut lumen into mucosal tissue and circulation (Rao and Samak, 2012).

Low concentrations of glutamine in plasma reflect reduced stores in muscle. This reduced availability of glutamine in catabolic states seems to correlate increased morbidity and mortality (Boelens *et al*., 2001); thus, it was hypothesized that supplementation with glutamine may counteract or attenuate metabolic catabolism during critical illness, trauma, sepsis and inflammatory states (Wernaman, 2008). When stress is prolonged, production of glutamine in skeletal muscle may not meet the demand in organs and lymphoid tissue and a deficiency occurs (Patrick, 2000); thus, the need for exogenous supplementation. Glutamine is applied under clinical conditions as an oral, parenteral or enteral supplement, either as a single amino acid or in the form of glutamine-containing dipeptides in critically-ill patients (Melis *et al*., 2004; Roth, 2008). This has been shown to counteract mucosal atrophy, reduce length of hospital stay and decrease mortality (Flaring *et al*., 2003). Glutamine is used as a constituent of most cell culture media, and has been shown to prevent apoptosis and stimulate cell growth (Field *et al*., 2002). The role of glutamine has been extensively studied in porcine, ovine and avian species (Kwon *et al*., 2003; Soltan *et al*., 2009; Wu *et al*., 2011; 2013; Jazideh *et al*., 2014), but there is paucity of information on the effects of its administration in the caprine species. The finding that glutamine is an intermediate amino acid in the synthesis of the antioxidant, glutathione suggests that it may also be beneficial in oxidative stress conditions in goats. There is virtually no report of adverse or harmful effects, involving glutamine supplementation by enteral or parenteral route at an

appropriate dose, both in short- and long-term administration (Wernaman, 2008; Wu *et al*., 2011; Lindinger and Anderson, 2014).

2.2.2 Glutamine and glutathione

The tripeptide glutathione (GSH, gamma-glutamyl-cysteinyl-glycine) is quantitatively the most important endogenous scavenger system, present at high concentrations in most mammalian cells (Flaring *et al*., 2003). GSH is synthesized from glutamate (which in turn is synthesized from glutamine), cysteine and glycine through reactions catalysed by two cytosolic enzymes; gama-glutamylcysteine ligase and glutathione synthase. It has many protective and metabolic functions within the cells, prominent among which is its role in the defence against oxidative stress and in the detoxification of xenobiotics (Humbert *et al*., 2005). Due to its thiol group, GSH protects cell membrane against ROS such as hydrogen peroxide, and is readily oxidized to glutathione disulfide (GSSG), which is lost from the cells (Morris *et al*., 2008). The intracellular GSH concentration is the final result of the dynamic balance between GSH synthesis and the combined rate of intracellular GSH consumption and efflux (Griffith, 1999). In addition, GSH maintains the thiol groups of many proteins in the reduced form, which is required for their normal function. Similarly in erythrocytes, GSH helps to maintain iron in its reduced form (Griffith *et al*., 1981; Wu *et al*., 2004). Extensive oxidation of intracellular GSH promotes oxidative damage to protein and lipids, and compromises the structural integrity of the cell. GSH depletion occurs in conditions characterized by oxidative damage, and it takes place parallel with a progressive reduction in glutamine concentration. Additionally, L-glutamine plays an antioxidant role through preservation of intracellular NADPH levels, required for glutathione recycling.

2.3 Goats

The goat is a member of the family *Bovidae*, closely related to the sheep, and both species are in the goat-antelope sub-family, *Caprinae*. There are over 300 distinct breeds of goats. Goats are one of the oldest domestic species (Linda *et al*., 2004; Fernandez *et al*., 2006). They fulfill multiple roles in the household nutrition and food security as they provide family with proteins as milk and meat of high biological value, and are also used for their skin, fibre and manure (Devendra, 2013). Goats are of great importance in many developed countries, for the production of high-quality products and the achievement of sustainable development of rural areas, and in developing countries to exploit marginal agricultural resources (Pariset *et al*., 2009). Goat meat production is of great potential for improving the nutritional and the socio-economic well-being of human populace of developing countries (Griffin *et al*.,1992; Gadiyaram and Kannan, 2004; Jibir *et al*., 2010). Goats are often dismissed as the "poor man's cow" (MacHugh and Bradley, 2001), and are considered to be adapted to water scarcity and food shortage. They have the ability to utilise various types of forages and tolerate harsh environmental conditions (Silanikove, 2000b). In most developing countries, including Nigeria, goats are kept primarily for meat, largely as part of subsistent agricultural system (Taneja, 1982; Terill, 1986). Goats represent a veritable resource for economic development and livelihood security in addition to major cultural importance. The population of goats in Nigeria was estimated at 58 million (FAO STAT, 2013); of which traditionally-reared stock contributes 99.97%, while 0.03% of the stock is commercially managed.

2.3.1 Domestication of goats

The origins of domestic goats (*Capra hircus*) remain uncertain and controversial, but archaeological evidence suggests that they were probably first domesticated in the Fertile Crescent region of the Near East about 10,000 years ago (Porter, 1996; Zeder and Hesse, 2000). In addition, accumulating archaeological evidence indicates that goats, in the form of their wild progenitor-the bezoar (*Capra aegagrus*), were the first wild herbivores to be domesticated (MacHugh and Bradley, 2001). Some studies hint that a second domestication in Pakistan could have given rise to the Cashmere breeds (Meadow, 1996; Porter, 1996). Others suggest that at least two wild species of *Capra* (Shackleton, 1997), apparently, contributed to the gene pool of domestic goats (Barker, 1983). This seems possible in the light of the enormous morphological diversity among the more than 300 goat breeds. Goats are the most adaptable and geographically wide-spread livestock species, ranging from the mountains of Siberia to the deserts and tropics of Africa. They are the main economic resource in numerous developing countries, and are growing in economic importance in western countries. Domestic goats might have played a central role in the Neolithic agricultural revolution and the spread of human civilisation around the globe (Porter, 1996; Pringle, 1998). From an agricultural stand-point, the world's 700 million goats provide reliable access to meat, milk, skins, and fibre for small farmers, particularly in developing countries.

2.3.2 Breeds of goats in Nigeria

Indigenous goats in Nigeria belong to three distinct breeds: The long-legged Sahel, found in the arid and Sahel regions; the relatively small body-sized Red Sokoto, found in the Savanna zone; and the hardy, short-legged West African Dwarf, restricted to the

high altitude areas and humid forest of the south and the Southern Guinea Savanna zone (Adu *et al*., 1979). The Sahel goat originated in the Sahelian North-West Africa, and is found along the northern fringes of the country (Mason, 1988). The Red Sokoto goat originated in Northern Nigeria and is the predominant breed in the Northern part of the country. The West African Dwarf goats are trypano-tolerant (Hoste *et al*., 1988), and are able to inhabit the trypano-endemic humid zones of southern Nigeria (Akpa *et al*., 1998). Goats possess the ability to tolerate harsh climate, remarkable capacity from drought and suitability to traditional systems due to small size, short generation interval (Odubote, 1996; Silanikove, 2000b). They have the ability to thrive on poor-quality diets, resulting from scarce grazing on marginal land (Hoste *et al*., 1988), which make the animals strategic in increasing livestock productivity in rural agricultural systems (Fitzugh *et al*., 1992).

2.3.2.1 The Red Sokoto goat

The Red Sokoto goat is characterised by a uniformly dark-red coat colour, short and horizontal ears, and horns in both sexes. The goats are mostly reared under the traditional extensive management system, with little or no shelter throughout the year. They serve as a good source of meat. The high-quality skin obtained from the goats is used in leather industries, and the goats provide an income to the small-scale farmers. Males are heavier (average of 27 kg) than the females (average of 25 kg). The breed is well adapted to the climate of the West and North African regions, including the Northern Guinea Savannah zone of Nigeria, which has contributed to its great population density in the regions (Ayo *et al*., 2009).

2.3.3 Thermoneutral zone in goats

Thermoneutral zone is the range of ambient temperatures without regulatory changes in metabolic heat production or evaporative heat loss (Kingma *et al*., 2012). It is a convenient means to describe schematically the interrelationships between an animal and its environment. The lower critical ambient temperature range point and the upper critical ambient temperature points define the limits of the thermoneutral zone (Robertshaw, 1981; Silanikove, 2000a). Although the lower critical temperature for goats is not specified, the limits of thermoneutrality for goats may be taken as a climatic environment having an air temperature of 13 - 27 °C, relative humidity of 60 - 70 %, wind velocity of 5 - 8 km/h, and a medium level of radiation (Misra and Puneet, 2009). Environmental conditions outside these ranges are considered stressful for goats. Generally, the thermoneutral zone depends on age, species, breed, feed intake, diet composition, previous state of temperature acclimation or acclimatization, tissue insulation (fat, skin), and the behaviour of the animal (Yousef, 1985). The farther an animal moves away from its preferred body temperature, the more detrimental temperature becomes to productive processes. Thus, a stable body temperature in the thermoneutral zone is a pre-requisite for maximal livestock productivity (Kadzere *et al*., 2002).

2.3.4 Effect of heat stress on goats

Environmentally-induced hyperthermia compromises efficient animal production and jeopardizes animal welfare. Heat stress is one of the costliest issues facing progressive animal producers and one of the primary impediments to efficient animal production in developing countries. Heat stress negatively impacts on productive parameters,

including milk yield and composition, growth, reproduction, and carcass traits. In addition, heat load increases health-care costs, and animals often succumb to severe stress, those reared without shade (Baumgard and Rhoads, 2013). Heat stress is a constant challenge to goat farming under tropical climate because of greater exposure to solar radiation for most of the day, while grazing with exaggeration by nutritional constraints. Menacing global rise in surface temperature has compelled more farmers to be focused on measures to mitigate heat stress (Sharma *et al*., 2013). Although goats are resistant to thermal stress at a greater extent, they often suffer from heat and cold stresses beyond their comfort zone (Mishra, 2009). Browsing of goats to open fields during most of the day hours makes them susceptible to environmental stress (Sharma *et al*., 2013).

High ambient temperature and high relative humidity, resulting in heat stress, which causes increased mortality, morbidity, loss in live mass, dehydration and poor meat quality, and often result in considerable economic losses (Minka and Ayo, 2007b). When subjected to heat stress, homeothermic animals employ several thermoregulatory mechanisms to control heat gain by an equivalent loss to maintain their core body temperature and attain thermal equilibrium (Darcan *et al*., 2009).

Heat production is directly controlled by the nervous and endocrine systems through modifications of appetite, digestive process and, indirectly, by alterations of the activity of respiratory enzymes and protein synthesis (Yousef, 1985). Exposure of animals to heat stress activates the hypothalamo-pituitary-adrenal axis (Abilay *et al*., 1975). At endocrinal level, plasma thyroid and adrenal hormonal secretions, which have impact on the productivity of animals, are significantly altered in heat stress. Secretion of thyroid

hormones decreases under heat stress, thereby hampering the productivity of the animal, for which optimum levels of thyroid hormones are required (Todini, 2007). High environmental temperature challenges the animal's ability to maintain energy, thermal, water, hormonal and mineral balance. Heat stress is one of the major causes of oxidative stress. Oxidative stress is defined as an imbalance of pro-oxidants and antioxidants, or a disruption of redox signaling and control (Jones, 2006; Lin *et al*., 2006; Azad *et al*., 2010). Heat stress stimulates excessive production of reactive oxygen species (ROS), including superoxide anion radicals, hydroxyl radical, hydrogen peroxide and singlet oxygen, which are continuously generated in the course of normal aerobic metabolism (Bernabucci *et al*., 2002). Behavioural, physiological and molecular adjustments occur during heat stress (Etches *et al*., 1995; Chowdhury *et al*., 2012) to prevent ROS damage to healthy cells, which may occur if ROS are not eliminated.

2.3.5 Effect of heat stress on haematological parameters of goats

Blood is an important and reliable medium for assessing the health status of individual animals. Values of haematological parameters are important in evaluating the responses of animals to various physiologic conditions (Zakari *et al*., 2014). Blood is frequently used for assessing threat to homeostasis (Kim *et al*., 2011b). The blood is particularly sensitive to changes in the environmental temperature, being important indicator of physiological responses to stressing agent in goats (Okoruwa, 2014). Apart from their main role in transporting oxygen and carbon dioxide, erythrocytes play also an important role in organism antioxidative defence. Direct exposure to reactive oxygen species (ROS) results in shortening of their half-life, even by 50% (Olszewska *et al*., 2012). The effects of heat stress on haematological parameters depend on its duration and severity (Belhadj Slimen *et al*., 2015). There are conflicting reports on the effects of

heat stress on the haematology of animals with some authors observing decrease, increase or no effect of heat stress on haematology. Alam *et al*. (2011) reported increase in PCV, HB and RBC in Bengal goats following exposure to ambient temperature of 28.74 °C and relative humidity of 87% for 8 h. Broucek *et al*. (2009) also did not find the effect of hot temperature on the RBC of cattle. Changes in haematological parameters of goats due to heat stress occur mostly as a result of oxidative alterations, mediated through reactive oxygen species (Minka and Ayo, 2010). Excessive generation of ROS leads to lipoperoxidation of erythrocyte membrane causing haemolysis consequently decreasing circulating erythrocytes. Borges *et al*. (2003) reported that heat stress reduces leucocyte counts in animals.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental Site

The experiment was performed at the Livestock Research Pen, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria (11° 10′N, 07° 38′E), located in the Northern Guinea Savannah zone of Nigeria.

3.2 Animals and Management

The experimental subjects were 28 apparently healthy adult Red Sokoto goats, including 14 bucks (7 experimental and 7 control) and 14 non-pregnant does (7 experimental and 7 control) of about 1-year-old. The goats were aged based on canine teeth eruption as described by Hassan and Hassan (2003). They were purchased from a local goat market at Makarfi (11° 23′ N, 7° 53′ E), located about 52 km from the experimental site. The goats were housed in four communal pens; one for each group, side by side. The pens were made of concrete floor and cement block wall, wire-mesh and asbestos roofing with ceiling. The pens measured 1.75 m × 7.88 m wide and 1.13 m high of wire-mesh from the floor, which provided adequate ventilation. The goats were allowed to move freely inside the pens. They were fed with maize offals, groundnut and premier digitaria (*Digitaria smutsi*) hays, and water was provided *ad libitum*. Salt lick was also provided *ad libitum*. The goats were preconditioned for two weeks before commencing the experiment. During this period, they were screened for haemoparasites, helminthes and ectoparasites after which they treated prophylactically with albendazole at a dose rate of 10 mg/kg *per os* and long-acting oxytetracycline at 20 mg/kg by deep intramuscular injection. The pens were kept clean throughout the experimental period. At 06:00 h,

each goat in the experimental group was administered with L-glutamine at 0.2 g/kg body weight (Wu *et al*., 2011) dissolved in 10 mL distilled water *per os*, every day for a period of three weeks. Simultaneously, each goat in the control group was administered with 10 mL of distilled water *per os*. The L-glutamine was obtained from PhD Nutrition Ltd., Hull, UK. The experiment was conducted between 28th April and 11th of June, 2014.

3.3 Experimental Measurements

3.3.1 Thermal environmental parameters

The dry- and wet-bulb temperatures were recorded at 6:00, 13:00 and 18:00 h before commencing, during and after L-glutamine administration, using the dry- and wet-bulb thermometer (Esal Scientific Industries, Delhi, India). The relative humidity (RH) was obtained using Osmon's hygrometric table. The temperature-humidity index (THI) was calculated using the following formula (Silanikove, 2000a):

$$THI = 0.72(W°C + D°C) + 40.6$$

Where THI = temperature-humidity index for goats, D°C = dry-bulb temperature and W°C = wet-bulb temperature.

3.3.2 Determination of liveweight of goats

The performance of the goats was assessed in terms of liveweight gain. The liveweight was obtained weekly in the morning before the animals were fed, and throughout the experimental period, using a calibrated weighing scale (Carmry Premium, China).

3.3.3 Measurement of rectal temperature

The RT, an indication of the body temperature was obtained using a digital thermometer (Hartmann Company, UK), inserted about 4 cm into the rectum through the anus. The thermometer was inclined so that it made contact with the wall of the rectum. The RT measurements were obtained at 6.00 h, 13.00 h and 18.00 h on experimental days 0, 7, 14, 21 and 28. The values obtained were expressed to the nearest 0.1^{o}C (Minka and Ayo, 2012). Experimental day 0, represented before; while days 7, 14 and 21, represented during the period of L-glutamine administration; and 28 represented the period after L-glutamine administration.

3.3.4 Collection of blood samples

Eight milliliters (8 mL) of blood sample (2 mL for erythrocyte osmotic fragility, 3 mL for haematology and 3 mL for serum) was collected on a weekly basis from the jugular vein of all the treated and control goats, with an anticoagulant, sodium ethylenediamintetraacetate. The blood for serum was dispensed in sterile test tubes without anticoagulant. The samples were allowed to clot at room temperature for 1 h. Serum was separated by centrifugation at 3,000 x g for 10 min, after which it was harvested and kept frozen at -20 °C until analysis (Ramnath *et al.*, 2008).

3.3.5 Evaluation of haematologic parameters

3.3.5.1 Determination of packed cell volume

Packed cell volume was determined using microhaematocrit method according to Dacie and Lewis (1991). Briefly, the blood samples were mixed well by gentle inversion of

the sample bottles several times. Heparinized capillary tubes (John Poulten Limited®, Essex, England) were filled to 75% of their length by capillary action. The empty ends of the tubes were sealed with flames. The tubes were loaded onto microhaematocrit centrifuge (Hawksley®, Lancing, England) and centrifuged at 3000 x g for 10 minutes. The PCV values were read with the aid of microhaematocrit reader.

3.3.5.2 Determination of total erythrocyte count

The total erythrocyte count was evaluated using haemocytometer method. Blood was drawn to 0.5 mark of the haemocytometer and diluted with 0.9 % normal saline, which was drawn to the 101 mark. After gentle mixing of the blood in the pipette, the diluted blood was discharged into haemocytometer counting chamber and allowed to settle. The high dry objective lens of the microscope was employed in determining the total erythrocyte count. The total number of cells in five squares in the centre of counting chamber was determined and multiplied by 10,000. This value represented the total number of erythrocytes per microlitre (Dacie and Lewis, 1991).

3.3.5.3 Determination of haemoglobin concentration

The haemoglobin concentration was determined using cyanmethaemoglobin method of Baker and Silverton (1985). Briefly, 0.02 mL of blood was obtained into clean cuvette and then diluted with 5 mL of cyanmethaemoglobin reagent (modified Drabkin fluid). After the diluent was added, it was stoppered and inverted 2-3 times and allowed to stand for 10 minutes for maximum conversion of Hb to cyanmethaemoglobin. The absorbance of the resulting mixture was read using spectrophotometer (Beckham Coulter, Model B U520, Austria) at wavelength of 540 nm against reagent blank. The

optical density at 540 nm was recorded and compared with the reading, obtained using a standard solution of cyanmethaemoglobin.

3.3.5.4 Determination of erythrocytic indices

The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from Hb, PCV and erythrocyte counts as described by Schalm *et al*. (1975; 2010) using the following formulae, respectively:

$$\text{MCV (fl)} = \frac{\text{PCV} \times 10}{\text{Number of erythrocytes per µL blood} \times 10^{-6}}$$

$$\text{MCH (ρ)} = \frac{\text{Haemoglobin (g/dL)} \times 10}{\text{Number of erythrocytes per µL blood} \times 10^{-6}}$$

$$\text{MCHC (g/dL)} = \frac{\text{Haemoglobin (g/dL)} \times 100}{\text{PCV (mL/dL)}}$$

3.3.5.5 Determination of total leucocyte count

Haemocytometer method, as decribed by Dacie and Lewis (1991), was employed for total leucocyte count. Briefly, the pipette was filled with blood up to 0.5 mark with the aid of a plastic mouth piece that was attached to the stem end of the pipette above the bulb. Excess blood clinging to the exterior of the pipette was removed by wiping with a piece of tissue paper. The diluting fluid (2 % glacial acetic acid, coloured with two drops of gentian violet) was then drawn to the 11.0 mark, and the blood and the diluting fluid were mixed by shaking the pipette vigorously for two to three minutes. Six drops of the

diluting fluid were removed by releasing the thumb intermittently. The haemocytometer was then filled by capillary action. This was examined under the microscope at x 40 magnification. The leucocytes in the four squares of the haemocytometer were counted, and the value obtained was multiplied by the factor 50 to obtain the total leucocyte count.

3.3.5.6 Determination of differential leucocyte counts

The method used to determine the differential leucocyte count was described by Dacie and Lewis (1991). Briefly, a thin blood smear was made, air-dried and fixed in methanol for four minutes before staining with Giemsa for 30 minutes. Thereafter, the smear was rinsed with distilled water and dried with blotting paper. The stained and dried blood smear was later viewed under microscope in oil immersion at x 100 magnification. Examination of slide began at the thin end of the smear, where erythrocytes were well separated and leucocytes were thinly spread. From the thin end, systematic meadering system of slow, careful and detailed examination of the leucocytes was made. For a differential leucocyte count, a minimum of 100 cells were differentiated and counted.

3.3.6 Determination of serum total protein and albumin

The serum total protein was determined using Biuret method as described by Kohn and Allen (1995). Briefly, blood serum (0.1 mL) was mixed with 5 mL of Biuret reagent. Measurements were made after 30 min at the wavelength of 546 nm. The serum albumin was determined using Bromocresol green (BCG) method (Peter *et al*., 1982). This method involves the binding of bromocresol green to protein under acidic

condition. The binding of the dye to protein is accomplished by the appearance of a strong absorbance at 610 nm, which is almost linear over the range of 10 - 80 µg (Flores, 1978). The reaction of serum samples with bromocresol green occurs in two steps. Albumin is involved in the faster (less than 1 minute) reaction and the slower reaction (30 minutes) reaction is a measure of "acute phase reactants" in serum. Serum was mixed with bromocresol green reagent and the absorbance was measured twice, immediately and at 60 minutes (Gustafsson, 1976).

3.3.7 Determination of erythrocyte osmotic fragility

The EOF test was performed as described by Oyewale (1992). Briefly, 0.02 mL of blood was added to tubes, containing increasing concentrations of phosphate-buffered sodium chloride (NaCl) solution at pH 7.4 (0, 0.1, 0.3, 0.5, 0.7, 0.9%). The tubes were gently mixed and incubated at room temperature (25 - 26 °C) for 30 minutes. The content in each tube was centrifuged at 1500 x g per minute for 10 minutes and supernatant was decanted. Optical density of the supernatant was determined spectrophotometrically at 540 nm using a spectrophotometer (Spectrolab 23A®, Labomed Incorporated, Carlifornia, USA). Haemolysis in each tube was expressed as a percentage, taking haemolysis in distilled water (0% NaCl) as 100%.

