University of Fort Hare
Together in Excellence

WATER RESTRICTION AND VITAMIN C SUPPLEMENTATION ON XHOSA GOATS DURING SUMMER: EFFECT ON GROWTH PERFORMANCE, BLOOD BIOCHEMICAL PROFILE, NUTRIENT BALANCE, CARCASS CHARACTERISTICS AND MEAT QUALITY

OLUWAKAMISI FESTUS, AKINMOLADUN

2020

University of Fort Hare
Together in Excellence

WATER RESTRICTION AND VITAMIN C SUPPLEMENTATION ON XHOSA GOATS DURING SUMMER: EFFECT ON GROWTH PERFORMANCE, BLOOD BIOCHEMICAL PROFILE, NUTRIENT BALANCE, CARCASS CHARACTERISTICS AND MEAT QUALITY

BY

OLUWAKAMISI FESTUS, AKINMOLADUN
(201821379)
MSc (Animal Science, University of Ibadan)
B.Agric.Tech. [Hons] (Animal Production and Health, Federal University of Technology, Akure)

A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY IN ANIMAL SCIENCE
DEPARTMENT OF LIVESTOCK AND PASTURE SCIENCES
FACULTY OF SCIENCE AND AGRICULTURE
UNIVERSITY OF FORT HARE
ALICE, 5700
SOUTH AFRICA
SUPERVISOR: DR. C.T. MPENDULO
CO-SUPERVISORS: DR. F.N. FON; PROF. O. OKOH

DECLARATION

I, the undersigned, declare that this thesis ‘*water restriction and vitamin c supplementation on Xhosa goats during summer: effect on growth performance, blood biochemical profile, nutrient balance, carcass characteristics and meat quality’* submitted to the University of Fort Hare for the degree of Doctor of Philosophy in Animal Science in the Faculty of Science and Agriculture is my original work and that the work has not been submitted to any other university partially or entirely for the award of any degree or examination purposes.

Name: Oluwakamisi Festus, Akinmoladun

Signature ………… ………

Date: ……31/08/2020………………

DECLARATION ON PLAGIARISM

I, **Oluwakamisi Festus, Akinmoladun,** with student number: 201821379, hereby declare that I am fully aware of the University of Fort Hare's policy on plagiarism, and I have taken every precaution to comply with the regulations.

Signature…… ………… Date……31/08/2020……………………..

DECLARATION ON RESEARCH ETHICAL CLEARANCE

I, **Oluwakamisi Festus, Akinmoladun**, student number: 201821379, hereby declare that I am fully aware of the University of Fort Hare’s policy on research ethics, and I have taken every precaution to comply with the regulations. I confirm that my research was ethically cleared for experimentation (Reference No: MUC011SAKI01).

Signature…… …..   Date…31/08/2020……………

CERTIFICATION

This thesis is entitled '*water restriction and vitamin c supplementation on Xhosa goats during summer: effect on growth performance, blood biochemical profile, nutrient balance, carcass characteristics and meat quality'* meets the regulation governing the award of the degree of Doctor of Philosophy of the University of Fort Hare and is approved for its contribution to scientific knowledge.

………   ……………5/10/2021…………

Dr. C.T. Mpendulo   Date

Supervisor

……………………………..   …………30/08/2020…………

Dr. F.N. Fon   Date

Co-supervisor

.. ..   …………28/08/2020……………

Prof. O. Okoh   Date

Co-supervisor

DEDICATION

This thesis is dedicated to God Almighty, the God who is too faithful to fail. Thank you for everything.

ACKNOWLEDGMENT

I want to specially thank my main host and supervisor, late Prof. Voster Muchenje, for accepting to supervise my PhD thesis at such short notice and request. Your recommendation and motivation were instrumental in securing the NRF-TWAS Africa Renaissance Doctoral Scholarship for my doctoral program. Your contributions to the success of this program cannot be quantified. May your gentle soul rest in perfect peace. I also want to appreciate my co-supervisors, Drs. Conference Mpendulo, Fabian Fon and Prof. Omobola Okoh for their guidance, time, and dedication to this program's success.

Special thanks to Drs Goke Adeniji, Ojemaye Mike, Falowo Dele and Olisah Chijioke for your selfless role in improving my manuscripts' quality even when it was not convenient. Sincere appreciation to a man of God and colleague, Dr. Ikusika Segun, for making this PhD journey less difficult and standing with me during the most challenging moments in this program. Also, to Dr. Osunla Charles, thanks for the motivation to put in for the TWAS scholarship for the third time, which later won me the award and also for making my settling down in Fort Hare very easy. You are part of my success story. My appreciation to Dr. (Mrs) Akanji Funke; I cannot forget our friendship and the struggles to bag these PhD degrees.

I cannot but appreciate my academic mentors in Nigeria; Profs. Awoniyi, T.A.M. and Akanbi, M. for their fatherly roles. Your words of advice and motivation contributed immensely to my success as an academic.

Special thanks to the National Research Foundation of South Africa and The World Academy of Science (NRF-TWAS) for providing full sponsorship for this doctoral program.