3.3.8 Determination of malondialdehyde concentration

Serum lipid peroxidation was evaluated as described by Janero (1990). Briefly, serum sample (100 µL) was mixed with sodium dodecyl sulfate-acetate buffer (pH 3.5) and aqueous solution of thiobarbituric acid. After heating at 95 °C for 60 minutes, the red pigment produced was extracted with n-butanol-pyrdine mixture and estimated by

measuring spectrophotmetrically (Spectrolab 23A®, Labomed Incorporated, Carlifornia, USA) the absorbance at 532 nm. Tetramethoxy-propane was used as an external standard and lipid peroxidation level was expressed in nmol/L.

3.3.9 Determination of serum antioxidant enzyme activities

3.3.9.1 Superoxide dismutase activity

The activity of superoxide dismutase (SOD) was measured using the Northwest Life Science Specialties SOD kit (NWLSS™ NWK-SOD02), based on the method of monitoring the auto-oxidation rate of haematoxylin, originally described by Martin (Jr) *et al*. (1987) and modified to enhance reliability. Briefly, 230 µL of assay buffer was added to wells of the microplate. Then, 10 µL of assay buffer (for blank) and 10 µL of serum sample were added. The wells were properly shaken, mixed, and incubated for 2 minutes. A multi-channel pipette was used to add 10 µL of haematoxylin reagent to begin the reaction. The content of each well was quickly mixed using instrument's shaker function; and, immediately, the absorbance at 560 nm was recorded. The SOD activity was calculated as: SOD U/mL = 1.25 x % inhibition.

3.3.9.2 Glutathione peroxidase activity

Glutathione peroxidase activity (GPx) was measured using the Northwest Life Science Specialist, Vancouver, Canada (NWLSS™) glutathione peroxidase assay kits protocol NWK-GPx01. Briefly, for standard procedure for microplate assay, all reagents were brought to room temperature (25°C). Diluted serum sample (50 µL) was added to the wells and then 50 µL of working nicotinamide adenine dinucleotide phosphate (NADPH) added to each well. Working H_2O_2 (50 µL) was also added to each well.

After waiting for 1 minute, microplate was placed in plate reader and read at 340 nm wavelength (Flohe and Gunzler, 1984).

To calculate GPx concentration using the NADPH absorption coefficient, the GPx concentration, expressed as mU/mL, was calculated using the GPx activity definition.

$$[GPx] = \frac{2(mRate_s - mRate_b) \cdot V_{Rkm}}{2.74 \cdot V_s} \cdot df$$

Where $mRate_s$ = -1,000 ΔA340/min of sample; $mRate_b$ = -1,000 ΔA340/min of blank; 2.74 = NADPH 340 nm millimolar absorption coefficient at 1 cm path length; V_{Rxm} = volume of reaction mixture; V_s = volume of sample; 2 = correction for 2 moles reduced glutathione, oxidized to 1 mole glutathione disulfide (oxidized glutathione) per mole NADPH oxidized; df = sample dilution factor.

3.3.9.3 Catalase activity

The catalase activity was measured as described by Abei (1984) using a catalase kit, purchased from Abcam PLC, 330 Cambridge Science Park, UK. Briefly, 12 µL of fresh 1 mM H_2O_2 was added into each well of serum samples, positive control solution and sample high control (HC) to start the reaction. Then, the samples were incubated at 25 ℃ for 30 minutes, and 10 µL of stop solution added into each sample well to stop the reaction. For each well, 50 µL of developer mix containing: 46 µL of assay buffer, 2 µL of OxiRed probe, and 2 µL of horseradish peroxidase solution was prepared. Fifty microlitres of the developer mix was then added to each test sample, control and standard. The samples were mixed well and incubated at 25 ℃ for 30 minutes, and the optical density at 570 nm was measured in a plate reader.

Calculation: Signal change by catalase in the sample was ΔA = AHC - Asample. AHC was the reading of sample HC; sample A was the reading of sample in 30 minutes. H_2O_2 standard curve was plotted, and the ΔA was applied to the H_2O_2 standard curve to get B nmol of H_2O_2 decomposed by catalase in 30 minutes reaction. Catalase activity was then calculated as follows:

$$\text{Catalase activity} = \frac{B}{30 \times V} \times \text{Sample dilution factor} = \text{nmol/min/mL} = \text{mU/mL}$$

Where B was the decomposed H_2O_2 amount from H_2O_2 standard curve (in nmol); V was the pretreated sample volume added into the well (in mL); 30 is the reaction time in 30 minutes; one unit of catalase was the amount of catalase that decomposed 1.0 μmol of H_2O_2 per minute at pH 4.5 at 25 °C.

3.4 Data Analyses

Data obtained were expressed as mean ± standard error of mean (mean ± SEM). The values obtained were subjected to statistical analysis and compared using one-way analysis of variance (ANOVA), followed by Tukey's *post-hoc* test. Values of $P < 0.05$ were considered significant (Snedecor and Cochran, 1994), using GraphPad prism version 5.0 (San Diego, California, USA) for Windows. Sexual comparisons between bucks and does were done using Student's *t*-test.

CHAPTER FOUR

RESULTS

4.1 Thermal Environmental Parameters

During the study period, the DBT, RH, and THI values ranged between 24.5 - 35.2 ℃, 41.7 - 84.0 % and 74.1 - 84.0, respectively. The overall mean values of DBT were 29.17 ± 2.52, 31.44 ± 1.78 and 29.17 ± 2.74 ℃ before, during and after treatment with L-glutamine respectively. The mean RH values before, during and after treatment were 66.33 ± 9.60 %, 51.22 ± 6.88 % and 65.33 ± 9.94 %, respectively. The mean THI before, during, and after treatment were 79.00 ± 2.44, 80.20 ± 1.63, and 78.88 ± 2.62, respectively (Table 4.1). The DBT value was lowest at 6.00 h and highest at 13:00 h. The highest THI value (84.04) was recorded at 13:00 h during the period of L-glutamine administration, while the lowest value (74.08) was recorded at 6:00 h after L-glutamine administration (Table 4.1).

Table 4.1: Thermal environmental parameters in the pen during the study period

	Before treatment			During treatment			Day 7 After treatment		
Time of day	DBT (°C)	RH (%)	THI	DBT (°C)	RH (%)	THI	DBT (°C)	RH (%)	THI
06:00	25.50	84.00	75.88	26.50	67.67	75.52	24.50	79.00	74.08
13:00	34.00	51.00	83.80	35.17	41.67	84.04	34.00	46.00	83.08
18:00	28.00	64.00	77.32	32.67	44.33	81.04	29.00	71.00	79.48
Mean ± SEM	29.17 ± 2.52	66.33 ± 9.60	79.00 ± 2.44	31.44 ± 1.78	51.22 ± 6.88	80.20 ± 1.63	29.17 ± 2.74	65.33 ± 9.94	78.88 ± 2.62

DBT = Dry-bulb temperature; RH = relative humidity; THI = temperature humidity index

4.2 Effect of Treatment on Liveweight of the Goats during the Study Period

The mean liveweight of the experimental group ranged between 10.21 ± 0.52 and 11.43 ± 0.68 kg while that of the conrol group ranged between 10.64 ± 0.37 and 10.93 ± 0.53 kg during the study period. There was no significant ($P < 0.05$) difference between the liveweght of the experimental group when compared to those of the control group. However, the liveweight of the experimental group was consistently higher than those of the control group during and after L-glutamine administration (Figure 4.1).

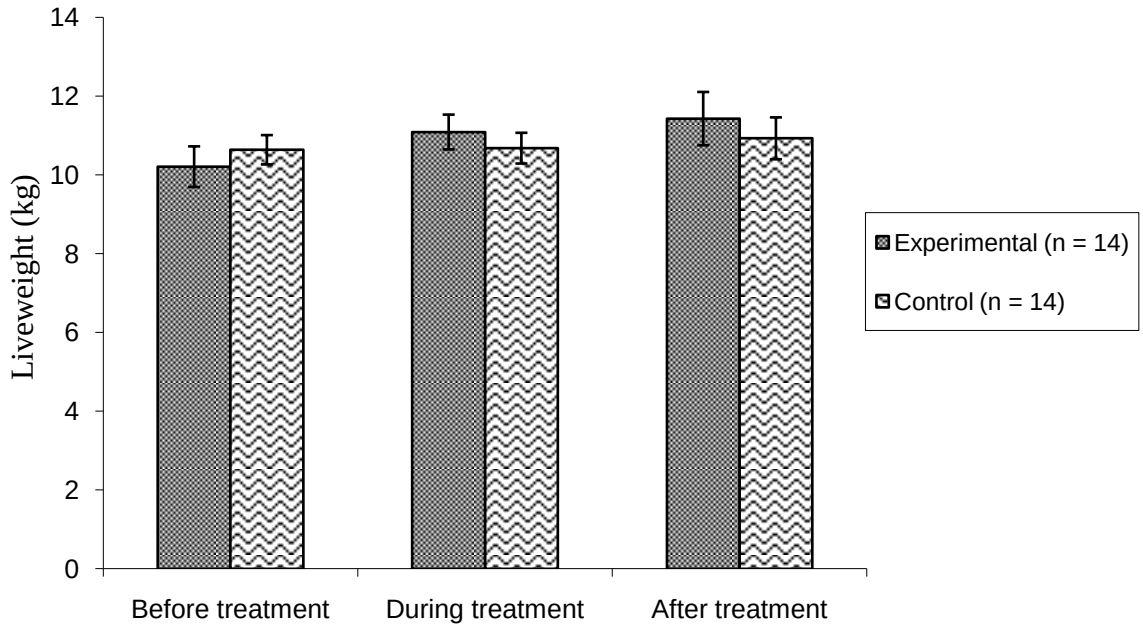


Figure 4.1: Effect of treatment with L-glutamine on liveweight of the goats during the study period

4.3 Fluctuations in Rectal Temperature of Red Sokoto Goats administered with L-glutamine during the Hot-dry Season

The RT value obtained at 6:00 h was significantly lower ($P < 0.05$) than the corresponding values recorded at 13:00 h and 18:00 h, respectively, during the study period (Table 4.2). There was no significant difference in RT values between experimental and control goats before L-glutamine administration (Figure 4.2), and the overall mean values of RT in experimental and control goats before L-glutamine administration were 39.04 ± 0.08 °C and 39.02 ± 0.08 °C, respectively ($P > 0.05$) (Table 4.2).

At each hour of recording during the period of L-glutamine administration, RT values in experimental goats were significantly ($P < 0.05$) lower than the corresponding values obtained in control goats. The overall mean values of RT in experimental and control goats were 38.78 ± 0.06 °C and 38.93 ± 0.06 °C, respectively ($P < 0.05$). The RT values in experimental goats were consistently lower than those of the control goats during L-glutamine administration at 6:00 h, 13:00 h and 18:00 h (Figure 4.3).

At day 7 after L-glutamine administration, there was no significant difference between RT values in experimental and control goats, except at 6:00 h, when the value in controls was still higher ($P < 0.05$) than that of the experimental goats (Figure 4.4).

Table 4.2: Fluctuations in Rectal temperature (°C) of Red Sokoto goats administered with L-glutamine during the hot-dry season (mean ± SEM, n = 14)

	Before treatment		During treatment		Day 7 after treatment	
Time of day	Experimental	Control	Experimental	Control	Experimental	Control
06:00 h	$38.68 \pm 0.09^{a,i}$	$38.67 \pm 0.09^{a,i}$	$38.05 \pm 0.08^{a,ii}$	$38.25 \pm 0.08^{a,ii}$	$38.28 \pm 0.12^{a,i}$	$38.59 \pm 0.10^{a,iii}$
	(38.1 - 39.2)	(38.2 - 39.3)	(37.1 - 39.5)	(37.0 - 39.8)	(37.1 - 38.8)	(37.9 - 39.2)
13:00 h	39.34 ± 0.09^{b}	39.34 ± 0.09^{b}	39.15 ± 0.06^{b}	39.29 ± 0.06^{b}	39.01 ± 0.08^{b}	39.03 ± 0.09^{b}
	(38.7 - 39.8)	(38.6 - 39.8)	(38.6 - 40.5)	(38.6 - 40.2)	(38.4 - 39.01)	(38.6 - 39.7)
18:00 h	39.09 ± 0.08^{b}	39.06 ± 0.08^{b}	39.15 ± 0.06^{b}	39.26 ± 0.05^{b}	39.22 ± 0.08^{b}	39.26 ± 0.06^{b}
	(38.7 - 39.7)	(38.6 - 39.7)	(38.5 - 39.8)	(38.6 - 39.9)	(38.6 - 39.8)	(39.0 - 39.6)
Overall mean ± SEM	39.04 ± 0.08	39.02 ± 0.09	38.78 ± 0.06^{a}	38.93 ± 0.06^{b}	38.84 ± 0.10	38.96 ± 0.08
	(38.68 - 39.34)	(38.67 - 39.34)	(38.05 - 39.15)	(38.25 - 39.29)	(38.28 - 39.22)	(38.59 - 39.26)

Values in parenthesis are minimum - maximum

a,b = means in the same column with different superscript letters are significantly ($P < 0.05$) different

i, ii, iii = means in the same row with different superscript numerals are significantly ($P < 0.05$) different

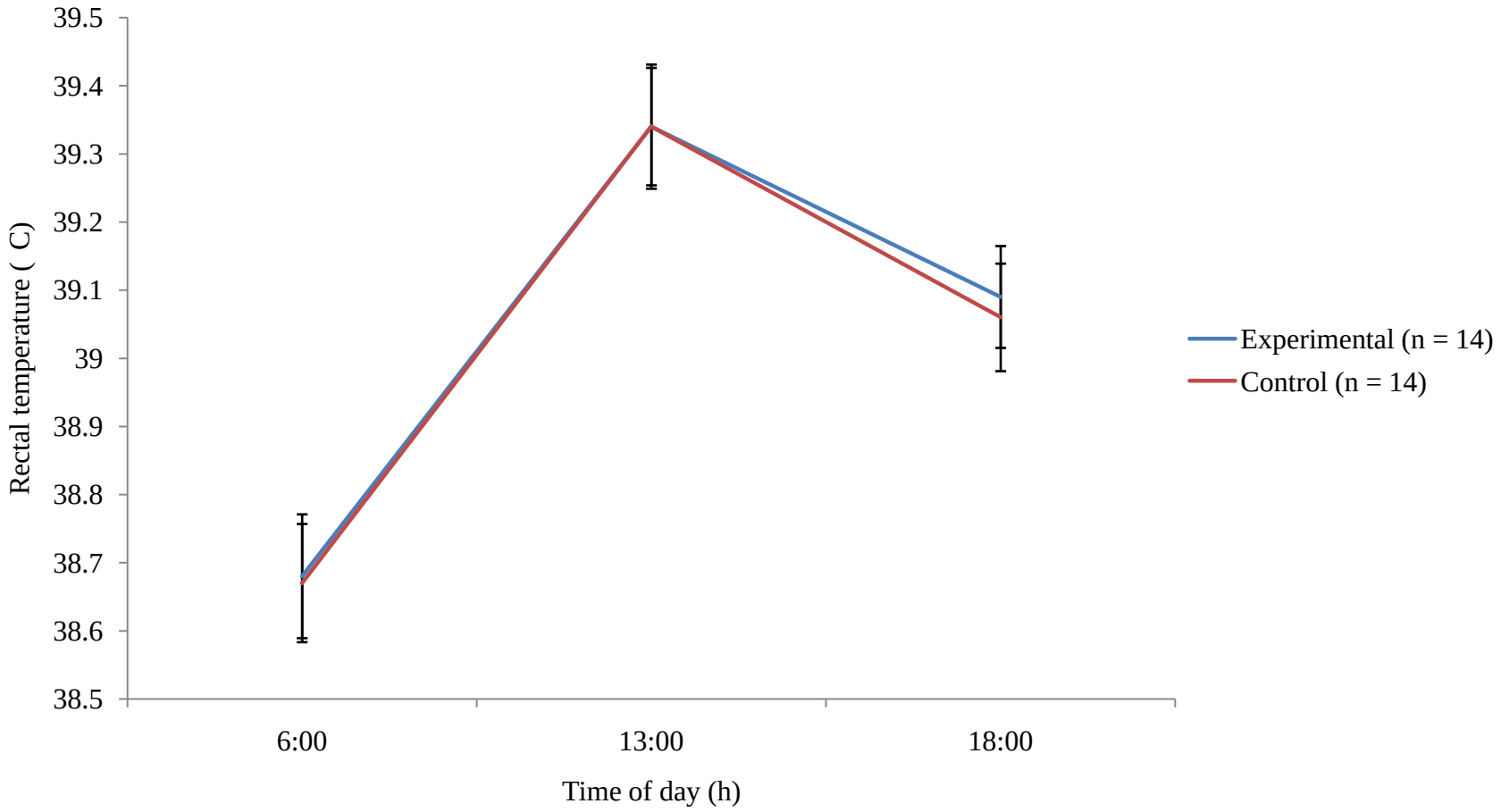


Figure 4.2: Diurnal fluctuations in rectal temperature (°C) of Red Sokoto goats before L-glutamine administration in the hot-dry season

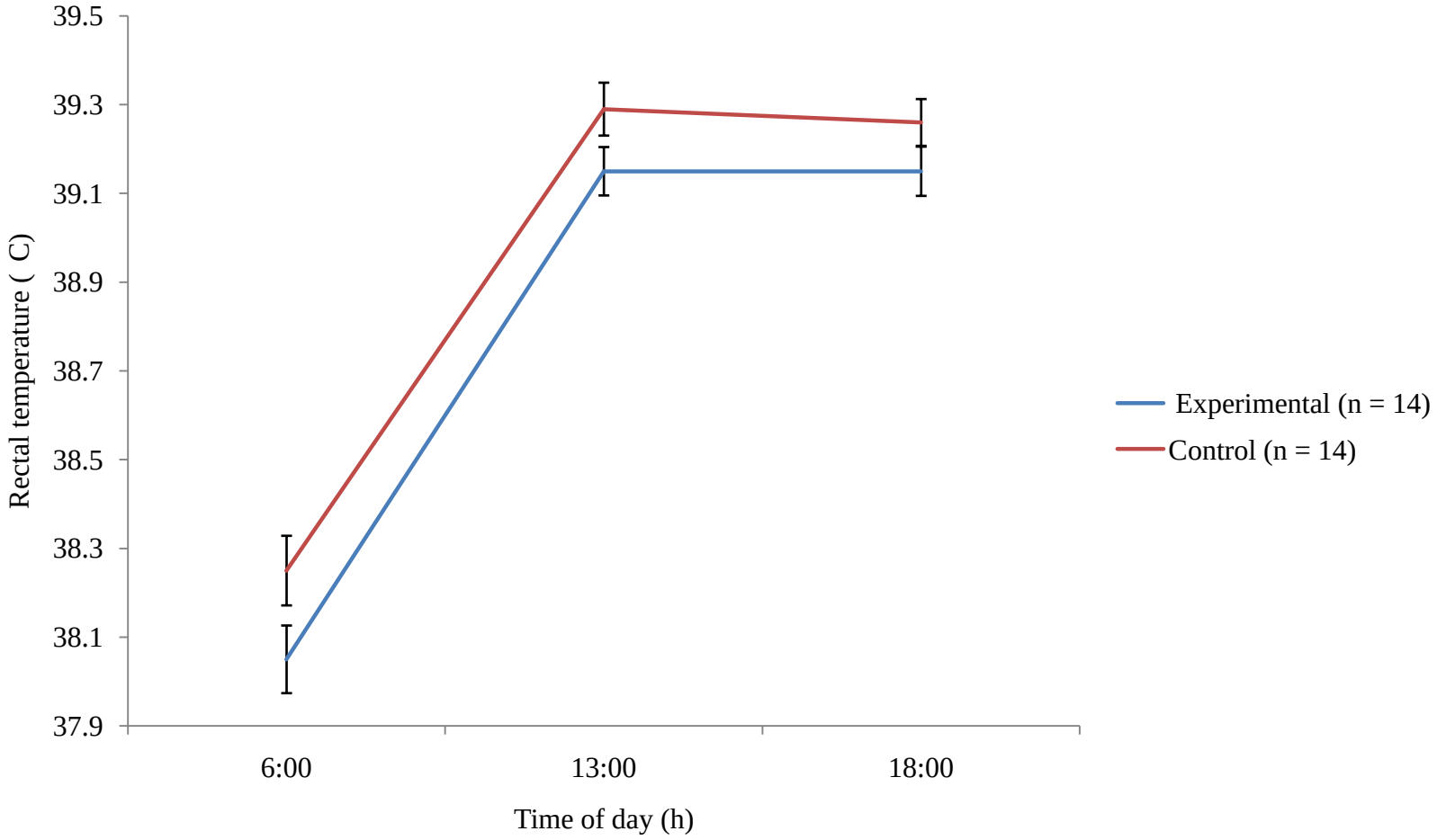


Figure 4.3: Diurnal fluctuations in rectal temperature (°C) of Red Sokoto goats during three weeks of L-glutamine administration in the hot-dry season

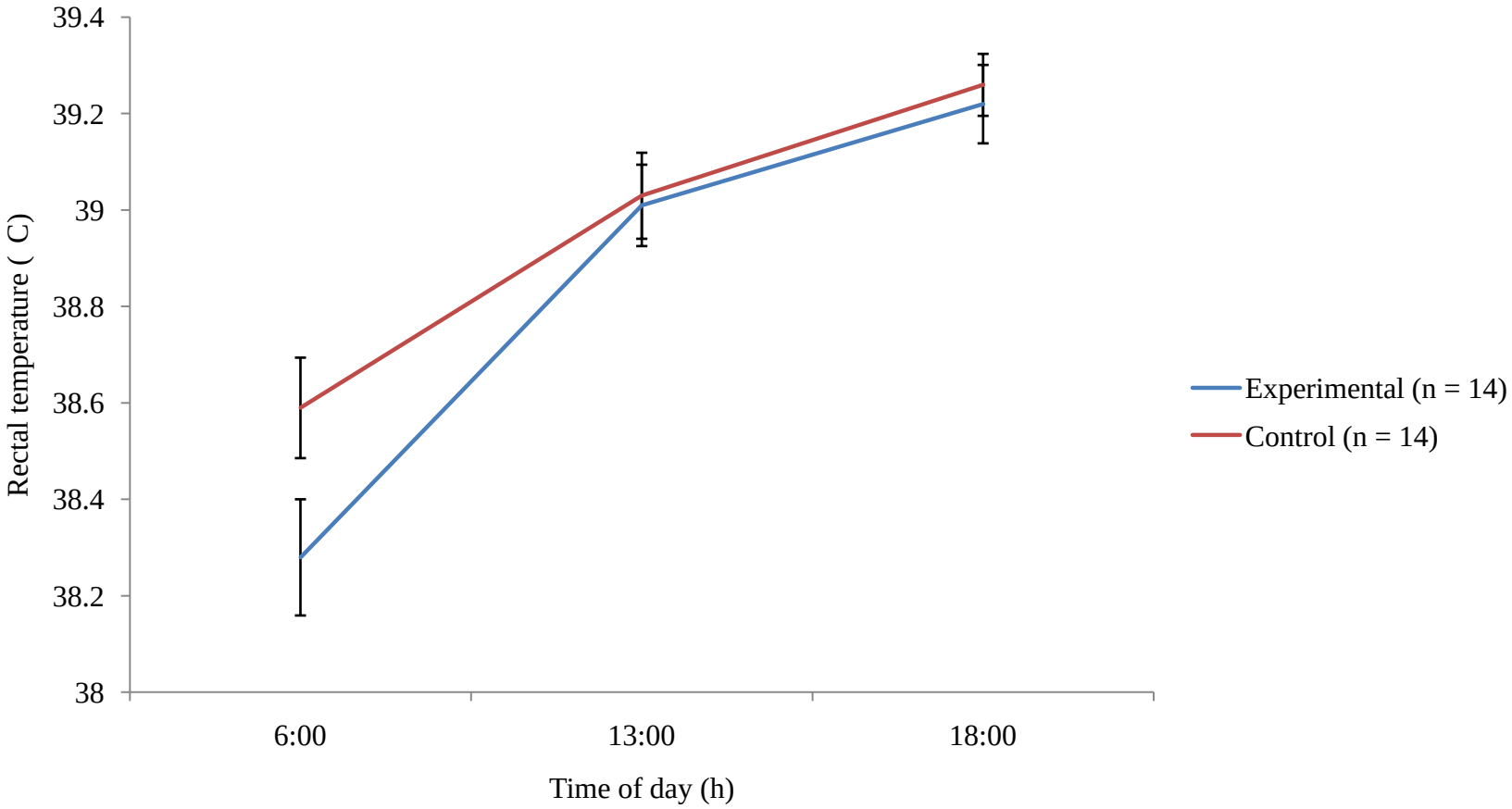


Figure 4.4: Diurnal fluctuations in rectal temperature (°C) of Red Sokoto goats day 7 after L-glutamine administration in the hot-dry season

4.4 Effect of L-glutamine Administration on Haematological Parameters

4.4.1 Effect of L-glutamine administration on packed cell volume

The PCV obtained in Red Sokoto goats before treatment with L-glutamine in the experimental and control groups were similar ($P > 0.05$) (28.73 ± 1.07 % and 28.91 ± 1.20 %, respectively). The mean PCV values of 28.57 ± 0.53 % and 28.90 ± 0.76 % recorded during and after L-glutamine administration in experimental group were significantly higher ($P < 0.05$) than the corresponding mean values of 24.17 ± 0.67 % and 26.57 ± 0.84 % recorded during and after L-glutamine administration, respectively, in control group. Although the mean PCV values obtained in the experimental group before, during and after treatment with L-glutamine were not significantly ($P > 0.05$) different during the hot-dry season, the PCV recorded in the control group during (24.17 ± 0.67 %) administration of L-glutamine differed significantly ($P < 0.05$) when compared with the values obtained before (28.19 ± 1.20 %) and after (26.57 ± 0.84 %) L-glutamine administration (Table 4.3).

4.4.2 Effect of L-glutamine administration on haemoglobin concentration

The Hb concentrations recorded before, during and after L-glutamine administration in the experimental group were similar ($P > 0.05$) (9.04 ± 0.38 g/dL, 9.21 ± 0.20 g/dL and 8.57 ± 0.31 g/dL, respectively). In the control group, the Hb concentrations before, during and after the treatment were significantly ($P < 0.05$) different (9.29 ± 0.20 g/dL, 6.86 ± 0.21 g/dL and 9.04 ± 0.39 g/dL, respectively). The Hb concentration obtained during the treatment in the experimental group (9.21 ± 0.20 g/dL) was significantly ($P < 0.05$) higher than that of the controls (6.86 ± 0.21 g/dL). (Table 4.3).

4.4.3 Effect of L-glutamine administration on red blood cell count

The RBC counts obtained in the experimental and control groups before treatment were similar ($P > 0.05$) ($9.04 \pm 0.38 \times 10^6/\mu L$ and $9.29 \pm 0.44 \times 10^6/\mu L$, respectively) (Table 4.3). During the treatment, the RBC count in the experimental group ($11.45 \pm 0.24 \times 10^6/\mu L$) was higher ($P < 0.05$), when compared to that recorded in the control group ($8.75 \pm 0.21 \times 10^6/\mu L$). The RBC counts obtained before, during and after treatment in the experimental group were not significantly ($P > 0.05$) different. The RBC counts recorded in the control group before ($11.56 \pm 0.50 \times 10^6/\mu L$) and after ($10.87 \pm 0.48 \times 10^6/\mu L$) treatment were higher ($P < 0.05$), when compared to that obtained during the treatment ($8.75 \pm 0.20 \times 10^6/\mu L$).

4.4.4 Effect of L-glutamine administration on mean corpuscular volume

The MCV obtained before treatment in the experimental and control groups were similar ($P > 0.05$) (24.30 ± 0.41 fL and 24.59 ± 0.40 fL, respectively) (Table 4.3). During treatment, the MCV obtained in the experimental group (24.45 ± 0.31 fL) was higher ($P < 0.05$) than that recorded in the control group (19.99 ± 0.38 fL). The MCV values obtained in the experimental group before, during and after treatment with L-glutamine were not significantly ($P > 0.05$) different.

4.4.5 Effect of L-glutamine administration on mean corpuscular haemoglobin

The MCH values obtained before treatment in the experimental and control groups were 7.73 ± 0.14 ρg and 7.79 ± 0.13 ρg ($P > 0.05$), respectively (Table 4.3). In the experimental and control groups, the values recorded during treatment were 7.69 ± 0.09 ρg and 6.92 ± 0.15 ρg ($P < 0.05$), respectively. The MCV values in the experimental

group before, during and after treatment with L-glutamine were not significantly ($P > 0.05$) different (Table 4.3).

4.4.6 Effect of L-glutamine administration on mean corpuscular haemoglobin concentration

The MCHC recorded in Red Sokoto goats during the hot-dry season before, during and after treatment with L-glutamine in the experimental group were 31.48 ± 0.49 %, 31.68 ± 0.35 % and 31.05 ± 0.54 % ($P > 0.05$), respectively. During the treatment, the MCHC obtained in the experimental group (31.68 ± 0.35 %) was higher ($P < 0.05$) than that of the control group (28.13 ± 0.44 %) (Table 4.3).

4.4.7 Effect of L-glutamine administration on total white blood cell count

The mean WBC counts recorded before treatment in the experimental and control groups were 8.91 ± 0.2 x 10^3/µL and 9.14 ± 0.35 x 10^3/µL, respectively. The WBC counts in the experimental and control groups during treatment were 10.89 ± 0.17 x 10^3/µL and 6.93 ± 0.27 x 10^3/µL ($P < 0.001$), respectively. The WBC counts recorded after treatment in the experimental group (11.00 ± 0.27 x 10^3/µL) was also higher ($P < 0.001$) than that recorded in the control group (6.04 ± 0.54 x 10^3/µL). The WBC counts in the experimental group before treatment was significantly ($P < 0.05$) lower than the count during or after treatment (Table 4.4).

4.4.8 Effect of L-glutamine administration on neutrophil count

The neutrophil counts obtained before treatment with L-glutamine in the experimental group (3.85 ± 0.24 x 10^3/µL), was lower ($P < 0.05$) than the count recorded during (5.53

± 0.15 x 10^3/µL) and after (5.91 ± 0.22 x 10^3/µL) treatment. During treatment, the neutrophil count obtained in the experimental group (5.53 ± 0.15 x 10^3/µL) was higher ($P < 0.05$) than the count recorded in the control group (2.83 ± 0.15 x 10^3/µL). The neutrophil counts obtained before, during and after treatment in the experimental group were not significantly ($P > 0.05$) different. The neutrophil count obtained in the control group during (2.83 ± 0.15 x 10^3/µL) and after (2.18 ± 0.22 x 10^3/µL) the treatment were lower ($P < 0.05$) than that recorded before treatment (3.81 ± 0.28 x 10^3/µL) (Table 4.4).

4.4.9 Effect of L-glutamine administration on lymphocyte count

The lymphocyte count obtained before treatment in the experimental and control groups were 3.96 ± 0.23 x 10^3/µL and 4.52 ± 0.25 x 10^3/µL, respectively. The lymphocyte count obtained during treatment in the experimental group (5.63 ± 0.14 x 10^3/µL) was higher ($P < 0.05$) than the count recorded in the control group (3.00 ± 0.14 x 10^3/µL). The lymphocyte count recorded after treatment with L-glutamine in the experimental group was 6.27 ± 0.14 x 10^3/µL, and 2.33 ± 0.21 x 10^3/µL for the control group ($P < 0.01$). During treatment, the lymphocyte count obtained in the experimental group (5.63 ± 0.14 x 10^3/µL) was higher ($P < 0.05$) than the count recorded in the control group (3.00 ± 0.14 x 10^3/µL). The lymphocyte count obtained in the experimental group before treatment (3.96 ± 0.23 x 10^3/µL) was lower ($P < 0.05$), when compared to the count obtained during (5.63 ± 0.14 x 10^3/µL) and after (6.27 ± 0.14 x 10^3/µL) treatment (Table 4.4).

4.4.10 Effect of L-glutamine administration on neutrophil:lymphocyte ratio

The mean neutrophil:lymphocyte ratio during L-glutamine supplementation was higher ($P < 0.05$) in experimental (1.00 ± 0.04) group than in the control (0.88 ± 0.04) group (Table 4.4).

Table 4.3: Erythrocytic parameters and serum protein of Red Sokoto goats administered with L-glutamine during the hot-dry season (mean ± SEM, n = 14)

	Before treatment		During treatment		Day 7 after treatment	
Parameters	Experimental	Control	Experimental	Control	Experimental	Control
Packed cell volume, %	28.73 ± 1.07[a]	28.91 ± 1.20[a]	28.57 ± 0.53[a]	24.17 ± 0.67[b]	28.9 ± 0.76[a]	26.57 ± 0.84[a]
Haemoglobin concentration, g/dL	09.04 ± 0.38[a]	09.29 ± 0.44[a]	09.21 ± 0.20[a]	06.86 ± 0.21[b]	08.57 ± 0.31[a]	09.04 ± 0.39[a]
Red blood cell count, x 10^6/µL	11.4 ± 0.54[a]	11.56 ± 0.50[a]	11.45 ± 0.24[a]	08.75 ± 0.21[b]	10.56 ± 0.49[a]	10.87 ± 0.48[a]
Mean corpuscular volume, fL	24.3 ± 0.41[a]	24.59 ± 0.40[a]	24.45 ± 0.31[a]	19.99 ± 0.38[b]	27.81 ± 0.91[c]	24.79 ± 0.91[a]
Mean corpuscular haemoglobin, ρg	07.73 ± 0.14[a]	07.79 ± 0.13[a]	07.69 ± 0.09[a]	06.92 ± 0.15[b]	07.83 ± 0.15[a]	07.66 ± 0.18[a]
Mean corpuscular haemoglobin concentration, %	31.48 ± 0.49[a]	32.09 ± 0.42[a]	31.68 ± 0.35[a]	28.13 ± 0.44[b]	31.05 ± 0.54[a]	31.63 ± 0.47[a]
Total protein, g/L	64.79 ± 0.9[a]	65.14 ± 0.7[a]	67.61 ± 0.6[a]	66.50 ± 0.6[a]	68.14 ± 0.8[b]	64.21 ± 0.9[a]
Albumin, g/L	33.79 ± 0.7[a]	34.79 ± 0.7[a]	38.39 ± 0.6[b]	36.18 ± 0.6[c]	37.50 ± 0.9[b]	36.71 ± 1.0[c]

Values in the same row with different superscripts are significantly ($P < 0.05$) different

Table 4.4: Leucocyte counts in Red Sokoto goats administered with L-glutamine during the hot-dry season (mean ± SEM, n = 14)

	Before treatment		During treatment		Day 7 after treatment	
Parameters	Experimental	Control	Experimental	Control	Experimental	Control
WBC count (x 10^3/µL)	8.91 ± 0.20^a	9.14 ± 0.35^a	10.89 ± 0.17^b	6.93 ± 0.27^c	11.00 ± 0.27^b	06.04 ± 0.54^c
Neutrophil count (x 10^3/µL)	3.85 ± 0.24^a	3.81 ± 0.28^a	5.53 ± 0.15^b	2.83 ± 0.15^c	5.91 ± 0.22^b	2.18 ± 0.22^c
Lymphocyte count (x 10^3/µL)	3.96 ± 0.23^a	4.52 ± 0.25^a	5.63 ± 0.14^b	3.00 ± 0.14^c	6.27 ± 0.14^b	2.33 ± 0.21^c
Neutrophil:Lymphocyte ratio	0.94 ± 0.07	0.90 ± 0.07	1.00 ± 0.04^a	0.88 ± 0.04^b	0.95 ± 0.05	0.90 ± 0.07
Monocyte count (x 10^3/µL)	0.39 ± 0.12	0.37 ± 0.07	0.29 ± 0.04	0.48 ± 0.14	0.15 ± 0.03	0.09 ± 0.04
Eosinophil count (x 10^3/µL)	0.07 ± 0.02	0.15 ± 0.00	0.05 ± 0.00	0.36 ± 0.21	0.05 ± 0.00	0.00 ± 0.00
Basophil count (x 10^3/µL)	0.00 ± 0.00	0.08 ± 0.00	0.00 ± 0.00	0.14 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Band cell count (x 10^3/µL)	0.26 ± 0.13	0.18 ± 0.09	0.18 ± 0.04	0.18 ± 0.02	0.00 ± 0.00	0.06 ± 0.02

Values in the same row with different superscripts are significantly ($P < 0.05$) different
Monocyte, eosinophil, basophil and band cell counts were not compared

4.5 Effect of L-glutamine on Biomarkers of Oxidative Stress in Red Sokoto Goats during the Hot-dry Season

4.5.1 Erythrocyte osmotic fragility in Red Sokoto goats during the study period

The EOF in both experimental and control goats decreased as NaCl concentrations increased from 0 to 0.9 %. There was no significant ($P > 0.05$) difference in EOF between the experimental and control goats before and after L-glutamine administration (Figure 4.5; 4.7). The EOF in experimental goats was significantly ($P < 0.05$) lower than that in the control goats at 0.5 % and 0.7 % concentrations of NaCl during the three-week period of L-glutamine administration (Figure 4.6).

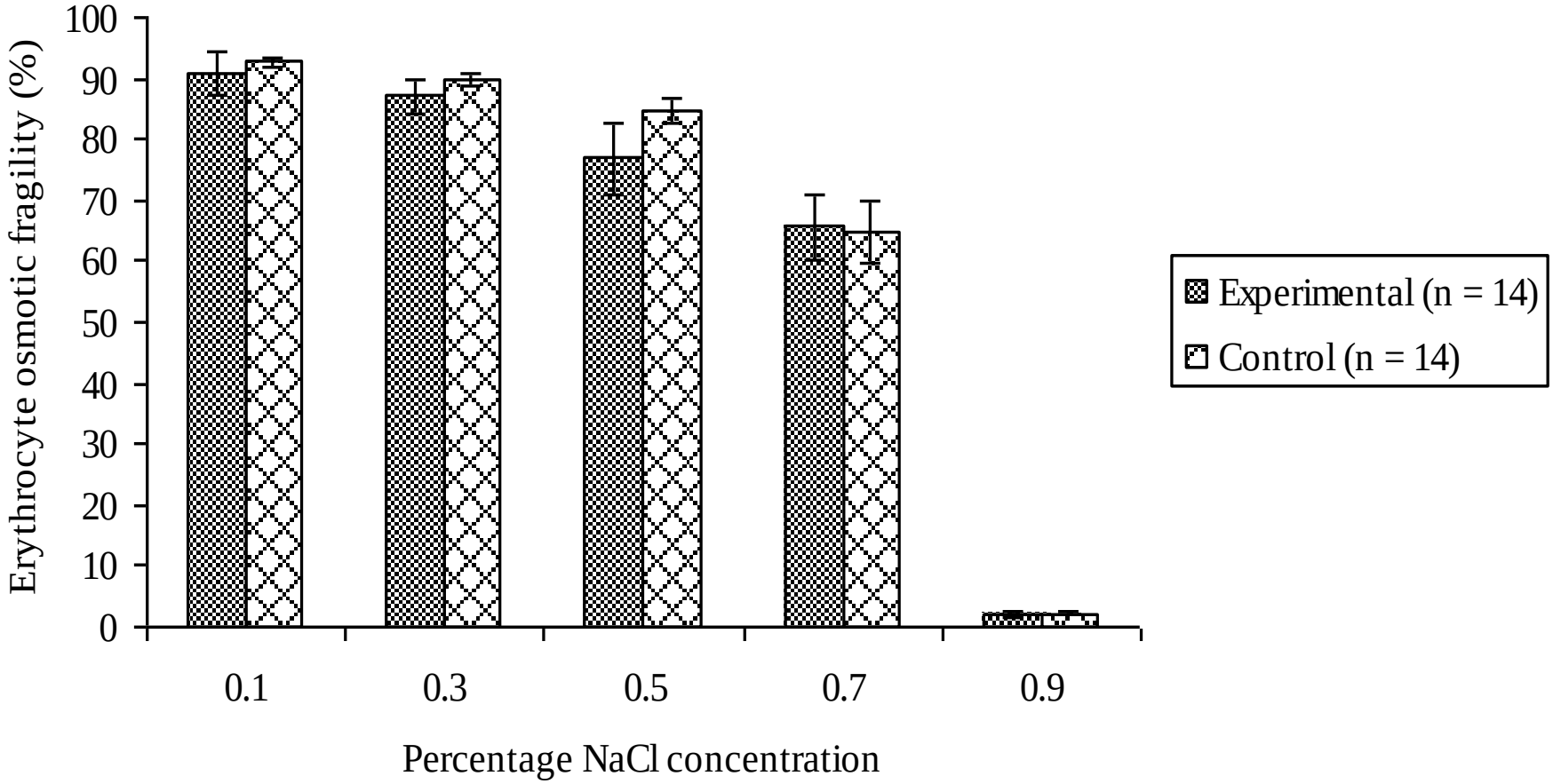


Figure 4.5: Dynamics of changes in erythrocyte osmotic fragility in Red Sokoto goats before L-glutamine administration during the hot-dry season

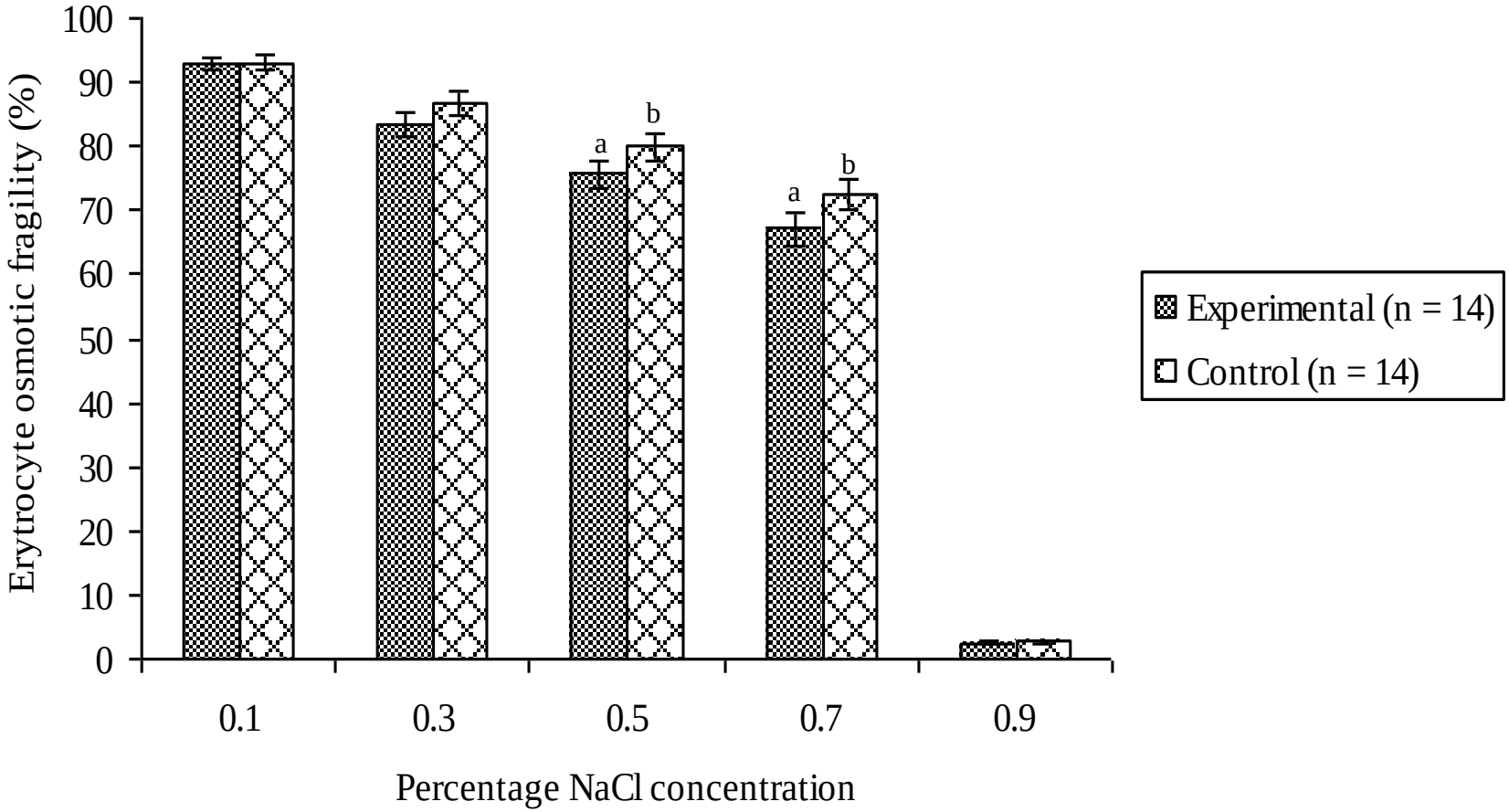


Figure 4.6: Dynamics of changes in erythrocyte osmotic fragility in Red Sokoto goats during 3-week L-glutamine administration in the hot-dry season. a, b = $P < 0.05$

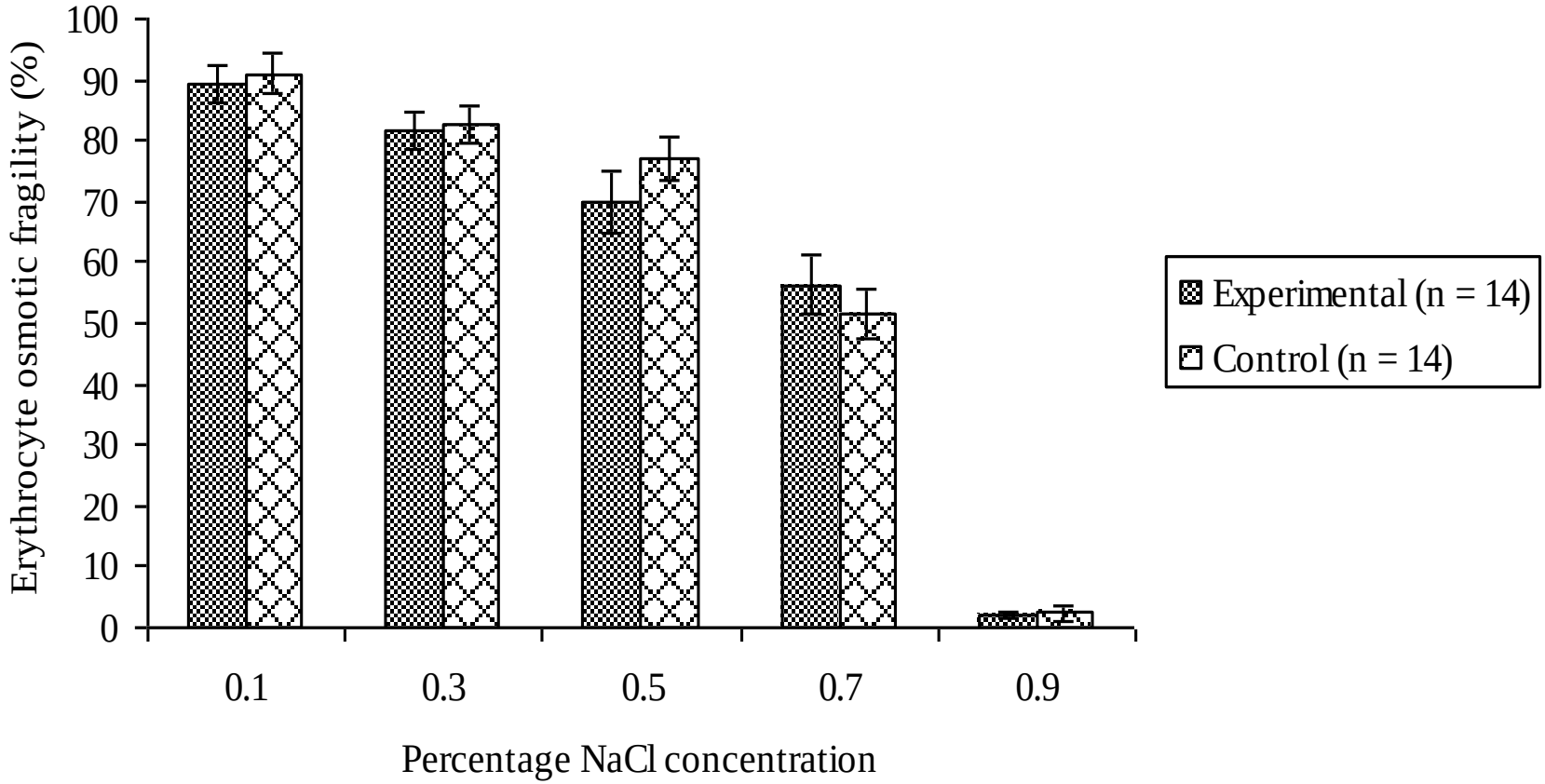


Figure 4.7: Dynamics of changes in erythrocyte osmotic fragility in Red Sokoto goats day 7 after L-glutamine administration during the hot-dry season

4.5.2 Variation in malondialdehyde concentration

The MDA concentration in the experimental goats (1.47 ± 0.1 nmol/L) was significantly ($P < 0.05$) lower than that recorded in the control goats (1.75 ± 0.1 nmol/L) during the period of L-glutamine administration. There was no significant difference in MDA concentrations between the experimental and control goats before and after L-glutamine administration. The concentrations of MDA recorded in both experimental and control goats were higher during the period of L-glutamine administration than before or after its administration (Figure 4.8).

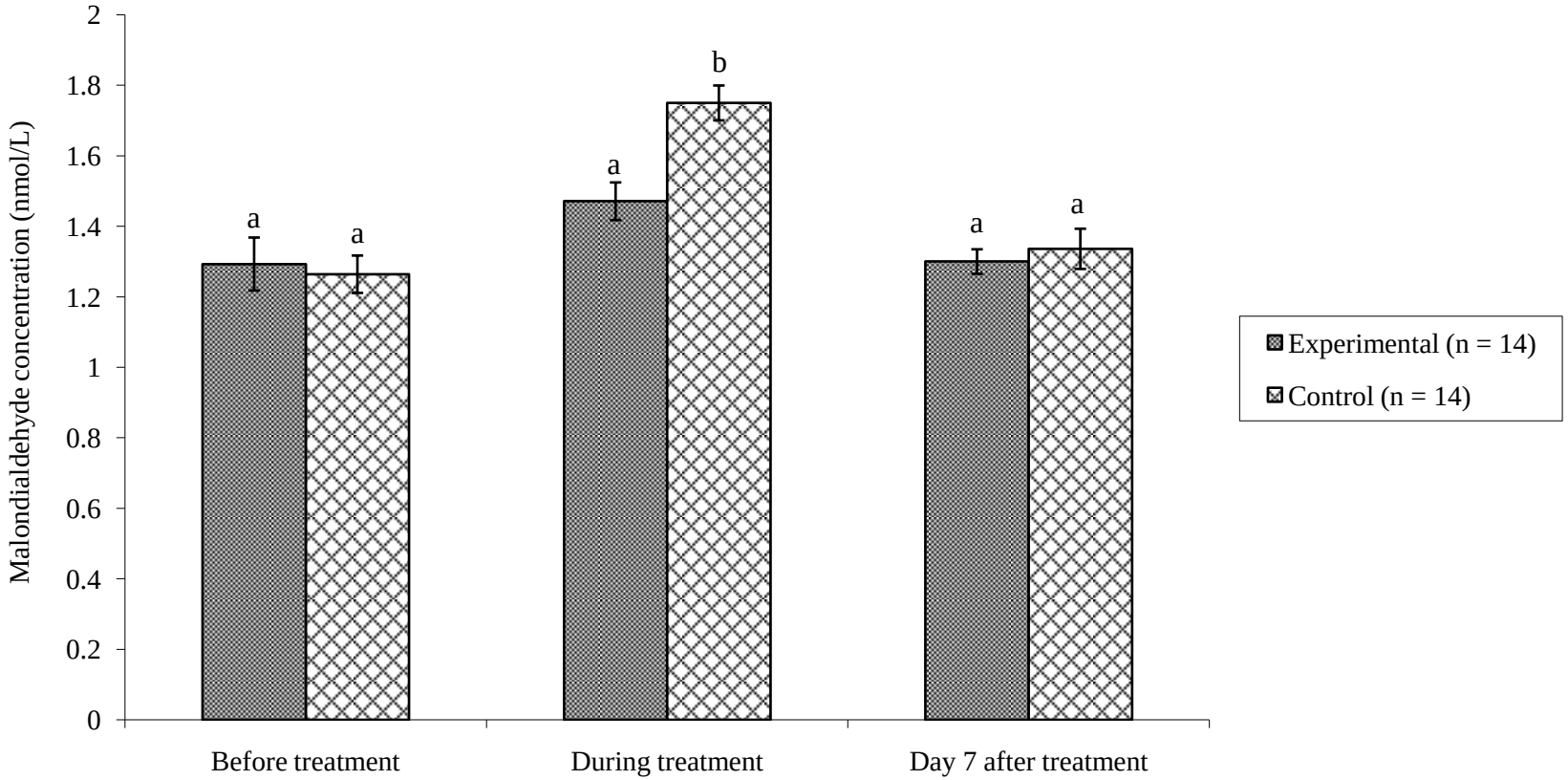


Figure 4.8: Effect of L-glutamine administration on serum malondialdehyde concentration in Red Sokoto goats during the hot-dry season. a, b = $P < 0.05$

4.5.3 Variation in activities of antioxidant enzymes

4.5.3.1 Superoxide dismutase activity

Before L-glutamine administration, there was no significant ($P > 0.05$) difference in SOD activity in the experimental goats, when compared with the control goats. However, during the period of L-glutamine administration, SOD activity in the experimental goats (2.29 ± 0.04 IU/L) was significantly ($P < 0.05$) higher that the values obtained in the control goats (2.10 ± 0.04 IU/L). There was no significant difference in SOD activities between experimental and control goats after L-glutamine administration (Figure 4.9).

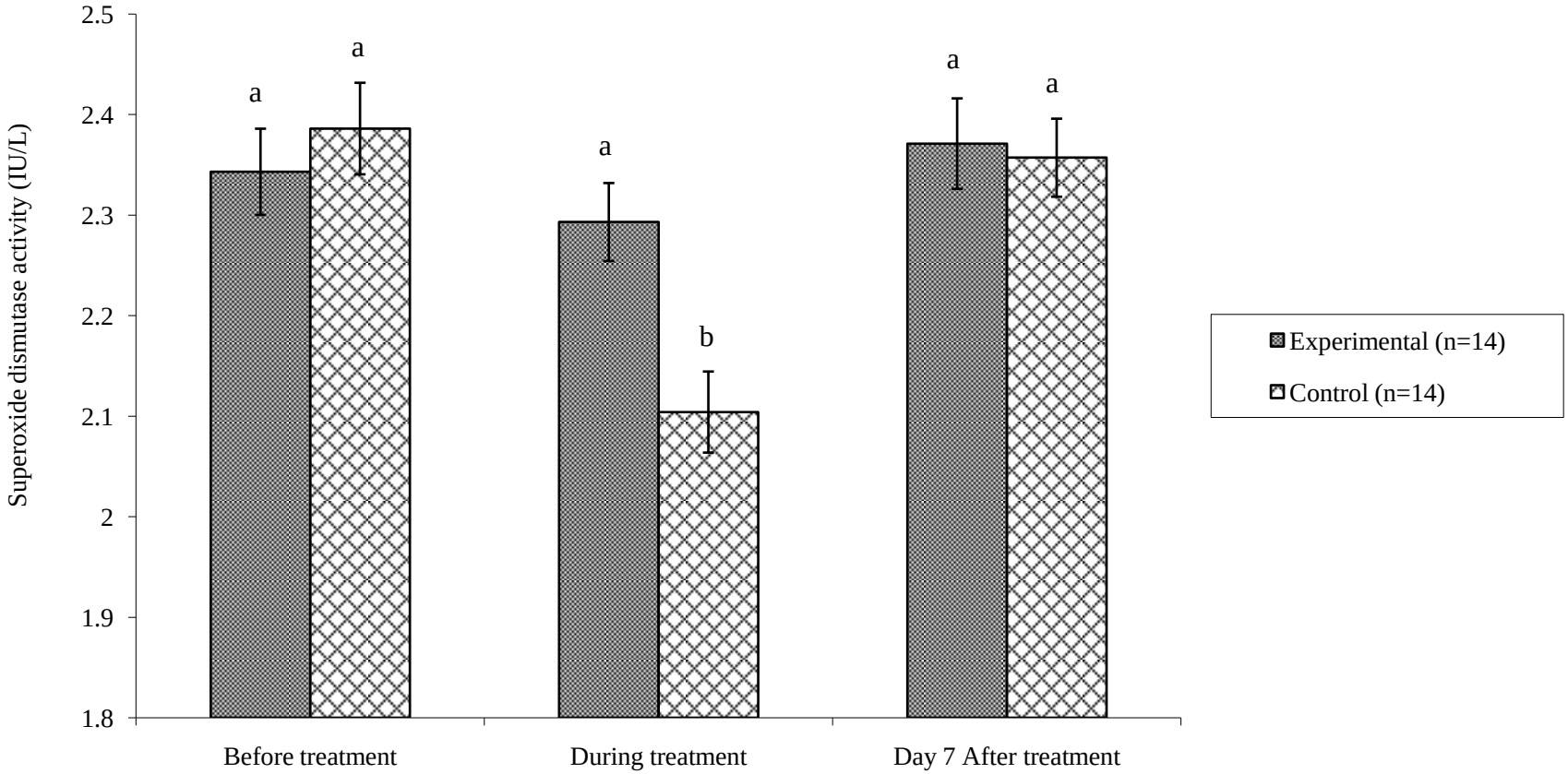


Figure 4.9: Effect of L-glutamine administration on serum superoxide dismutase activity in Red Sokoto goats during the hot-dry season. a, b = $P < 0.05$

4.5.3.2 Glutathione peroxidase activity

There was no significant ($P > 0.05$) difference in gluthathione peroxidase activity between experimental and control goats before and after L-glutamine administration. However, activity of glutathione peroxidase recorded during L-glutamine administration in experimental goats (44.82 ± 0.98 IU/L) was higher ($P < 0.05$) than that recorded in the control goats (42.26 ± 0.65 IU/L) (Figure 4.10). Activities recorded in experimental and control goats before (54.14 ± 1.07 and 53.79 ± 1.17 IU/L, respectively) L-glutamine administration were significantly ($P < 0.05$) higher than those recorded during (44.82 ± 0.98 and 42.46 ± 0.65 IU/L, respectively) or after (45.86 ± 0.88 and 44.86 ± 0.88 IU/L, respectively) L-glutamine administration.

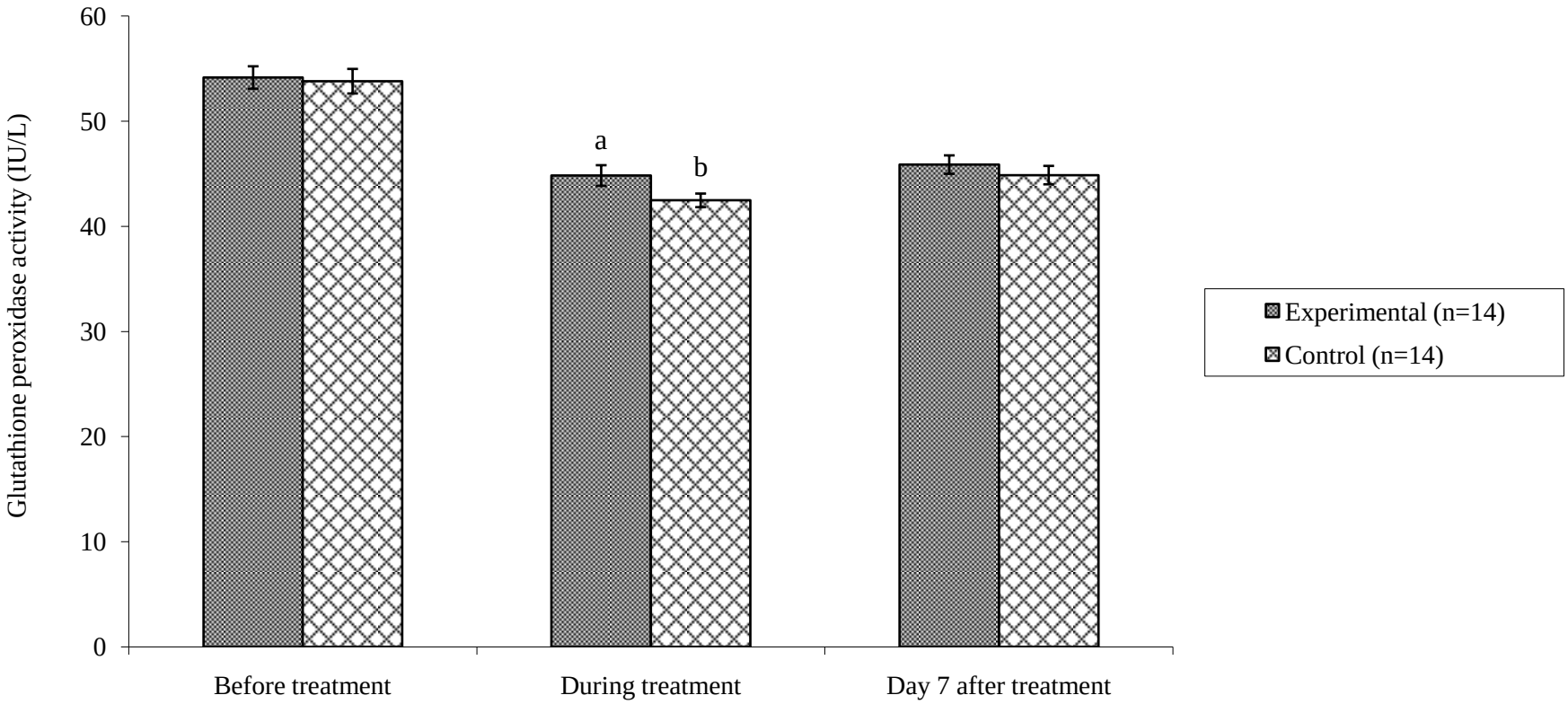


Figure 4.10: Effect of L-glutamine administration on serum glutathione peroxidase activity in Red Sokoto goats during the hot-dry season. a, b = $P < 0.05$

4.5.3.3 Catalase activity

The catalase activity recorded in the experimental goats (48.96 ± 0.9 IU/L) was significantly ($P < 0.05$) higher than the activity recorded in the control goats (44.04 ± 0.9 IU/L) during the period of L-glutamine administration (Figure 4.11). There was no significant difference in the catalase activities between experimental and control goats before L-glutamine administration. The catalase activity in experimental goats was significantly ($P < 0.05$) higher than in control goats after L-glutamine administration (Figure 4.11).

4.6 Sexual variation

There were no significant differences between bucks and does for all the parameters (Values not shown).

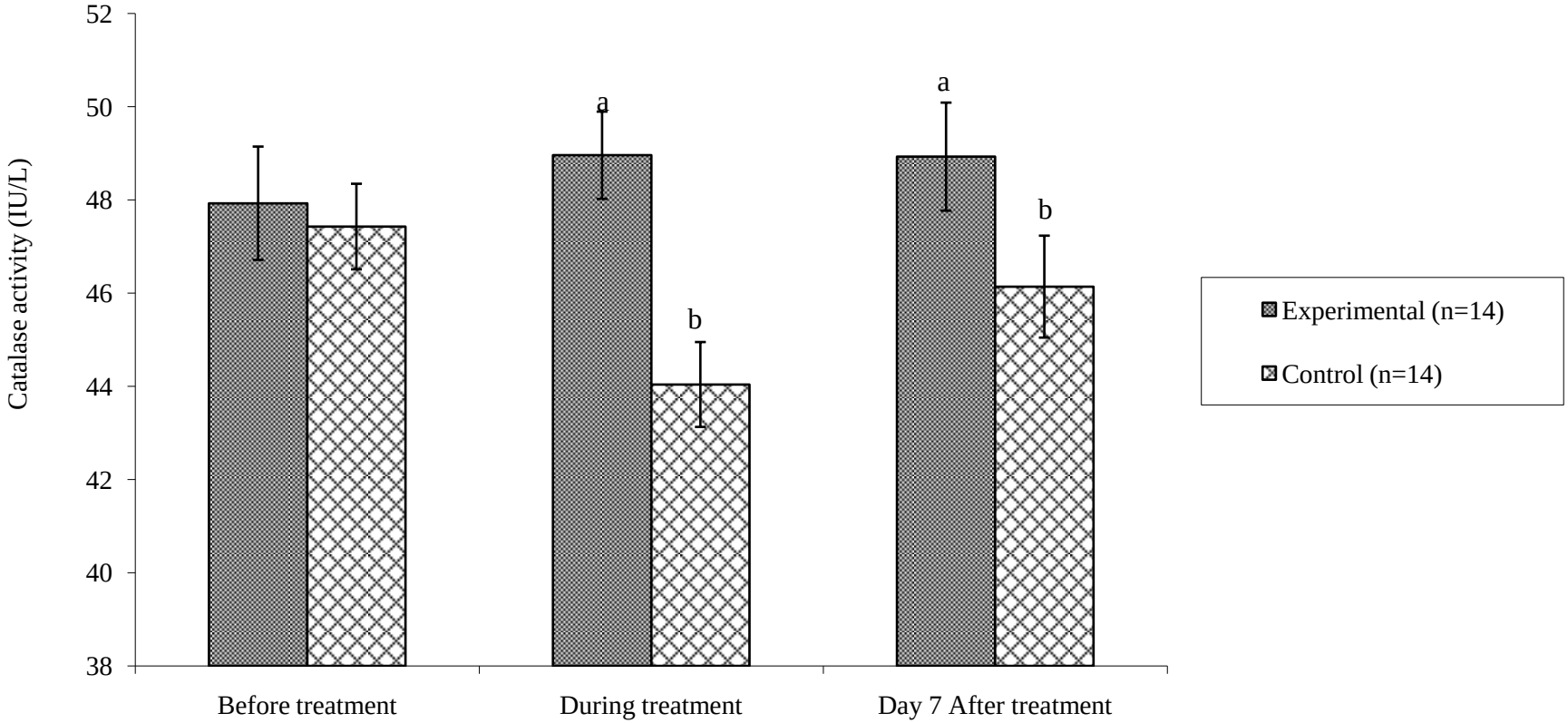


Figure 4.11: Effect of L-glutamine administration on serum catalase activity in Red Sokoto goats during the hot-dry season

a, b = $P < 0.05$

CHAPTER FIVE

DISCUSSION

5.1 Thermal Environmental Parameters

The DBT values recorded during the study period were predominantly outside the thermoneutral values of 13 - 27 °C established for goats in tropical zone (Misra and Puneet, 2009). Similarly, the RH values recorded were predominantly outside the optimal range of 65 - 75 % (Minka and Ayo, 2010) for goats; but are similar to those of Minka and Ayo, (2014), who obtained 26.2 - 37.7 °C for DBT and 50 - 80 % for RH during the hot-dry season in the Northern Guinea Savanna zone of Nigeria. The high RH further compounded the deleterious effects of high AT as it reduces evaporative heat loss; thus, inducing heat stress. The results of the present study further indicate that the hot-dry season (March - May) in the Northern Guinea Savanna zone is thermally stressful to the goats.

The THI, which is a parameter widely used to describe the heat load in an animal, is a good indicator of how thermally stressful thermal environment conditions are (Tao and Xin, 2003). The overall mean THI values before, during and after treatment with L-glutamine (Table 4.1) were above the normal value of 70 for ruminants (Silanikove, 2000a), indicating that the goats were exposed to high heat load and thus, under heat stress. THI values of 70 or less are considered comfortable; 75 - 78 stressful; and values greater than 78 cause extreme distress, and animals are unable to maintain thermoregulatory mechanisms or normal body temperature. The high values of THI recorded during this study, especially at 13:00 h show that the goats were under extreme distress. THI values were also generally higher during L-glutamine administration than before or after the administration, which also suggest that the period was most thermally

stressful to the goats. Minka and Ayo (2012) showed that high heat load was thermally stressful to Red Sokoto goats that were transported for 12 h during the hot-dry season. Generation of ROS is excessively high during heat stress, resulting in oxidative stress, shown to reduce productivity, increase susceptibility to diseases and increase mortality. This is because antioxidant capacity is overwhelmed (Bohmanova *et al*., 2007; Celi, 2011; Adenkola and Ayo, 2013).

5.2 Fluctuations in Rectal Temperature of Red Sokoto Goats administered with L-glutamine during the Hot-dry Season

In the present study, the RT values showed diurnal fluctuations in both experimental and control goats before, during and after L-glutamine administration. The result agrees with the findings that RT in goats varies with the hour of the day (Minka and Ayo, 2012). The RT is controlled by a biological "clock", located in the suprachiasmatic nucleus of the brain. The significantly ($P < 0.05$) lower values of RT recorded in the experimental goats between 06:00 h and 18:00 h during L-glutamine administration showed that L-glutamine decreased the RT in the goats. This result agrees with the finding of Hsu *et al*. (2012), who reported that L-glutamine lowered RT in lipopolysaccharide-challenged pigs. The finding of the present study differs from that of Lindinger and Anderson (2014), who found no significant difference in RT of horses supplemented with L-glutamine-containing diet for nine weeks.

Although the mechanism by which L-glutamine reduced the RT in the experimental goats was not investigated in the present study, Hsu *et al*. (2012) suggested that L-glutamine prevents rise in RT by modulating the expression of plasma tumour necrotic factor-alpha, thus reducing inflammation. The mechanism by which L-glutamine

exerted this effect has not been fully elucidated, but it could be through enhancement of glutathione production. L-glutamine is also involved in signaling pathways and may be involved in regulation of heat-shock proteins, which facilitate the adaptation of animals to high environmental temperature (Mohanarao *et al*., 2014). The difference in the present finding and that of Lindinger and Anderson (2014) may be due to different experimental subjects used, mode of L-glutamine administration, and the environmental conditions in which the horses were kept. It is hypothesized that L-glutamine exerts RT lowering effect through antioxidant mechanism by increasing glutathione production (Roth *et al*. 2002) and, thus, its administration may be beneficial in heat stress alleviation in goats. Further investigations into the molecular mechanism of L-glutamine effect on RT are warranted.

5.3 Effect of L-glutamine Administration on Haematological Parameters

The haematological values, recorded in the experimental and control groups of Red Sokoto goats were within the normal reference range established for goats (Ambore *et al*., 2009; McManus *et al*., 2009; Schalm *et al*., 2010; Hashem, 2014). In the present study, values of the haematological parameters decreased in the control group, that were not treated with L-glutamine, and exposed to environmental heat stress. This finding suggests that heat stress decreased haematological parameters of the goats, and this is in agreement with the result obtained by Sivakumar *et al*. (2010) in goats, and in sheep subjected to heat stress (Srikandakumar *et al*., 2003). The reduction in RBC, PCV, Hb, MCV and MCH obtained in the present study may be due to increased attack of ROS on the RBC membrane and, consequently, resulting in RBC lysis (haemolysis). Heat stress significantly alters haematological parameters, and antioxidant supplementation has been shown to ameliorate its negative effects on the parameters in Malpura ewes (Sejian

et al., 2014). The reduction in haematological parameters in the control group, recorded in the presented study also agreed with the result obtained by Kumar *et al*. (2011), who observed that Beetal goats exposed to heat stress exhibited lower PCV, RBC and Hb, and the use of antioxidant vitamins C and E ameliorated the adverse effects. Hashem (2014) observed increased haematological values in sheared Balady and Damascus goats, exposed to heat stress during summer, which disagreed with the result of the present study. The difference in the results may be due to season and breed.

The finding that values of the erythrocytic parameters in the present study were higher in the group administered with L-glutamine, may suggest that L-glutamine enhanced erythropoiesis in the goats. Furthermore, L-glutamine, as an antioxidant, apparently reduced haemolysis and preserved the RBC integrity by reducing the oxidative effect of ROS, generated in excess during heat stress. Likewise, antioxidants such as ascorbic acid and vitamin E are ROS scavengers, which protect the body defence systems against ROS-induced damages during heat stress (Sivakumar *et al*., 2010; Olaifa *et al*., 2012). As an antioxidant and immunonutrient (El-Sheikh and Khalil, 2011), L-glutamine, like other antioxidants, apparently delays autoxidation by inhibiting the formation of ROS or by interrupting the propagation of ROS (Brewer, 2011).

The PCV, Hb, MCHC values obtained in the present study were similar to those recorded in West African Dwarf goats in South-Eastern Nigeria (Opara *et al*., 2010), although the MCV, MCH and WBC were however slightly higher, in the West African Dwarf goats than those of the present study. Similarly, the present study showed that the PCV, RBC, Hb, MCHC and WBC values were lower, as compared to those obtained by Elitok (2012) in Saanen goats of Turkey, however, the MCV and MCH values were higher in the present study. The differences observed in the present study may be due to geographical location, breed and nutrition (Frelich *et al*. 2006; Tripathi *et al*. 2008;

Temizel *et al*., 2009). The variations in physiological and haematological parameters in heat-adapted goats may be due to their adaptation to different environmental and geographical conditions, essential for their survival (Banerjee *et al*., 2014). Haematological values in the experimental and control groups were lower than those reported by Temizel *et al*. (2009) in Saanen goats, but values obtained by Habibu *et al*. (2014) in male Red Sokoto goats were higher than those recorded in both experimental and control groups in the present study. The difference in the result of the present study with that of Habibu *et al*. (2014) may be due to age difference between animals of the present study and those of Habibu *et al*. (2014).

The increase in differential leucocyte count and erythrocytic parameters observed in the experimental group of Red Sokoto goats was similar to the finding of Soltan (2009), who observed that glutamine supplementation significantly increased red blood cell and white blood cell counts in birds. The results of the present study indicate that L-glutamine is important for lymphocyte proliferation, cytokine production, phagocytosis and secretion by macrophages (Sakamoto *et al*., 2006). Glutamine acts as immunonutrients by potentiating immunocytes such as lymphocytes, macrophages and neutrophils, preserving the immune-competence of the body following major surgical procedures and promoting wound healing conditionally (Caroprese *et al*., 2012; Hu *et al*., 2013). Oral supplementations with glutamine are effective in attenuating oxidative stress and the pro-inflammatory responses induced by endotoxaemia (Cruzat *et al*., 2014). In the present study, L-glutamine supplementation increased the total and differential leucocyte counts in the peripheral blood, and this finding may be related to the fact that glutamine is essential to cell division processes, including haemopoiesis in the bone marrow (Li *et al*., 2009; Wu, 2010; Xi *et al*., 2010; Ruth and Field, 2013). The decrease in leucocyte and neutrophil counts observed in the control group agreed with

the finding of Wojtas *et al*. (2014) who observed a decrease in leucocyte and neutrophil counts in heat-stressed Merino sheep,. The leucocyte counts obtained in the experimental and control groups in the present study, were lower than those recorded by Piccione *et al*. (2008) in Valle del Belice ewes of Palermo, Italy, subjected to heat stress; Decampos *et al*. (2013) in West African Dwarf sheep; and by Okoruwa (2014) in West African Dwarf goats in Southern Nigeria. The leucocyte, neutrophil and lymphocyte counts obtained in the present study (Table 4.4) were similar to the corresponding counts (10.60 ± 0.25 x 10^3/µL, 4.60 ± 0.18 x 10^3/µL and 4.94 ± 0.15 x 10^3/µL, respectively), recorded by Piccione *et al*. (2010) in Girgentana goats. The leucocyte count recorded by Mubi *et al.* (2015) in Yankassa sheep (6.79 ± 4.5 x 10^3/µL) was lower, when compared to the count obtained in the present study (8.82 ± 0.83 x 10^3/µL).

5.4 Erythrocyte Osmotic Fragility in Red Sokoto Goats during the Study Period

The result showed that L-glutamine administration protected the integrity of the caprine erythrocyte membrane during the hot-dry season. Thus, L-glutamine has antioxidant potentials in the amelioration of heat stress in goats. The significantly lower EOF at 0.5 and 0.7 % NaCl, in the experimental group during the three-week period of L-glutamine administration showed that L-glutamine exerted protective effect on the integrity of the erythrocyte membrane. The erythrocytes of goats are relatively resistant to osmotic perturbation which may due to the nature of their cell membrane, and high level of stress is needed to deform its membranes (Minka and Ayo, 2010; Schalm *et al*., 2010). The higher osmotic fragility recorded in the control group indicated that the meteorological conditions were stressful to the goats during the study period. The

findings of the present study agrees with that of Minka and Ayo (2010), who reported that antioxidant vitamin, ascorbic acid, protects the erythrocytes of goats, subjected to 12-h transportation and heat stresses during the hot-dry season.

5.5 Variation in Malondialdehyde Concentration

The result showed that L-glutamine mitigated heat stress-induced increase in MDA concentration in the experimental goats. The finding agrees with the result obtained by Yang *et al*. (2011) and Cruzat *et al*. (2014), who, independently showed that L-glutamine reduces MDA concentrations in *Haemophilus parasuis*-infected mice and lipopolysaccharide-challenged mice, respectively. MDA is a sensitive biomarker of oxidative stress. The reduction observed in this study may be as a result of the activities of glutathione-dependent antioxidant systems. L-glutamine is one of the precursors of glutathione (Cavalcante and de Vasconcelos, 2015), and its supplementation preserves glutathione during stress. The higher MDA concentrations recorded in both experimental and control goats during the period of L-glutamine supplementation, suggests strongly that the period was more thermally stressful to the goats than any other period of the study. This was evidenced by the highest THI recorded during the period of L-glutamine administration (Table 4.1).

5.6 Variation in Antioxidant Enzyme Activities

The result also showed that L-glutamine increased the activities of serum antioxidant enzymes during the period of L-glutamine administration. The finding agrees with the result of Hu *et al*. (2014), who showed that L-glutamine improves repair activity in fish

erythrocytes after challenge with H_2O_2, by restoring H_2O_2-induced decrease in glutathione concentration, activities of glutamine peroxidase and superoxide dismutase.

The finding that L-glutamine relatively sustained the SOD, glutathione peroxidase and catalase activities in the experimental goats suggests that its administration during heat stress and other stressful conditions may be beneficial to goats reared in the Northern Guinea Savanna zone of Nigeria, especially during the hot-dry season. L-glutamine, as a major respiratory fuel for rapidly-dividing cells, has been shown to improve immune status and gut health by maintaining redox status (Wu *et al*., 2011; Rao and Samak, 2012). It enhances the antioxidant status of animals by increasing ROS-scavenging activity of the antioxidant enzymes. This finding was demonstrated by the significantly higher antioxidant enzyme activities in the experimental goats, when compared to that of the control group.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

It is concluded that:

1) The hot-dry season imposed heat load on the goats, which adversely affected their rectal temperature, haematological and oxidative stress parameters during the hot-dry season.

2) The administration of L-glutamine to Red Sokoto goats at 0.2 g/kg body weight *per os* ameliorated the adverse effects of heat stress on their rectal temperature, haematological and oxidative stress parameters during the hot-dry season.

6.2 Recommendations

It is recommended that:

1) L-glutamine should be administered to goats (at 0.2 g/kg body weight) during the hot-dry season to ameliorate heat stress, improve their health, and, consequently, productivity.

2) Rectal temperature and indices of oxidative stress, such as erythrocyte osmotic fragility, malondialdehyde levels and activities of antioxidant enzymes, may be useful diagnostic tools for evaluating effects of heat stress in goats during the hot-dry season.

3) Further investigations should be carried out to ascertain the optimal dose of L-glutamine in goats during the hot-dry season.

4) Further studies are also required to elucidate the mechanism by which L-glutamine ameliorates oxidative changes in goats subjected to heat stress.

5) Studies on inclusion of L-glutamine into the diet of heat-stressed goats are suggested.

REFERENCES

Abei, H. (1984). Catalase *in vitro*. *Methods in Enzymology*, 105: 121-126.

Abilay, T. A., Johnson, H. D. and Madan, M. (1975). Influence of environmental heat on peripheral plasma progesterone and cortisol during the bovine estrous cycle. *Journal of Dairy Science*, 58(12): 1836-1840.

Adenkola, A. Y. and Ayo, J. O. (2013). Physiological and behavioural responses of livestock to road transportation stress: A review. *African Journal of Biotechnology*, 9(31): 4845-4856.

Adu, I. F., Buvanendran, V. and Lakpini, C. A. M. (1979). The reproductive performance of Red Sokoto goats in Nigeria. *The Journal of Agricultural Science*, 93(03): 563-566.

Akinwande, A. I. and Adebule, A. O. A. (2003). Ascorbic acid and beta-carotene alleviate oxidative effect of London King Size® cigarette smoke on tissue lipids. *Nigerian Journal of Health and Biomedical Sciences*, *2*(1): 12-15.

Akpa, G. N., Duru S. and Amos T. T. (1998). Influence of strain and sex on estimation of within-age-group body weight of Nigerian Maradi goats from their linear body measurement. *Tropical Agriculture (Trinidad)*, 74: 462-467.

Alam, M. M., Hashem, M. A., Rahman, M. M., Hossain, M. M., Haque, M. R., Sobhan, Z. and Islam, M. S. (2011). Effect of heat stress on behavior, physiological and blood parameters of goat. *Progressive Agriculture*, 22(1-2): 37-45.

Al-Haidary, A. A. (2006). Seasonal variation in thermoregulatory and some physiological responses of Arabian camel (Camelus dromedaries). *Journal of the Saudi Society of Agricultural Sciences*, 5: 30-41.

Alicigüzel, Y., Özdem, S. N., Özdem, S. S., Karayalçin, Ü., Siedlak, S. L., Perry, G. and Smith, M. A. (2001). Erythrocyte, plasma, and serum antioxidant activities in untreated toxic multinodular goiter patients. *Free Radical Biology and Medicine*, 30(6): 665-670.

Altan, Ö., Pabuçcuoğlu, A., Altan, A., Konyalioğlu, S. and Bayraktar, H. (2003). Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. *British Poultry Science*, 44(4): 545-550.

Ambali, S. F., Abubakar, A. T., Shittu, M., Yaqub, L. S., Kobo, P. I. and Giwa, A. (2010). Ameliorative effect of zinc on chlorpyrifos-induced erythrocyte fragility in wistar rats. *New York Science Journal*, 3: 117-122.

Ambore, B., Ravikanth, K., Maini, S. and Rekhe, D. S. (2009). Haematological profile and growth performance of goats under transportation stress. *Veterinary World*, 5: 195-198.

Ames, B. N., Shigenaga, M. K. and Hagen, T. M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences*, 90(17): 7915-7922.

Andreyev, A. Y., Kushnareva, Y. E. and Starkov, A. A. (2005). Mitochondrial metabolism of reactive oxygen species. *Biochemistry (Moscow)*, 70(2): 200-214.

Asala, O. O., Ayo, J. O., Rekwot, P. I., Minka, N. S., Omoniwa, D. O. and Adenkola, A. Y. (2011). Effect of ascorbic acid administration on erythrocyte osmotic fragility of pigs transported by road during the hot-dry season. *Veterinary Research Communications*, 35:245-54.

Ayo, J. O., Minka, N. S., Sackey, A. K. B. and Adelaiye, A. B. (2009). Responses of serum electrolytes of goats to twelve hours of road transportation during the hot-dry season in Nigeria, and the effect of pretreatment with ascorbic acid. *Onderstepoort Journal of Veterinary Research*, 76(4): 409-418.

Ayo, J. O., Oladele, S. B. and Fayomi, A. (1998). Stress and its adverse effects on modern swine production. *Pig News and Information*, 19(2): 51N-56N.

Ayo, J. O., Oladele, S. B., Fayomi, A., Jumbo, S. D. and Hambolu, J. O. (1988). Body temperature, respiration and heart rates in the Red Sokoto goat during the harmattan season. *Bulletin of Animal Health and Production in Africa*, 46: 161-166.

Azad, M. A., Kikusato, M., Maekawa, T., Shirakawa, H. and Toyomizu, M. (2010). Metabolic characteristics and oxidative damage to skeletal muscle in broiler chickens exposed to chronic heat stress. *Comparative Biochemistry Physiology, Part A: Molecular and Integrative Physiology*, 155: 401-406.

Baker, F. J. and Silverton, R. E. (1985). *Introduction to Medical Laboratory Technology*, 6th Edition, Butterworth and Co., Publishers Limited, UK, 408 pp.

Banerjee, A. K., Mandal, A., Chanda, D. and Chakraborti, S. (2003). Oxidant, antioxidant and physical exercise. *Molecular and Cellular Biochemistry*, 253(1-2): 307-312.

Banerjee, D., Upadhyay, R. C., Chaudhary, U. B., Kumar, R., Singh, S., Ashutosh, Das, T.K. and De, S. (2014). Seasonal variations in physio-biochemical profiles of Indian goats in the paradigm of hot and cold climate. *Biological Rhythm Research*, 46(2): 221-236.

Barker, G. (1983). Review of J. Clutton-Brock 'Domesticated Animals from Early Times'. *The Antiquaries Journal*, 63: 138-139.

Baumgard, L. H. and Rhoads Jr, R. P. (2013). Effects of heat stress on postabsorptive metabolism and energetics. *Annual Review of Animal Biosciences*, 1(1): 311-337.

Belhadj Slimen, I., Najar, T., Ghram, A. and Abdrrabba, M. (2015). Heat stress effects on livestock: molecular, cellular and metabolic aspects, a review. *Journal of Animal Physiology and Animal Nutrition* DOI: 10.1111/jpn.12379.

Bellavance, M. A. and Rivest, S. (2014). The HPA–immune axis and the immunomodulatory actions of glucocorticoids in the brain. *Frontiers in Immunology*, 5: 136-148.

Bernabucci, U., Ronchi, B., Lacetera, N. and Nardone, A. (2002). Markers of oxidative status in plasma and erythrocytes of transition dairy cows during the hot season. *Journal of Dairy Science*, 8(9): 2173-2179.

Bianca, W.K. (1976). The significance of meteorology in animal production. *International Journal of Biometeorology*, 20: 139-156.

Bitla, A. R., Reddy, P., Sambasivaih, K., Suchitra, M. M., Reddy, S. and Rao, P. S. (2011). Evaluation of plasma malondialdehyde as a biomarker in patients with carcinoma of stomach. *Biomedical Research*, 22: 63-68.

Boelens, P. G., Nijveldt, R. J., Houdijk, A. P., Meijer, S. and van Leeuwen, P. A. (2001). Glutamine alimentation in catabolic state. *The Journal of Nutrition*, 131(9): 2569-2577.

Bohmanova, J., Misztal, I. and Cole, J. B. (2007). Temperature-humidity indices as indicators of milk production losses due to heat stress. *Journal of Dairy Science*, 90(4): 1947-1956.

Borges, S. A., Da Silva, A. F., Ariki, J., Hooge, D. M. and Cummings, K. R. (2003). Dietary electrolyte balance for broiler chickens exposed to thermoneutral or heat-stress environments. *Poultry Science*, 82(3): 428-435.

Boveris, A. and Chance, B. (1973). The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. *Biochemical Journal*, 134: 707-716.

Brewer, M. S. (2011). Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. *Comprehensive Reviews in Food Science and Food Safety*, 10(4): 221-247.

Broucek, J., Kisac, P. and Uhrincat, M. (2009). Effect of hot temperatures on the hematological parameters, health and performance of calves. *International Journal of Biometeorology*, 53(2): 201-208.

Broucek, J., Ryba, S., Mihina, S., Uhrincat, M. and Kisac, P. (2007). Impact of thermal-humidity index on milk yield under conditions of different dairy management. *Journal of Animal and Feed Sciences*, 16(3): 329-344.

Brzezińska-Ślebodzińska, E. (2001). Erythrocyte osmotic fragility test as the measure of defence against free radicals in rabbits of different age. *Acta Veterinaria Hungarica*, 49(4): 413-419.

Candiani, D., Salamano, G., Mellia, E., Doglione, L., Bruno, R., Toussaint, M. and Gruys, E. (2008). A combination of behavioral and physiological indicators for assessing pig welfare on the farm. *Journal of Applied Animal Welfare Science*, 11(1): 1-13.

Caroprese, M., Albenzio, M., Marino, R., Santillo, A. and Sevi, A. (2012). Immune response and milk production of dairy cows fed graded levels of rumen-protected glutamine. *Research in Veterinary Science*, 93: 202-209.

Cavalcante, A. A. M. and de Vasconcelos, P. R. L. (2015). Enteral Nutrition Supplemented with l-glutamine in Patients with Sepsis. *In*: *Glutamine in Clinical Nutrition*, Springer, New York, Pp. 169-179.

Celi, P. (2011). Biomarkers of oxidative stress in ruminant medicine. *Immunopharmacology and Immunotoxicology*, 33(2): 233-240.

Chatterjee, M., Saluja, R., Kanneganti, S., Chinta, S. and Dikshit, M. (2007). Biochemical and molecular evaluation of neutrophil NOS in spontaneously hypertensive rats. *Cellular and Molecular Biology*, 53(1): 84-93.

Chauhan, S. S., Celi, P., Leury, B. J., Clarke, I. J. and Dunshea, F. R. (2014). Dietary antioxidants at supranutritional doses improve oxidative status and reduce the negative effects of heat stress in sheep. *Journal of Animal Science*, 92(8): 3364-3374.

Chowdhury, V. S., Tomonaga, S., Nishimura, S., Tabata, S. and Furuse, M. (2012). Physiological and behavioral responses of young chicks to high ambient temperature. *The Journal of Poultry Science*, 49(3): 212-218.

Collier, R. J., Collier, J. L., Rhoads, R. P. and Baumgard, L. H. (2008). Invited review: genes involved in the bovine heat stress response. *Journal of Dairy Science*, 91(2): 445-454.

Cruzat, V. F., Bittencourt, A., Scomazzon, S. P., Leite, J. S. M., de Bittencourt, P. I. H. and Tirapegui, J. (2014). Oral free and dipeptide forms of glutamine supplementation attenuate oxidative stress and inflammation induced by endotoxemia. *Nutrition*, 30: 602-611.

Cucuzza, L. S., Riondato, F., Macchi, E., Bellino, C., Franco, G., Biolatti, B. and Cannizzo, F. T. (2014). Haematological and physiological responses of Piemontese beef cattle to different housing conditions. *Research in Veterinary Science*, 97(2): 464-469.

Curi, R., Lagranha, C. J., Sellitti, D. F., Procopio, J., Pithon-Curi, T. C., Corless, M. and Newsholme, P. (2005). Molecular mechanisms of glutamine action. *Journal of Cellular Physiology*, 204(2): 392-401.

Dacie, J. V. and Lewis, S. M. (1991). *Practical Haematology*, 7th Edition, Churchill Livinstone, London, Pp. 659-661.

Dangi, S. S., Gupta, M., Nagar, V., Yadav, V. P., Dangi, S. K., Shankar, O., Chouhan, V. S., Kumar, P., Singh, G. and Sarkar, M. (2014). Impact of short-term heat stress on physiological responses and expression profile of HSPs in Barbari goats. *International Journal of Biometeorology*, 58(10): 2085-2093.

Darcan, K. D., Cankaya, S. and Karakok, S. G. (2009). The effects of skin pigmentation on physiological factors of thermoregulation and grazing behaviour of dairy goats in a hot and humid climate. *Asian-Australasian Journal of Animal Science*, 22(5): 727-732.

Decampos, J. S., Ikeobi, C. O., Olowofeso, O., Smith, O. F., Adeleke, M. A., Wheto, M. and Amusan, S. A. (2013). Effects of coat colour genes on body measurements, heat tolerance traits and haematological parameters in West African Dwarf sheep. *Open Journal of Genetics*, 3: 280-284.

Devendra, C. (2001). Small ruminants: Imperative for productivity enhancement, improved livelihoods and rural growth - A review. *Asian-Australian Journal of Animal Sciences*, 14(10): 1483-1496.

Devendra, C. (2013). Investments on pro-poor development projects on goats: Ensuring success for improved livelihoods. *Asian-Australasian Journal of Animal Sciences*, 26(1): 1-18.

Devendra, C. and Burns, M. (1983). Goat Production in the Tropics. *In*: *Commonwealth Agricultural Bureaux*, Slough. UK.: 183pp.

Donaldson, M. S. (2004). Nutrition and cancer: a review of the evidence for an anti-cancer diet. *Nutrition Journal*, 3(1): 19-25.

Dorđevic, N. Z., Barbic, G. M., Markovic, S. D., Ognjanovic, B. K., Štajn, A. S., Žikic, R. V. and Saicic, Z. S. (2008). Oxidative stress and changes in antioxidative defense system in erythrocytes of pre-eclampsia in women. *Reproductive Toxicology*, 25: 213-18.

Dröge, W. (2002). Free radicals in the physiological control of cell function. *Physiological Reviews*, 82(1): 47-95.

Ďuračková, Z. (2010). Some current insights into oxidative stress. *Physiological Research*, 59: 459-469.

Earley, B., Buckam-Sporer, K., Gupta, S., Pang, W. and Ting, S. (2010). Biologic response of animals to husbandry stress with implications for biomedical models. *Open Access Animal Physiology*, 2: 25-42.

Elitok, B. (2012). Reference values for hematological and biochemical parameters in Saanen goats breeding in Afyonkarahisar province. *Kocatepe Veterinary Journal*, 5: 7-11.

El-Sheikh, N. M. and Khalil, F. A. (2011). L-Arginine and L-glutamine as immunonutrients and modulating agents for oxidative stress and toxicity induced by sodium nitrite in rats. *Food and Chemical Toxicology*, 49: 758-762.

Esonu, B. O., Emenalom, O. O., Udedebie, U., Herbert, D. F., Ekpori, I. C. and Iheukwuemere, F.C. (2001). Performance and blood chemistry of weaner pigs fed raw mucuna bean (velvet bean) meal. *Tropical Animal Production and Investigation*, 4: 141 - 150.

Etches, R., John, J. M. and Gibbins, A. M. V. (1995). Behavioural, physiological, endocrine and molecular responses to heat stress. In: Daghir, N.J. (Ed.), Poultry Production in Hot Climates. CAB International, Wallingford, Pp. 31-65.

Etim, N. N., Williams, M. E., Evans, E. I. and Offiong, E. E. (2013). Physiological and behavioural responses of farm animals to stress: Implications to animal productivity. *American Journal of Advanced Agricultural Research*, 1(2): 53-61.

Ewing, S. A., Lay, D. C. and Von Borell, E. (1999). *Farm animal well-being: Stress physiology, animal behavior, and environmental design*. Prentice Hall, Upper Saddle River, NJ, USA, Pp. 1-357.

FAOSTAT (2013): http://faostat.fao.org/default.aspx

Fazio, E. and Ferlazzo, A. (2003). Evaluation of stress during transport. *Veterinary Research Communications*, 27(1): 519-524.

Fernández, H., Hughes, S., Vigne, J. D., Helmer, D., Hodgins, G., Miquel, C., Hanni, C., Luikart, G. and Taberlet, P. (2006). Divergent mtDNA lineages of goats in an Early Neolithic site, far from the initial domestication areas. *Proceedings of the National Academy of Sciences*, 103(42): 15375-15379.

Field, C. J., Johnson, I. R. and Schley, P. D. (2002). Nutrients and their role in host resistance to infection. *Journal of Leucocyte Biology*, 71(1): 16-32.

Fitzugh, H.A., Ehui, S.K. and Lahlou-Kassi, A. (1992). Research strategies for development of animal agriculture. *World Animal Review*, 72: 9-19.

Flaring, U. B., Rooyackers, O. E., Wernerman, J. and Hammarqvist, F. (2003). Glutamine attenuates post-traumatic glutathione depletion in human muscle. *Clinical Science*, 104(3): 275-282.

Flohe, L. and Günzler, W. A. (1984). Assays of glutathione peroxidase. *Methods in Enzymology*, 105: 114-121.

Flores, R. (1978). A rapid and reproducible assay for quantitative estimation of proteins using bromophenol blue. *Analytical Biochemistry*, 88(2): 605-611.

Frelich J., Pecharova E., Klimeš F., Šlachta M., Hakrova P. and Zdražil M. (2006). Landscape management by means of cattle pasturage in the submountain area of the Czech Republic. *Ekologia (Bratislava)*, 25: 116-124.

Fridovich, I. (1995). Superoxide radical and superoxide dismutases. *Annual Review of Biochemistry*, 64(1): 97-112.

Gadiyaram, K. M. and Kannan, G. (2004). Comparison of textural properties of low-fat chevon, beef, pork, and mixed-meat sausages. 34 (Supplement 1) ©South African Society for Animal Science Peer reviewed paper: 8th International Conference on Goats, 212 pp.

Ganaie, A. H., Shanker, G., Bumla, N. A., Ghasura, R. S., Mir, N. A., Wani, S. A. and Dudhatra, G. B. (2013). Biochemical and physiological changes during thermal stress in bovines. *Journal of Veterinary Science and Technology*, 3(3): 423-430.

Gebremedhin, K. G., Hillman, P. E., Lee, C. N., Collier, R. J., Willard, Arthington, J.D. and Brown-Brandl, T. M., (2008). Sweating rate of dairy cows and beef heifers in hot conditions. *Transactions of American Society of Agricultural and Biological Engineers*, 51: 2167-2178.

Georgieva, N. V. (2005). Oxidative stress as a factor of disrupted ecological oxidative balance in biological systems - a review. *Bulgarian Journal of Veterinary Medicine*, 8(1): 1-11.

Gleeson, M. (2008). Dosing and efficacy of glutamine supplementation in human exercise and sport training. *The Journal of Nutrition*, 138(10): 2045-2049.

Griffin, C. L., Orcutt, M. W., Riley, R. R., Smith, G. C., Savell, J. W. and Shelton, M. (1992). Evaluation of palatability of lamb, mutton and chevon by sensory panels of various cultural backgrounds. *Small Ruminant Research*, 8: 67-74.

Griffith, O. W. (1999). Biologic and pharmacologic regulation of mammalian glutathione synthesis. *Free Radical Biology and Medicine*, 27(9): 922-935.

Griffith, O. W., Bridges, R. J. and Meister, A. (1981). Formation of gamma-glutamycyst (e) ine *in vivo* is catalyzed by gamma-glutamyl transpeptidase. *Proceedings of the National Academy of Sciences of the United States of America*, 78(5): 2777-2781.

Gultekin, F., Delibas, N., Yasar, S. and Kilinc, I. (2001). *In vivo* changes in antioxidant systems and protective role of melatonin and a combination of vitamin C and

vitamin E on oxidative damage in erythrocytes induced by chlorpyrifos-ethyl in rats. *Archives of Toxicology*, 75: 88-96.

Gupta, M., Kumar, S., Dangi, S. S. and Jangir, B. L. (2013a). Physiological, biochemical and molecular responses to thermal stress in goats. *International Journal of Livestock Research*, 3(2): 27-38.

Gupta, R. K. and Singh, N. (2013). Morinda citrifolia (Noni) alters oxidative stress marker and antioxidant activity in cervical cancer cell lines. *Asian Pacific Journal of Cancer Prevention*, 14(8): 4603-4606.

Gupta, R. K., Patel, A. K., Shah, N., Chaudhary, A. K., Jha, U. K., Yadav, U. C., Gupta, P. K. and Pakuwal, U. (2013b). Oxidative stress and antioxidants in disease and cancer: a review. *Asian Pacific Journal of Cancer Prevention*, 15(11): 4405-4409.

Gustafsson, J. E. (1976). Improved specificity of serum albumin determination and estimation of " acute phase reactants" by use of the bromocresol green reaction. *Clinical Chemistry*, 22(5): 616-622.

Gwatibaya, S. Svotia, E. and Jambwa, D. (2007). Potential effects and management options for heat stress in dairy cows in Zimbabwe: A review. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, 6: 2066-2074.

Habibu, B., Kawu, M. U., Makun, H. J., Aluwong, T., Yaqub, L. S., Ahmad, M. S., Tauheed, M. and Buhari, H. U. (2014). Influence of sex, reproductive status and foetal number on erythrocyte osmotic fragility, haematological and physiologic parameters in goats during the hot-dry season. *Veterinarni Medicina*, 59(10): 479-490.

Halliwell, B. (1997). Antioxidants: the basics - what they are and how to evaluate them. *Advances in Pharmacology*, 38:3-20.

Halliwell, B. (2007). Biochemistry of oxidative stress. *Biochemical Society Transactions*, 35(5): 1147-1150.

Halliwell, B. (2012). Free radicals and antioxidants: updating a personal view. *Nutrition Reviews*, 70(5): 257-265.

Halliwell, B. and Chirico, S. (1993). Lipid peroxidation: its mechanism, measurement, and significance. *The American Journal of Clinical Nutrition*, 57(5): 715-724.

Halliwell, B. and Cross, C. E. (1994). Oxygen-derived species: their relation to human disease and environmental stress. *Environmental Health Perspectives*, 102(Suppl 10): 5-12.

Halliwell, B. H. and Gutteridge, J. M. C. (2007). *Free radicals in biology and medicine*. 4th Edition, Oxford University Press, Oxford, 704 pp.

Hanczakowska, E. and Niwińska, B. (2013). Glutamine as a feed supplement for piglets: a review. *Annals of Animal Science*, 13(1): 5-15.

Hanzawa, K. and Watanabe, S. (2000). Change in osmotic fragility of erythrocytes in athletic horses. *Journal of Equine Veterinary Science*, 11: 51-61.

Hashem, A. L. S. (2014). Effect of summer shearing on thermoregulatory, hematological and cortisol responses in Balady and Damascus goats in desert of Sinai, Egypt. *World Applied Sciences Journal*, 30: 521-533.

Hassan, A. Z. and Hassan, F. B. (2003). *An introduction to veterinary practice*. Ahmadu Bello University Press Limited, Zaria, Nigeria, 389 pp.

Ho, E., Galougahi, K. K., Liu, C. C., Bhindi, R. and Figtree, G. A. (2013). Biological markers of oxidative stress: applications to cardiovascular research and practice. *Redox Biology*, 1(1): 483-491.

Hoste, C. H., Chalon, E., D'leteren, G. and Trail, J. C. M. (1988). Trypanotolerant livestock in West and Central Africa. *FAO Animal Health and Production Papers*, 20(2): 56-67.

Hsu, C. B., Lee, J. W., Huang, H. J., Wang, C. H., Lee, T. T., Yen, H. T. and Yu, B. (2012). Effects of supplemental glutamine on growth performance, plasma parameters and LPS-induced immune response of weaned barrows after castration. *Asian-Australasian Journal of Animal Sciences*, 25(5): 674-681.

Hu, K., Feng, L., Jiang, W., Liu, Y., Jiang, J., Li, S. and Zhou, X. (2014). Oxidative damage repair by glutamine in fish enterocytes. *Fish Physiology and Biochemistry*, 40(5): 1437-1445.

Hu, Z. Y., Su, H. W., Li, S. L. and Cao, Z. J. (2013). Effect of parenteral administration of glutamine on autophagy of liver cell and immune responses in weaned calves. *Journal of Animal Physiology and Animal nutrition*, 97: 1007-1014.

Humbert, B., Nguyen, P., Martin, L., Dumon, H., Vallette, G., Maugere, P. and Darmaun, D. (2007). Effect of glutamine on glutathione kinetics *in vivo* in dogs. *The Journal of Nutritional Biochemistry*, 18(1): 10-16.

Igono, M. O., Molokwu, E. C. I. and Aliu, Y.O. (1982). Body temperature responses of Savannah Brown goat to harmattan and hot-dry season. *International Journal of Biometeorology*, 26: 225-230.

Janero, D. R. (1990). Malondialdehyde and thiobarbituric acid reactivity as diagnositic indexes of lipid peroxidation and peroxidative tissue injury. *Free Radical Biology and Medicine*, 9: 515-540.

Jazideh, F., Farhoomand, P., Daneshyar, M. and Najafi, G. (2014). The effects of dietary glutamine supplementation on growth performance and intestinal morphology of broiler chickens reared under hot conditions. *Turkish Journal of Veterinary and Animal Sciences*, 38(3): 264-270.

Jibir, M., Hassan, W. A., Maigandi1, S. A., Garba, S. and Adeyaniju, J. B. (2010). The effect of breed, age and fasting status on macro-nutrient composition of meat from goat breeds of North-Western Nigeria. *Nigerian Journal of Basic and Applied Science*, 18(2): 269-271.

Jindal, S. K. (1980). Effect of climate on goats: a review. *Indian Journal of Dairy Science*, 33: 285-293.

Jones, D. P. (2006). Redefining oxidative stress. *Antioxidants and Redox Signaling*, 8(9-10): 1865-1879.

Kadzere, C. T., Murphy, M. R., Silanikove, N. and Maltz, E. (2002). Heat stress in lactating dairy cows: a review. *Livestock Production Science*, 77(1): 59-91.

Kashinakunti, S. V., Sunitha, H., Gurupadappa, K., Shankarprasad, D. S., Suryaprakash, G., and Ingin, J. B. (2010). Lipid peroxidation and antioxidant status in preeclampsia. *Al-Ameen Journal of Medical Sciences*, 3(1): 38-41.

Keim, S. M., Guisto, J. A. and Sullivan Jr, J. B. (2002). Environmental thermal stress. *Annals of Agricultural and Environmental Medicine*, 9(1): 1-15.

Kim, J. Y., Burghardt, R. C., Wu, G., Johnson, G. A., Spencer, T. E. and Bazer, F. W. (2011a). Select nutrients in the ovine uterine lumen. VII. Effects of arginine, leucine, glutamine, and glucose on trophectoderm cell signaling, proliferation, and migration. *Biology of Reproduction*, 84(1): 62-69.

Kim, M. H., Yang, J. Y., Upadhaya, S. D., Lee, H. J., Yun, C. H. and Ha, J. K. (2011b). The stress of weaning influences serum levels of acute-phase proteins, iron-binding proteins, inflammatory cytokines, cortisol, and leukocyte subsets in Holstein calves. *Journal of Veterinary Science*, 12(2): 151-157.

Kingma, B., Frijns, A. and van Marken Lichtenbelt, W. (2012). The thermoneutral zone: implications for metabolic studies. *Frontiers in Bioscience* (*Elite Ed*.), 4: 1975-1985.

Kirschvink, N., de Moffarts, B. and Lekeux, P. (2008). The oxidant/antioxidant equilibrium in horses. *The Veterinary Journal*, 177(2): 178-191.

Kirton, A. H. (1988). Characteristics of goat meat including carcass quality and methods of slaughter. *Proceeding of the Workshop on Goat Meat Production in Asia*, March, 13-18, Tando Jam, Pakistan, Pp: 87-99.

Ko, Y. G., Kim, E. K., Kim, T., Park, H., Park, H. S., Choi, E. J. and Kim, S. (2001). Glutamine-dependent antiapoptotic interaction of human glutaminyl-tRNA synthetase with apoptosis signal-regulating kinase 1. *Journal of Biological Chemistry*, 276(8): 6030-6036.

Kohn, R. A. and Allen, M. S. (1995). Enrichment of Proteolysis activity relative to nitrogen in preparations from the human for *in vitro* studies. *Animal Feed Science and Technology*, 52: 1-4.

Koknaroglu, H. (2008). Effect of concentrate level on sustainability of beef cattle production. *Journal of Sustainable Agriculture*, 32(1): 123-136.

Koknaroglu, H. and Akunal, T. (2013). Animal welfare: an animal science approach. *Meat Science*, 95(4): 821-827.

Kolanjiappan, K., Manoharan, S. and Kayalvizhi, M, (2002). Measurement of erythrocyte lipids, lipid peroxidation, antioxidants and osmotic fragility in cervical cancer patients. *Clinica Chimica Acta*, 326: 143-49.

Kregel, K. C. (2002). Invited review: heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. *Journal of Applied Physiology*, 92(5): 2177-2186.

Kumar, M., Jindal, R. and Nayyar, S. (2011). Influence of heat stress on antioxidant status in Beetal goats. *Indian Journal of Small Ruminants*, 17: 178-181.

Kwon, H., Spencer, T. E., Bazer, F. W. and Wu, G. (2003). Developmental changes of amino acids in ovine fetal fluids. *Biology of Reproduction*, 68(5): 1813-1820.

Li, P., Mai, K., Trushenski, J. and Wu, G. (2009). New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. *Amino Acids*, 37: 43-53.

Li, P., Yin, Y. L., Li, D., Kim, S. W. and Wu, G. (2007). Amino acids and immune function. *British Journal of Nutrition*, 98(02): 237-252.

Li, X., Rezaei, R., Li, P. and Wu, G. (2011). Composition of amino acids in feed ingredients for animal diets. *Amino Acids*, 40(4): 1159-1168.

Lin, H., Decuypere, E. and Buyse, J. (2006). Acute heat stress induces oxidative stress in broiler chickens. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, 144(1): 11-17.

Linda, C., Hale, M. and Wells, M. (2004). Goats Sustainable Production Overview. *National Sustainable Agriculture Information Service. ATTRA Publication*, 23 pp.

Lindinger, M. I. and Anderson, S. C. (2014). Seventy day safety assessment of an orally ingested, l-glutamine-containing oat and yeast supplement for horses. *Regulatory Toxicology and Pharmacology*, 70(1): 304-311.

Lu, C. D. (1989). Effect of heat stress on goat production. *Small Ruminant Research*, 2: 151-162.

Lykkesfeldt, J. and Svendsen, O. (2007). Oxidants and antioxidants in disease: Oxidative stress in farm animals. *The Veterinary Journal*, 173: 502-511.

MacHugh, D. E. and Bradley, D. G. (2001). Livestock genetic origins: goats buck the trend. *Proceedings of the National Academy of Sciences*, 98(10): 5382-5384.

Mansour, S. A. and Mossa, A. H. (2009). Lipid peroxidation and oxidative stress in rat erythrocytes induced by chlorpyrifos and the protective effect of zinc. *Pesticide Biochemistry and Physiology*, 93: 34-39.

Marai, I. F. M., El-Darawany, A. A., Fadiel, A. and Abdel-Hafez, M. A. M. (2007). Physiological traits as affected by heat stress in sheep - A review. *Small Ruminant Research*, 71(1): 1-12.

Martin, J. P. Jr., Dailey, M. and Sugarman, E. (1987). Negative and positive assays of superoxide dismutase based on haematoxylin autoxidation. *Archives of Biochemistry and Biophysics*, 255: 329-336.

Mason, I. L. (1988). *A World Dictionary of Livestock Breeds*: *Types and Varieties*. C.A.B. International. 348 pp.

Mates, J. M. (2000). Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. *Toxicology*, 153: 83-104.

Mates, J. M., Segura, J. A., Martín-Rufián, M., Campos-Sandoval, J. A., Alonso, F. J. and Marquez, J. (2013). Glutaminase isoenzymes as key regulators in metabolic and oxidative stress against cancer. *Current Molecular Medicine*, 13(4): 514-534.

Matteri, R. L., Carroll, J. A. and Dyer, C. J. (2000). Neuroendocrine responses to stress. *In:* Moberg, G. P. and Mench, J. A. (editors). *The Biology of Animal Stress*. CAB International Publishing, Pp. 43-76.

McDowell, L. R., Wilkinson, N., Madison, R. and Felix, T. (2007). Vitamins and minerals functioning as antioxidants with supplementation considerations. *In*: *The Proceedings of the Florida Ruminant Nutrition Symposium. Best Western Gateway Grand. Gainesville, FL* (Pp. 30-31).

McManus, C., Paludo, G. R., Louvandini, H., Gugel, R., Sasaki, L. C. B. and Paiva, S. R. (2009). Heat tolerance in Brazilian sheep: physiological and blood parameters. *Tropical Animal Health and Production*, 41: 95-101.

Meadow, R. H. (1996) *In*: Harris, D. R. (Editor). *The Origins and Spread of Agriculture and pastoralism in Eurasia, Smithsonian Institution, Washington, DC,USA*, Pp. 390-412.

Melis, G. C., ter Wengel, N., Boelens, P. G. and van Leeuwen, P. A. (2004). Glutamine: recent developments in research on the clinical significance of glutamine. *Current Opinion in Clinical Nutrition and Metabolic Care*, 7(1): 59-70.

Mert, H., Mert, N. and Irak, K. (2014). Ascorbic Acid and Heat Stress in Laying Hens. *Journal of Animal and Veterinary Advances*, 13(3): 178-180.

Minka, N. S. and Ayo, J. O. (2007a). Effects of shade provision on some physiological parameters, behaviour and performance of pack donkeys (*Equinus asinus*) during the hot-dry season. *Journal of Equine Science*, 18(2): 39-46.

Minka, N. S. and Ayo, J. O. (2007b). Physiological responses of transported goats treated with ascorbic acid during hot-dry season. *Animal Science Journal*, 78(2): 164-72.

Minka, N. S. and Ayo, J. O. (2010). Physiological responses of erythrocytes of goats to transportation and the mondulatory role of ascorbic acid. *Journal of Veterinary Medical Science*, 72(7): 875-881.

Minka, N. S. and Ayo, J. O. (2011). Modulating role of vitamins C and E against transportinduced stress in pullets during the hot-dry conditions. *International Scholarly Research Network Veterinary Science*, Volume 2011, Article ID 497138, 7 pages.

Minka, N. S. and Ayo, J. O. (2012). Assessment of thermal load on transported goats administered with ascorbic acid during the hot-dry conditions. *International Journal of Biometeorology*, 56(2): 333-341.

Minka, N. S. and Ayo, J. O. (2014). Effects of transportation positions and orientations on muscular damage of goats transported by road for 12 h during the hot-dry conditions. *Journal of Veterinary Medicine and Animal Health*, 6(3): 75-82.

Minton, J. E. (1994). Function of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system in models of acute stress in domestic farm animals. *Journal of Animal Science*, 72(7): 1891-1898.

Mishra, R. P. (2009). Role of housing and management in improving productivity efficiency of goats. *In*: *Goat Production - processing of Milk and Meat*, First Edition, Pp. 45.

Misra, R. P. and Puneet, K. (2009). Improved shelter management, feeding and watering devices for goats. *Goat enterprises, CIRG, Mathura (UP), India*, 132.

Moberg, G. P. (2000). Biological response to stress: implications for animal welfare. *In*: Moberg, G. P. and Mench, J. A. (Editors). *The Biology of Animal Stress*. CAB International Publishing, Pp. 123-146.

Mohanarao, G. J., Mukherjee, A., Banerjee, D., Gohain, M., Dass, G., Brahma, B., Datta, T. K., Upadhyay, R. C. and De, S. (2014). HSP70 family genes and

HSP27 expression in response to heat and cold stress in vitro in peripheral blood mononuclear cells of goat (Capra hircus). *Small Ruminant Research*, 116(2): 94-99.

Morris, C. R., Suh, J. H., Hagar, W., Larkin, S., Bland, D. A., Steinberg, M. H., Vichinsky, E. P., Shigenaga, M., Ames, B., Kuypers, F. A. and Klings, E. S. (2008). Erythrocyte glutamine depletion, altered redox environment, and pulmonary hypertension in sickle cell disease. *Blood*, 111(1): 402-410.

Mostl, E. and Palme, R. (2002). Hormones as indicators of stress. *Domestic Animal Endocrinology*, 72: 1891-1898.

Mubi, A. A., Midau, A. and Asabe, S. M. (2015). Haematological parameters and live weight changes of Yankasa sheep fed diet containing cotton seed cake and maize offal before grazing. *Journal of Animal Production Advances*, 5(1): 579-584.

Nardone, A., Ronchi, B., Lacetera, N., Ranieri, M. S. and Bernabucci, U. (2010). Effects of climate changes on animal production and sustainability of livestock systems. *Livestock Science*, 130(1): 57-69.

Nazifi, S., Saeb, M., Baghshani, H. and Saeb, S. (2009). Influence of road transportation during hot-summer conditions on oxidative status biomakers in Iranian dromedary Camels (*Camelus dromedarius*). *African Journal of Biochemical Research*, 3: 282-287.

Newsholme, P., Lima, M. M. R., Procopio, J., Pithon-Curi, T. C., Bazotte, R. B. and Curi, R. (2003). Glutamine and glutamate as vital metabolites. *Brazilian Journal of Medical and Biological Research*, 36(2): 153-163.

Nienaber, J. A. and Hahn, G. H. (2007). Livestock production system management responses to thermal challenges. *International Journal of Biometeorology*, 52: 149-157.

Noori, S. (2012). An overview of oxidative stress and antioxidant defensive system. 1:413. doi:10.4172/scientificreports.413

Obernier, J. A., and Baldwin, R. L. (2006). Establishing an appropriate period of acclimatization following transportation of laboratory animals. *Institute for Laboratory Animal Research Journal*, 47(4): 364-369.

Odubote, I. K. (1996). Genetic analysis of the reproductive performance of West African Dwarf goats in the humid tropics. In *Small Ruminant Research and Development in Africa*, Lebbie, S. H. B. and Kagwini, E. (Editors). Proceedings of the Third Biennial Conference of the African Small Ruminant Research Network, Kampala, Uganda, 5-9 December 1994. Pp. 33-36.

Okoruwa, M. I. (2014). Effect of heat stress on thermoregulatory, live body weight and physiological responses of dwarf goats in Southern Nigeria. *European Scientific Journal*, 10(27): 255-264.

Olaifa, F., Ayo, J. O., Ambali, S. F. and Rekwot, P. I. (2012). Effect of packing on changes in erythrocyte osmotic fragility and malondialdehyde concentration in donkeys administered with ascorbic acid: original research. *Onderstepoort Journal of Veterinary Research*, 79(1): 1-5.

Olszewska, M., Wiatrow, J., Bober, J., Stachowska, E., Gołembiewska, E., Jakubowska, K., Stańczyk-Dunaj, M. and Pietrzak-Nowacka, M. (2012). Oxidative stress modulates the organization of erythrocyte membrane cytoskeleton. *Postepy Higieny i Medycyny Doswiadczalnej* (*Advances in Hygiene and Experimental Medicine*) *(Online)*, 66: 534-542.

Opara, M. N., Udevi, N. and Okoli, I. C. (2010). Haematological parameters and blood chemistry of apparently healthy West African Dwarf goats in Owerri, South Eastern Nigeria. *New York Science Journal*, 3: 68-72.

Otoikhian, C. S. O., Orheruata, J. A., Imasuen, J. A. and Akporhuarho, O. P. (2009). Physiological response of local (West African Dwarf) and adapted Switzerland (White Bornu) goat breed to varied climatic conditions in South-South Nigeria. *African Journal of General Agriculture*, 5(1): 1-6.

Oyewale, J. O. (1992). Effects of temperature and pH on osmotic fragility of erythrocytes of the domestic fowl (*Gallus domesticus*) and guinea-fowl (*Numida meleagris*). *Research in Veterinary Science*, 52(1): 1-4.

Oyewale, J., Dzenda, T., Yaqub, L., Akanbi, D., Ayo, J., Owoyele, O., Minka, N. and Dare, T. (2011). Alterations in the osmotic fragility of camel and donkey erythrocytes caused by temperature, pH and blood storage. *Veterinarski Arhiv*, 81(4): 459-470.

Pacher, P., Beckman, J. S. and Liaudet, L. (2007). Nitric oxide and peroxynitrite in health and disease. *Physiological Reviews*, 87(1): 315-424.

Padayatty, S.J., Katz, A., Wang, Y., Eck, P., Kwon, O., Lee, J., Chen, S., Dutta, A., Dutta, S.K. and Levine, M. (2003). Vitamin C as antioxidant: Evaluation of its role in disease prevention. A review. *Journal of American College of Nutrition*, 22: 18-35.

Pariset, L., Cuteri, A., Ligda, C., Ajmone-Marsan, P. and Valentini, A. (2009). Geographical patterning of sixteen goat breeds from Italy, Albania and Greece assessed by Single Nucleotide Polymorphisms. *BMC Ecology*, 9(1): 20, doi:10.1186/1472-6785-9-20.

Patrick, L. (2000). Nutrients and HIV: N-acetylcysteine, alpha-lipoic acid, L-glutamine, and L-carnitine. *Alternative Medicine Review*, 5(4): 60-71.

Perez-Crespo, M., Pintado, B. and Gutierrez-Adan, A. (2008). Scrotal heat stress effects on sperm viability, sperm DNA integrity, and the offspring sex ratio in mice. *Molecular Reproduction and Development*, 75: 40-47.

Peter, T., Biamonte, G. T. and Doumas, B. T. (1982). Protein (total protein) in serum, wine and cerebrospinal fluids: Albumin serum. Paulkner, W.R. and Meites, S. (Eds.). *In*: *Selected Method of Clinical Chemistry*, American Association for Clinical Chemistry, Washington, D.C. Pp. 9.

Piccione, G. and Caola, G. (2002). Biological rhythm in livestock. *Journal of Veteterinary Science*, 3: 145-157.

Piccione, G., Casella, S., Lutri, L., Vazzana, I., Ferrantelli, V. and Caola, G. (2010). Reference values for some haematological, haematochemical, and electrophoretic parameters in the Girgentana goat. *Turkish Journal of Veterinary and Animal Sciences*, 34: 197-204.

Piccione, G., Lutri, L., Casella, S., Ferrantelli, V. and Pennisi, P. (2008). Effect of shearing and environmental conditions on physiological mechanisms in ewes. *Journal Environmental Biology*, 29: 877-880.

Porter, V. (1996). *Goats of the World*. Farming Press, Ipswich, UK, 174 pp.

Powers, S. K. and Jackson, M. J. (2008). Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiological Reviews*, 88(4): 1243-1276.

Pringle, H. (1998). Neolithic agriculture: Reading the signs of ancient animal domestication. *Science*, 282(5393): 1448-1448.

Qwele, K., Hugo, A., Oyedemi, S. O., Moyo, B., Masika, P. J. and Muchenje, V. (2013). Chemical composition, fatty acid content and antioxidant potential of meat from goats supplemented with Moringa (Moringa oleifera) leaves, sunflower cake and grass hay. *Meat Science*, 93(3): 455-462.

Rahal, A., Kumar, A., Singh, V., Yadav, B., Tiwari, R., Chakraborty, S. and Dhama, K. (2014). Oxidative stress, prooxidants, and antioxidants: the interplay. *BioMed Research International*, http://dx.doi.org/10.1155/2014/761264.

Ramnath, V., Rekha, P. S. and Sujatha, K. S. (2008). Amelioration of heat stress-induced disturbances of antioxidant defence system in chickens by *Brahma rasyana*. *Evidence-Based Complementary and Alternative Medicine*, 5(1): 77-84.

Rao, R. K. and Samak, G. (2012). Role of glutamine in protection of intestinal epithelial tight junctions. *Journal of Epithelial Biology and Pharmacology*, 5(Suppl11-M7): 47-54.

Renaudeau, D., Collin, A., Yahav, S., De Basilio, V., Gourdine, J. L. and Collier, R. J. (2012). Adaptation to hot climate and strategies to alleviate heat stress in livestock production. *Animal*, 6(05): 707-728.

Rhoads, R. P., Baumgard, L. H. and Suagee, J. K. (2013). Metabolic priorities during heat stress with an emphasis on skeletal muscle. *Journal of Animal Science*, 91(6): 2094-2503.

Robertshaw, D. (1981). The environmental physiology of animal production. *In*: Clark, J.A. (Ed.), *Environmental Aspects of Housing for Animal Production*. Butterworth, London, Pp. 3-17.

Roth, E. (2008). Non-nutritive effects of glutamine. *The Journal of Nutrition*, 138(10): 2025-2031.

Roth, E., Oehler, R., Manhart, N., Exner, R., Wessner, B., Strasser, E. and Spittler, A. (2002). Regulative potential of glutamine-relation to glutathione metabolism. *Nutrition*, 18(3): 217-221.

Ruth, M. R. and Field, C. J. (2013). The immune modifying effects of amino acids on gut-associated lymphoid tissue. *Journal of Animal Science Biotechnology*, 4(1): 27-37.

Sakamoto, M. I., Murakami, A. E., Silveira, T. G. V., Fernandes, J. I. M. and de Oliveira, C. A. L. (2006). Influence of glutamine and vitamin E on the performance and the immune responses of broiler chickens. *Revista Brasileira de Ciência Avícola*, 8(4): 243-249.

Sakatani, M., Kobayashi, S. I. and Takahashi, M. (2004). Effects of heat shock on in vitro development and intracellular oxidative state of bovine preimplantation embryos. *Molecular Reproduction and Development*, 67(1): 77-82.

Salama, A. A. K., Caja, G., Hamzaoui, S., Badaoui, B., Castro-Costa, A., Facanha, D. A. E., Guilhermino, M.M. and Bozzi, R. (2014). Different levels of response to heat stress in dairy goats. *Small Ruminant Research*, 121(1), 73-79.

Sattar, A. and Mirza, R. H. (2009). Haematological parameters in exotic cows during gestation and lactation under subtropical conditions. *Pakistan Veterinary Journal*, 29(3): 129-132.

Schalm, O. W., Jam, N. C. and Carrol, E. G. (1975). *Veterinary Haematology*. Leo and Febiger, Philadelphia, Pp. 1402-1520.

Schalm, O.W., Jain, N.C. and Carrol, E. J. (2010). *Veterinary Haematology*, Lea and Febiger, Philadelphia, Pa, U.S.A., 6th Edition, 1176 pp.

Sejian, V., Singh, A. K., Sahoo, A. and Naqvi, S. M. K. (2014). Effect of mineral mixture and antioxidant supplementation on growth, reproductive performance

and adaptive capability of Malpura ewes subjected to heat stress. *Journal of Animal Physiology and Animal Nutrition*, 98: 72-83.

Selye H (1977). The conception of stress as we understand it in 1976. *In*: *Advances in Hormones and Mechanism of their Action*. Naukova Dumka, Kiev. Pp. 27-51 (in Russian).

Selye, H. (1946). The general adaptation syndrome and the diseases of adaptation 1. *The Journal of Clinical Endocrinology and Metabolism*, 6(2): 117-230.

Shackleton, D. M., (Ed.) (1997). *Wild Sheep and Goats and their Relatives: Status Survey and Conservation Action Plan for Caprinae*, IUCN, Gland, Switzerland. Pp. 21-26.

Sharma, B., Rai, D.K., Rai, P.K., Rizvi, S.I. and Watal, G. (2009). Determination of erythrocyte fragility as a marker of pesticide-induced membrane oxidative damage. *Methods in Molecular Biology*, 594: 123-128.

Sharma, S., Ramesh, K., Hyder, I., Uniyal, S., Yadav, V. P., Panda, R. P. Maurya, V.P., Singh, G., Kumar, P., Mitra, A. and Sarkar, M. (2013). Effect of melatonin administration on thyroid hormones, cortisol and expression profile of heat shock proteins in goats (Capra hircus) exposed to heat stress. *Small Ruminant Research*, 112(1): 216-223.

Sies, H. (1991). Oxidative stress: introduction. *In*: Sies, H. (Ed.). *Oxidative Stress: Oxidants and Antioxidants*, Academic Press, Padova, Italy, Pp. 15-21.

Silanikove N. (1992). Effects of water scarcity and hot environment on appetite and digestion in ruminants: a review. *Livestock Production Science*, 30: 175-194.

Silanikove, N. (2000a). Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livestock Production Science*, 67(1): 1-18.

Silanikove, N. (2000b). The physiological basis of adaptation of goats to scarcity of food and water in harsh environments. *Small Ruminant Research*, 35: 181-193.

Sivakumar, A. V. N., Singh, G. and Varshney, V. P. (2010). Antioxidants supplementation on acid base balance during heat stress in goats. *Asian-Australasian Journal of Animal Science*, 23: 1462-1468.

Snedecor, G. W. and Cochran, W. G. (1994). *Statistical Methods*, Oxford and IBH Publishing Corporation, Calcutta, 509 pp.

Soltan, M. A. (2009). Influence of dietary glutamine supplementation on growth performance, small intestinal morphology, immune response and blood parameters of broiler chickens. *International Journal of Poultry Science*, 8(1): 60-68.

Spurlock, M. E. and Savage, J. E. (1993). Effect of dietary protein and selected antioxidants on fatty liver hemorrhagic syndrome induced in Japanese quail. *Poultry Science*, 72(11): 2095-2105.

Srikandakumar, A., Johnson, E. H. and Mahgoub, O. (2003). Effect of heat stress on respiratory rate, rectal temperature and blood chemistry in Omani and Australian Merino sheep. *Small Ruminant Research*, 49(2): 193-198.

Stanger, K. J., Ketheesan, N., Parker, A. J., Coleman, C. J., Lazzaroni, S. M. and Fitzpatrick, L.A. (2005). The effect of transportation on the immune status of *Bos indicus* steers. *Journal of Animal Science*, 83: 2632 - 2636.

Stipanuk, M. H., Ueki, I., Dominy Jr, J. E., Simmons, C. R. and Hirschberger, L. L. (2009). Cysteine dioxygenase: a robust system for regulation of cellular cysteine levels. *Amino Acids*, 37(1): 55-63.

Strimbu, K. and Tavel, J. A. (2010). What are biomarkers?. *Current opinion in HIV and AIDS*, *5*(6): 463-468.

Sunil Kumar, B.V., Kumar, A. and Kataria, M. (2011). Effect of heat stress in tropical livestock and different strategies for its amelioration: a review. *Journal of Stress Physiology and Biochemistry*, 7(1): 45-54.

Taneja, G. C. (1982). Breeding goats for meat production. *In*: *Proceedings of the 3rd International Conference on Goat Production and Diseases*, January, 5-9. Tucson, Arizona, USA, Pp. 27-30.

Tao, X. and Xin, H. (2003). Acute synergistic effects of air temperature, humidity and velocity on homeostasis of market-size boilers. *Transactions of the American Society of Agricultural and Biological Engineers*, 46(2): 491-497.

Temizel, E. M., Senturk, S. and Kasap, S. (2009). Clinical, haematological and biochemical findings in Saanen goat kids with naturally occurring heat stroke. *Tierärztliche Praxis Großtiere*, 37: 236-241.

Terlouw, E. M., Schouten, W. G. P. and Ladewig, J. (1997). Physiology. *In*: Appleby, M. C. and Hughes, B. O. (Editors). *Animal Welfare*. Cambridge: CAB International, University Press, Pp. 143-58.

Terrill, C. E. (1986) Trends in sheep and goat production over the past 20 years. *In*: Timon, V.M. and Harahan, J.P. (Eds.). *Small Ruminant Production in Developing Countries. Proceedings of an Expert Consultative Forum*, held in Sofia, Bulgaria. July 8-12. *FAO Animal Production and Health Paper*, 58, FAO, Rome. Pp. 1-11.

Todini, L. (2007). Thyroid hormones in small ruminants: effects of endogenous, environmental and nutritional factors. *Animal*, 1(7): 997-1008.

Tripathi, M. K., Mondal, D. and Karim, S. A. (2008). Growth, haematology, blood constituents and immunological status of lambs fed graded levels of animal feed grade damaged wheat as substitute of maize. *Journal of Animal Physiology and Animal Nutrition*, 92(1): 75-85.

Uchendu, C., Ambali, S. F., Yakub, S. L., Lasisi, D. I. and Umosen, A. J. (2011). Comparative effects of vitamin C and acetyl-L-carnithine on subacute chlorpyrifos-induced erythrocyte osmotic fragility in Wister rats. *Advances in Applied Science Research*, 2(5): 297-302.

Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M. M. and Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions*, 160(1): 1-40.

Vani, R., Reddy, C. S. S. and Devi, S. A. (2010). Oxidative stress in erythrocytes: a study on the effect of antioxidant mixtures during intermittent exposures to high altitude. *International Journal of Biometeorology*, 54(5): 553-562.

von Borell, E. H. (2001). The biology of stress and its application to livestock housing and transportation assessment. *Journal of Animal Science*, 79: 260-267.

von Borell, E., Dobson, H. and Prunier, A. (2007). Stress, behaviour and reproductive performance in female cattle and pigs. *Hormones and Behaviour*, 52(1): 130-138.

Wernerman, J. (2008). Clinical use of glutamine supplementation. *The Journal of Nutrition*, 138(10): 2040-2044.

West, J. W. (2003). Effects of heat-stress on production in dairy cattle. *Journal of Dairy Science*, 86(6): 2131-2144.

Willcox, J. K., Ash, S. L. and Catignani, G. L. (2004). Antioxidants and prevention of chronic disease. *Critical Reviews in Food Science and Nutrition*, 44(4): 275-295.

Williams, C. A., Gordon, M. E., Betros, C. L. and McKeever, K. H. (2008). Apoptosis and antioxidant status are influenced by age and exercise training in horses. *Journal of Animal Science*, 86: 576-583.

Wojtas, K., Cwynar, P. and Kołacz, R. (2014). Effect of thermal stress on physiological and blood parameters in merino sheep. *Bulletin of the Veterinary Institute in Pulawy*, 58: 283-288.

Wu, G. (2009). Amino acids: metabolism, functions, and nutrition. *Amino acids*, 37(1): 1-17.

Wu, G. (2010). Functional amino acids in growth, reproduction, and health. *Advances in Nutrition*, 1(1): 31-37.

Wu, G., Bazer, F. W., Johnson, G. A., Knabe, D. A., Burghardt, R. C., Spencer, T. E., Li, X.L. and Wang, J. J. (2011). Triennial growth symposium: Important roles

for L-glutamine in swine nutrition and production. *Journal of Animal Science*, 89(7): 2017-2030.

Wu, G., Fang, Y. Z., Yang, S., Lupton, J. R. and Turner, N. D. (2004). Glutathione metabolism and its implication for health. *Journal of Nutrition*, 134: 489-492.

Wu, G., Wu, Z., Dai, Z., Yang, Y., Wang, W., Liu, C., Wang, B., Wang, J. and Yin, Y. (2013). Dietary requirements of "nutritionally non-essential amino acids" by animals and humans. *Amino Acids*: 44: 1107-1113.

Xi, P., Jiang, Z., Dai, Z., Li, X., Yao, K., Zheng, C., Lin, Y., Wang, J. and Wu, G. (2012). Regulation of protein turnover by L-glutamine in porcine intestinal epithelial cells. *The Journal of Nutritional Biochemistry*, 23(8): 1012-1017.

Xi, P., Jiang, Z., Zheng, C., Lin, Y. and Wu, G. (2010). Regulation of protein metabolism by glutamine: implications for nutrition and health. *Frontiers in Bioscience (Landmark edition)*, 16: 578-597.

Yang, G., Deng, J., Ren, W., Liu, G., Li, R. C., Wu, X. and Su, D. (2011). Effect of dietary L-arginine and L-glutamine supplementation on *H. parasuis* infection. *Journal of Food, Agriculture and Environment*, 9(3 and 4): 588-591.

Yaqub, L. S., Mshelia, W. P. and Ayo, J. O. (2014). Erythrocyte osmotic fragility and hematological responses of horses administered ascorbic acid and exposed to oad Transportation. *Journal of Equine Veterinary Science*, 34(11): 1324-1328.

Yarovan, N. I. (2008). Effect of zeolites on adaptation processes in cows. *Russian Agricultural Sciences*, 34(2): 120-122.

Young, V. R. and Ajami, A. M. (2001). Glutamine: the emperor or his clothes? *The Journal of Nutrition*, 131(9): 2449-2459.

Yousef, M. K. (1985). *Principles of bioclimatology and adaptation of livestock*, WAS, B5, Holland: Elsevier Publ. Pp. 17-31.

Yu, Q., Ma, X. and Xu, L. (2013). Solubility, dissolution enthalpy and entropy of l-glutamine in mixed solvents of ethanol + water and acetone + water. *Thermochimica Acta*, 558: 6-9.

Zakari, F. O., Ayo, J. O., Rekwot, P. I. and Kawu, M. U. (2014). Effect of age, sex, physical activity and meteorological factors on haematological parameters of donkeys (*Equus asinus*). *Comparative Clinical Pathology*, DOI: 10.1007/s00580-014-2026-3.

Zeder, M. A. and Hesse, B. (2000). The initial domestication of goats (*Capra hircus*) in the Zagros Mountains 10,000 years ago. *Science*, 287(5461): 2254-2257.

Zhang, F. J., Weng, X. G., Wang, J. F., Zhou, D., Zhang, W., Zhai, C. C., Hou, X.Y. and Zhu, Y. H. (2014). Effects of temperature-humidity index and chromium

supplementation on antioxidant capacity, heat shock protein 72, and cytokine responses of lactating cows. *Journal of Animal Science*, 92(7): 3026-3034.

Zhang, J., Hu, Z., Lu, C., Bai, K., Zhang, L. and Wang, T. (2015). Effect of the varying levels of dietary curcumin on meat quality and antioxidant profile of breast muscle in broilers. *Journal of Agricultural and Food Chemistry*, 63(15): 3880-3886.

APPENDICES

Appendix I: Mean ± SEM erythrocyte osmotic fragility in both experimental (n=14) and control (n=14) goats the study period

	Before treatment		During treatment		After treatment	
% NaCl	Experimental	Control	Experimental	Control	Experimental	Control
0.9	2.02 ± 0.41	2.276 ± 0.45	2.49 ± 0.27	2.62 ± 0.30	2.06 ± 0.57	2.355 ± 1.11
0.7	65.58 ± 5.56	64.82 ± 4.96	67.15 ± 2.48	72.44 ± 2.23	56.26 ± 4.80	51.46 ± 4.26
0.5	76.83 ± 5.69	84.78 ± 1.96	75.72 ± 2.13	79.91 ± 2.11	69.94 ± 5.15	76.92 ± 3.50
0.3	86.99 ± 2.96	89.77 ± 1.07	83.51 ± 1.78	86.78 ± 1.74	81.8 ± 3.01	82.62 ± 3.02
0.1	90.75 ± 3.58	92.64 ± 0.84	92.93 ± 0.94	93.11 ± 1.09	89.44 ± 2.97	90.92 ± 3.25

Appendix II: Dynamics of changes in oxidative stress parameters in Red Sokoto goats administered with L-glutamine during the hot-dry season (mean ± SEM)

	Before treatment		During treatment		Day 7 after treatment	
Parameters	Experimental	Control	Experimental	Control	Experimental	Control
Malondialdehyde concentration (nmol/L)	1.29 ± 0.08	1.26 ± 0.05	1.47 ± 0.05	1.75 ± 0.05	1.30 ± 0.04	1.34 ± 0.06
Superoxide dismutase activity(IU/L)	2.34 ± 0.04	2.39 ± 0.05	2.29 ± 0.04	2.10 ± 0.04	2.37 ± 0.05	2.36 ± 0.04
Glutathione peroxidase activity (IU/L)	54.14 ± 1.07	53.79 ± 1.17	44.82 ± 0.98	42.46 ± 0.65	45.86 ± 0.88	44.86 ± 0.88
Catalase activity (IU/L)	47.93 ± 1.22	47.43 ± 0.92	48.96 ± 0.93	44.04 ± 0.91	48.93 ± 1.16	46.14 ± 1.09

Appendix III: Rectal temperature (°C) of goats before L-glutamine administration

	6:00 h		13:00 h		18:00 h	
S/No.	Experimental	Control	Experimental	Control	Experimental	Control
1	37.1	39.5	39.5	39.8	39.3	39.0
2	37.3	38.8	39.5	38.9	39.3	39.4
3	37.1	38.4	39.8	39.0	39.4	39.0
4	38.5	38.9	39.0	39.5	38.8	39.5
5	38.2	39.0	39.4	39.4	38.7	38.9
6	37.9	38.4	38.9	39.7	38.8	38.9
7	38.5	38.2	38.7	39.4	38.9	39.1
8	38.5	39.3	39.0	39.6	39.3	39.7
9	38.3	38.6	39.5	39.3	39.1	39.0
10	39.0	38.9	39.5	39.4	39.0	39.0
11	39.0	38.6	39.3	39.5	39.7	39.1
12	38.6	38.5	39.3	39.6	39.0	38.8
13	39.0	38.2	39.6	39.0	39.0	38.8
14	39.2	38.6	39.1	38.6	38.9	38.6

S/No 1-7 = bucks and 8-14 = does (for appendices 3-17).

Appendix IV: Rectal temperature (°C) of goats week one of L-glutamine administration

	6:00 h		13:00 h		18:00 h	
S/No.	Experimental	Control	Experimental	Control	Experimental	Control
1	37.8	37.4	40.2	38.9	40.3	39.4
2	38.5	37.5	39.2	38.6	39.8	38.8
3	38.2	37.9	39.0	39.4	39.6	39.9
4	38.6	38.8	38.9	38.8	39.3	39.2
5	38.5	39.8	38.9	39.5	39.3	39.6
6	39.1	38.5	39.2	39.8	39.2	40.2
7	39.2	39.0	39.0	39.8	39.2	39.6
8	37.5	38.0	38.8	39.2	39.2	39.4
9	38.2	38.4	39.2	38.9	39.5	39.4
10	38.7	38.1	39.4	39.0	39.9	39.3
11	38.8	38.2	39.3	38.9	39.6	39.5
12	38.2	37.7	39.2	39.2	39.3	39.4
13	37.8	37.2	39.3	39.1	39.5	39.8
14	38.9	38.1	39.0	39.0	39.2	39.5

Appendix V: Rectal temperature (°C) of goats week two of L-glutamine administration

	6:00 h		13:00 h		18:00 h	
S/No.	Experimental	Control	Experimental	Control	Experimental	Control
1	37.8	37.4	39.6	39.7	39.8	39.8
2	37.2	38.5	39.0	38.8	39.6	38.8
3	37.2	38.4	39.0	40.2	39.4	40.2
4	37.7	37.6	39.1	39.7	39.6	38.9
5	38.0	38.6	39.0	39.7	39.3	39.6
6	37.3	37.9	39.3	40.0	39.3	39.6
7	37.8	38.0	38.8	39.7	39.5	39.6
8	37.1	38.6	39.4	39.3	39.5	39.3
9	38.4	38.7	39.1	39.9	39.0	39.8
10	38.0	38.6	39.3	39.4	39.4	39.3
11	38.7	38.8	39.3	39.4	39.3	39.4
12	38.7	38.2	39.7	39.3	39.7	39.4
13	37.7	38.2	39.2	39.2	39.4	39.6
14	39.5	38.5	40.5	38.9	39.8	39.0

Appendix VI: Rectal temperature (°C) of Red Sokoto goats week three of L-glutamine administration

	6:00 h		13:00 h		18:00 h	
S/No.	Experimental	Control	Experimental	Control	Experimental	Control
1	37.8	37.7	39.2	39.4	38.8	39.1
2	37.9	37	39.0	39.2	39.5	37.4
3	38.0	37.8	38.7	39.5	37.9	38.8
4	38.0	38.0	38.7	39.4	38.8	39.3
5	37.8	38.0	38.6	39.5	38	39.5
6	38.4	37.9	38.6	39.7	38.9	39.0
7	38.5	37.9	38.7	39.5	38.5	38.6
8	37.5	38.2	39.2	38.8	38.5	39.2
9	38.5	38.7	39.2	38.9	38.8	38.8
10	37.2	39.0	39.9	39.3	39.5	39.2
11	38.5	38.9	38.9	39	38.9	38.6
12	38.5	38.3	39.3	38.9	39.5	38.9
13	38.9	38.1	39.5	38.9	39.3	38.9
14	38.8	38.6	39.0	38.8	38.8	38.5

Appendix VII: Rectal temperature (°C) of goats week after L-glutamine administration

	6:00 h		13:00 h		18:00 h	
S/No.	Experimental	Control	Experimental	Control	Experimental	Control
1	38.0	38.5	39.9	38.7	39.2	39.1
2	37.1	38.6	38.7	38.6	39.8	39.2
3	38.2	38.6	39.1	39.3	38.9	39.3
4	38.2	38.5	39.1	39.4	39.2	39.0
5	38.8	39.0	39.2	39.7	39.3	39.5
6	38.0	38.0	38.9	38.7	38.9	39.0
7	38.5	39.0	38.9	39.0	39.2	39.3
8	37.8	38.2	39.1	38.8	39.4	39.0
9	38.7	38.5	39.0	38.7	39.3	39.0
10	38.5	39.2	39.3	39.0	39.3	39.1
11	38.6	39.1	39.1	39.5	39.0	39.6
12	38.7	38.6	39.5	39.0	39.6	39.6
13	38.4	37.9	39.1	39.0	39.4	39.6
14	38.4	38.5	38.5	39.0	38.6	39.4

Appendix VIII: Packed cell volume (%) in Red Sokoto goats during the study period

	Before treatment		Week 1 of treatment		Week 2 of treatment		Week 3 of treatment		After treatment	
S/No.	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
1	32.6	26.7	31.9	22.8	32.3	20.8	33.01	18.8	32.4	25.9
2	29.5	35.5	28.3	33.3	27.9	31.2	27.3	29.5	28.5	25.5
3	28.9	36.5	27.5	33.7	26.9	30.5	27.4	28.4	28.5	28.5
4	33.9	32.3	32.7	29.4	33.4	27.4	32.9	26.5	32.9	27.3
5	27.7	29.4	26.9	27.5	27.4	24.6	27.9	22.6	27.5	27.4
6	36.3	25.9	35.2	22.5	36.2	18.8	35.8	26.3	34.3	24.9
7	30.3	30.8	29.8	27.5	30.2	24.5	29.7	22.8	30.5	29.8
8	24.5	29.8	23.5	24.8	23.6	22.4	24.4	21.2	26.5	27.8
9	29.3	25.1	29.5	21.6	29.6	19.6	29.3	16.7	28.3	24.1
10	25.9	18.8	28.3	16.3	29.3	15.3	28.8	24.9	27.9	17.8
11	20.4	30.5	21.5	27.7	23.2	25.7	22.8	23.9	23.4	26.6
12	28.4	27.7	28.9	24.8	27.9	22.8	28.1	24.8	29.4	28.7
13	28.9	29.8	27.5	26.5	28.5	23.5	27.8	22.5	27.9	30.8
14	25.6	25.9	24.5	22.6	26.5	20.6	25.9	17.6	26.6	26.9

Appendix IX: Haemoglobin concentration (g/dL) in Red Sokoto goats during the study period

	Before treatment		Week 1 of treatment		Week 2 of treatment		Week 3 of treatment		After treatment	
S/No.	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
1	11.5	8.0	10.9	6.6	11.3	5.9	10.6	5.5	10.4	8.7
2	8.9	11.3	9.2	9.3	8.9	7.3	8.6	6.9	8.4	10.3
3	9.8	12.5	8.9	10.8	9.4	8.8	9.9	7.8	9.3	12.4
4	10.3	11.6	9.7	9.5	9.3	6.5	10.5	6.3	9.5	10.6
5	8.4	9.3	8.6	8.3	9.6	7.7	11.2	7.2	8.5	8.3
6	11.7	7.9	12.2	6.3	11.8	5.5	12.2	7.5	10.7	6.9
7	9.6	9.9	9.3	8.5	8.5	6.5	9.3	5.7	8.9	9.6
8	7.6	9.6	7.4	8.3	7.7	6.7	8.4	5.7	7.4	9.3
9	8.6	7.9	8.3	6.3	8.8	5.8	9.3	4.8	8.3	7.4
10	7.8	6.3	7.4	5.5	8.4	4.5	7.9	7.4	7.3	7.3
11	6.9	9.3	6.8	7.6	7.1	6.8	8.3	6.1	6.4	8.3
12	8.3	8.8	8.7	6.9	8.4	5.9	8.9	5.2	8.2	9.8
13	8.9	9.4	8.7	8.3	9.3	7.3	10.3	7.3	8.4	8.8
14	8.3	8.3	8.7	6.9	8.5	5.7	9.4	4.7	8.3	8.9

Appendix X: Red blood cell count (x 10^6/µL) in Red Sokoto goats during the study period

	Before treatment		Week 1 of treatment		Week 2 of treatment		Week 3 of treatment		After treatment	
S/No.	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
1	13.5	9.8	13.6	8.3	12.8	8.9	12.4	8.4	12.9	8.8
2	12.9	13.7	12.5	11.4	11.9	10.9	12.1	10.1	11.9	12.7
3	12.5	15.4	13.3	12.6	12.7	10.6	13.6	10.2	11.8	14.4
4	14.4	13.7	13.8	10.7	12.8	8.5	12.3	8.3	13.4	12.7
5	10.3	11.6	9.8	9.4	9.9	8.4	10.3	8.6	9.3	10.6
6	14.8	10.5	13.9	8.3	13.5	9.6	14.0	9.3	12.8	9.5
7	12.9	12.3	12.5	9.6	12.6	7.6	11.9	7.8	11.9	10.3
8	9.5	11.7	10.3	9.7	9.9	9.0	11.3	8.4	9.4	10.6
9	10.9	10.5	11.2	8.5	11.2	7.7	12.2	7.4	9.7	9.5
10	8.9	8.7	9.4	6.5	9.9	9.2	9.6	8.2	7.9	7.9
11	9.5	12	9.9	9.5	8.9	8.5	8.9	8.1	8.5	11.0
12	9.7	9.5	10.3	7.6	11.0	6.6	12.7	6.4	8.7	9.7
13	9.8	12.6	9.5	10.0	9.7	8.9	11.2	8.3	9.7	11.6
14	10	9.9	9.7	6.8	10.4	7.1	11.5	7.5	9.9	12.9

Appendix XI: Absolute white blood cell count (x 10^3/µL) in Red Sokoto goats during the study period

	Before treatment		Week 1 of treatment		Week 2 of treatment		Week 3 of treatment		After treatment	
S/No.	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
1	8.8	11.2	9.5	10.3	10.5	9.7	11.1	9.0	10.4	8.6
2	8.7	10.6	9.8	9.6	10.4	8.6	10.9	8.1	10.3	7.9
3	9.8	11.1	11.6	9.1	12.0	8.5	12.6	7.9	11.6	7.9
4	8.9	9.1	9.2	8.8	10.6	7.3	11.4	6.8	10.4	7.4
5	9.6	9.9	11.2	7.8	9.9	7.2	10.4	6.5	9.4	6.1
6	8.6	10.2	10.6	8.5	12.4	7.5	13.2	7.2	12.9	6.8
7	10.8	9.6	11.5	8.0	11.4	7.5	12.5	9.9	11.7	9.6
8	8.1	7.5	9.6	5.6	10.9	4.5	11.5	4.2	11.2	4.1
9	9.2	8.0	11.0	6.5	12.2	5.4	12.9	5.1	12.4	4.9
10	8.4	7.4	9.5	5.8	10.0	4.5	11.1	3.9	11.1	3.4
11	8.8	8.7	9.2	8.0	10.3	6.8	11.3	5.8	10.7	5.3
12	8.2	8.0	9.0	7.5	9.8	6.4	10.4	5.9	9.5	5.2
13	9.0	8.1	10.5	7.6	11.0	5.8	11.7	4.6	10.7	4.2
14	7.8	8.5	9.2	5.1	11.2	4.6	12.2	3.8	11.7	3.2

Appendix XII: Absolute neutrophil count (x 10^3/µL) in Red Sokoto goats during the study period

	Before treatment		Week 1 of treatment		Week 2 of treatment		Week 3 of treatment		After treatment	
S/No.	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
1	5.2	3.7	6.3	2.8	6.6	2.4	7.1	2.0	6.9	1.6
2	3.5	4.9	4.4	3.9	4.7	3.4	5.6	3.1	5.2	3.0
3	3.3	5.5	5.1	4.5	5.4	4.1	6.1	3.6	6.0	3.2
4	4.8	4.5	6.3	4.8	7.0	4.2	7.6	3.5	7.1	3.1
5	4.5	3.8	5.6	3.8	6.1	3.3	7.2	3.0	7	2.8
6	3.6	5.0	4.6	4.6	5.2	4.1	6.0	3.7	5.5	3.3
7	3.8	4.5	4.6	3.7	5.1	3.1	6.3	2.7	6.0	2.4
8	3.1	3.2	4.3	2.9	4.9	2.2	5.3	2.1	5.1	2.0
9	3.8	2.5	4.5	1.4	5.2	1.1	5.6	0.9	5.2	0.9
10	4.6	1.9	5.5	2.1	5.9	1.9	6.4	1.5	6.1	1.4
11	5.2	3.7	5.8	3.4	6.4	2.8	7.5	2.3	7.2	2.0
12	2.6	4.1	3.9	3.0	4.3	2.7	5.0	2.6	5.0	2.3
13	3.3	3.5	3.9	2.8	4.3	2.3	5.4	1.7	5.4	1.3
14	2.6	2.6	4.3	2.1	5.7	1.6	5.3	1.2	5.0	1.2

Appendix XIII: Absolute lymphocyte count (x 10^3/µL) in Red Sokoto goats during the study period

	Before treatment		Week 1 of treatment		Week 2 of treatment		Week 3 of treatment		After treatment	
S/No.	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
1	4.6	5.4	5.0	4.6	5.7	4.2	6.2	3.9	6.0	3.2
2	4.5	5.6	5.6	4.6	6.3	4.1	7.2	3.4	7.1	3.3
3	5.3	4.2	4.8	3.8	5.5	3.2	5.6	3.0	5.2	3.1
4	3.7	4.3	4.5	4.0	5.2	3.5	6.3	2.9	6.1	2.7
5	3.8	3.6	4.3	3.2	5.4	3.0	6.1	2.5	5.7	2.5
6	3.5	3.6	5.0	3.0	5.7	2.5	6.4	2.0	6.1	1.9
7	3.5	3.8	4.7	2.9	5.4	2.0	6.7	1.5	6.2	1.4
8	5.8	4.5	6.3	3.9	6.8	3.3	7.4	2.3	7.3	2.1
9	4.2	4.2	5.4	3.1	5.9	2.9	6.5	1.4	6.4	1.2
10	3.6	2.8	4.2	2.0	5.5	1.7	6.4	3.5	6.2	3.1
11	2.4	5.3	3.3	4.2	5.0	3.8	6.2	3.4	6.1	3.0
12	3.7	5.4	4.5	4.3	5.4	4.0	6.4	2.9	6.2	2.2
13	3.3	4.6	4.3	2.6	5.5	2.4	7.0	2.0	6.8	1.9
14	3.6	6.0	4.3	1.9	5.7	1.4	6.9	1.2	6.4	1.0

Appendix XIV: Serum malondialdehyde concentration (nmol/L) in goats during the study period

	Before treatment		Week 1 of treatment		Week 2 of treatment		After treatment	
S/No.	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
1	1.0	0.9	1.3	1.7	1.2	1.7	1.3	1.4
2	1.1	1.3	1.2	1.2	1.6	1.3	1.3	1.0
3	1.3	1.2	1.5	1.5	1.3	1.6	1.4	1.5
4	1.0	1.1	1.1	1.7	1.3	1.8	1.3	1.2
5	1.2	1.5	1.3	1.9	1.1	2.1	1.2	1.1
6	0.8	1.4	1.3	1.9	1.6	2.2	1.3	1.6
7	1.1	1.3	1.1	1.7	1.3	1.9	1.4	1.3
8	1.5	1.2	1.6	1.3	2.0	1.6	1.3	1.0
9	1.3	1.1	1.5	1.5	1.9	1.6	1.4	1.3
10	1.6	1.6	1.8	1.5	2.0	1.8	1.5	1.3
11	1.6	1.3	1.2	1.9	1.3	2.0	1.0	1.3
12	1.3	1.0	1.5	1.7	1.7	1.8	1.3	1.5
13	1.8	1.3	1.3	2.1	1.5	2.2	1.1	1.7
14	1.5	1.5	1.7	1.8	2.0	2.0	1.4	1.5

Appendix XV: Superoxide dismutase activity (IU/L) in Red Sokoto goats during the study period

	Before treatment		Week 1 of treatment		Week 2 of treatment		After treatment	
S/No.	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
1	2.2	2.3	2.6	2.1	2.1	2.2	2.2	2.3
2	2.4	2.5	2.2	2.0	2.3	2.3	2.5	2.5
3	2.4	2.2	2.5	1.9	2.1	2.5	2.4	2.2
4	2.1	2.4	2.2	2.2	2.0	2.0	2.1	2.4
5	2.4	2.6	2.5	2.0	2.2	2.2	2.4	2.6
6	2.2	2.1	2.0	2.2	2.6	2.4	2.2	2.1
7	2.1	2.4	2.4	1.9	2.1	2.3	2.6	2.4
8	2.5	2.6	2.0	2.2	2.2	2.2	2.5	2.3
9	2.5	2.4	2.4	2.1	2.4	2.4	2.5	2.4
10	2.3	2.5	2.2	1.8	2.0	2.0	2.3	2.2
11	2.2	2.4	2.6	1.7	2.5	2.0	2.2	2.4
12	2.5	2.6	2.4	2.0	2.6	2.1	2.5	2.6
13	2.6	2.1	2.1	2.2	2.2	2.4	2.6	2.3
14	2.4	2.3	2.3	1.6	2.5	2.0	2.2	2.3

Appendix XVI: Glutathione peroxidase activity (IU/L) in Red Sokoto goats during the study period

	Before treatment		Week 1 of treatment		Week 2 of treatment		After treatment	
S/No.	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
1	58	51	56	47	51	44	50	45
2	61	57	50	45	47	46	49	49
3	56	55	49	42	51	49	47	44
4	57	58	42	40	44	44	41	51
5	55	56	52	39	49	40	50	47
6	60	52	50	41	48	42	42	45
7	50	44	50	42	47	45	45	46
8	55	58	41	36	44	33	49	39
9	53	60	43	41	46	45	44	45
10	49	55	40	44	41	42	46	47
11	50	51	39	40	42	46	49	42
12	49	52	37	40	40	41	41	41
13	51	56	39	41	36	44	43	41
14	54	48	40	47	41	43	46	46

Appendix XVII: Catalase activity (IU/L) in Red Sokoto goats during the study period

	Before treatment		Week 1 of treatment		Week 2 of treatment		After treatment	
S/No.	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
1	54	49	55	44	59	44	54	49
2	56	45	51	46	46	51	56	53
3	47	50	54	47	51	46	55	50
4	50	53	50	35	49	50	50	43
5	53	42	56	53	51	47	47	50
6	51	51	55	45	54	38	51	47
7	42	46	53	47	52	44	50	43
8	42	45	44	41	46	42	49	45
9	44	44	47	46	48	47	44	42
10	45	52	43	47	46	33	41	41
11	46	50	45	44	44	47	46	47
12	48	44	41	39	45	45	50	50
13	50	48	55	48	41	38	44	47
14	43	45	43	36	47	43	48	39