My appreciation to my colleagues and friends; Eunice, Yawa, Denis and Drs. Chika, Tutu and Nantapo. Thanks for your contributions and for making the department very lively. My sincere appreciations to Messrs Dumisani Pepe and Sizwe Tikwayo for your various technical assistance on and off the research farm during my experimental field trial.

To the Redeemed Christian Church of God's family, Fountain of Life Parish, thank you for the spiritual atmosphere that helped me remain focused and undistracted.

To my darling wife, Seun, and my little angels, Feyikemi and Toluwani, your loving thoughts, prayers, and support kept me going. We all did it together. My sincere appreciation goes to my parents, Mr and Mrs Akinmoladun, for your prayers and support; my siblings, Kemi, Rotimi, Funmi and Joshua, for believing in me; and my In-laws, for your unwavering support.

LIST OF PUBLICATIONS FROM THIS THESIS

Published journal articles

i. **Akinmoladun**, **O.F.,** Muchenje, V., Fon, F.N. and Mpendulo, C.T. (2019). Small ruminants: Farmers' hope in a world threatened by water scarcity. *Animals*. 9, 456; doi:10.3390/ani9070456

ii. **Akinmoladun, O.F**., Fon, F.N., Mpendulo, C.T. and Okoh, O. (2020). Performance, heat tolerance and blood metabolites of water restricted Xhosa goat supplemented with vitamin C. *Transl. Anim. Sci.* 4(2), 1-15; doi**:** 10.1093/tas/txaa044

iii. **Akinmoladun O.F**., Fon, F.N. and Mpendulo, C.T. (2020). Stress indicators, carcass characteristics and meat quality of Xhosa goats subjected to different watering regimen and vitamin C supplementation. *Livest. Sci.* 238, 104083**;** doi: 10.1016/j.livsci.2020.104083

iv. **Akinmoladun O.F**., Fon, F.N., Mpendulo, C.T. and Okoh, O. (2021). Intake, nutrient digestibility, nitrogen and mineral balance of water restricted goats supplemented with vitamin C. *Open Agric.* 6, 168-177; doi:10.1515/opag-2020-0190

v. **Akinmoladun O.F**., Fon, F.N., Mpendulo, C.T., Hugo, A., Falowo, A.B. and Nantapo C.T.W. (2022). Fatty acid profile, oxidative stability of lipids and sensory attributes of water restricted Xhosa goat meat supplemented with vitamin C. *Anim. Prod. Sci.* 62(1), 67-77**;** doi:10.1071/AN20238

Manuscript under review

i. Vitamin C has the potential to modulate stress in ruminants. A review.

LIST OF CONFERENCE PRESENTATION

Akinmoladun O.F., Fon, F.N., Mpendulo, C.T. and Okoh, O. (2020). Performance and meat quality of water restricted Xhosa goats supplemented with vitamin C. 66th International Congress of Meat Science and Technology (ICoMST) and 73rd Reciprocal Meat Conference (RMC) hosted by the American Meat Science Association held between August 3rd-6th 2020, Florida, USA.

LIST OF ACRONYMS

^{o}C	Degree centigrade
DMI	Dry matter intake
WI	Water intake
VC	Vitamin C
AA	Ascorbic acid
TMR	Total mixed ration
BCs	Body condition scores
THI	Temperature humidity index
ALT	Alanine aminotransferase
AST	Aspartate amino transferase
FSH	Follicle stimulating hormone
LH	Luteinizing hormone
SBW	Shrunk body weight
EBW	Empty body weight
HCW	Hot carcass weight
CCW	Cold carcass weight
CCY	Cold carcass yield
TBY	True biological yield
CCI	Carcass compactment index
FFDM	Free fat dry matter
CLA	Conjugated linolenic acid
AI	Atherogenicity index
DI	Desaturase index
i.m	Intramuscular
i.v	Intravenous
s.c	Subcutaneous

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

GENERAL ABSTRACT

This study aimed to evaluate the effect of single and extra vitamin C (VC) supplementation on water-stressed Xhosa goats during the summer by evaluating their growth performance, blood biochemical profile, nutrient balance, carcass characteristics and meat qualities. The objectives were to assess the effect of water restriction and vitamin C supplementation by evaluating: firstly, growth performance, heat tolerance responses, and blood metabolites; secondly, intake, nutrient digestibility, nitrogen and mineral balance; thirdly, stress indices, carcass characteristics and meat qualities parameters and lastly, fatty acid profile, oxidative stability of lipids and sensory attributes were evaluated. To achieve these objectives, forty-two (42) goats were divided equally into seven treatments (75-day trial); without water restriction (W0, control); water restriction of 70% of *ad libitum* water intake WI (W70); water restriction of 50% *ad libitum* WI (W50); water restriction of 70% of *ad libitum* WI plus 3g VC daily (W70^{+}); water restriction 50% of *ad libitum* WI plus 3g VC daily (W50^{+}); water restriction of 70% of *ad libitum* WI plus 3g VC and extra 5gVC given every eight-day (70^{++}); water restriction of 50% of *ad libitum* WI plus 3g VC and extra 5gVC given every eight-day (W50^{++}). In the first objective, growth performance parameters, body condition scores, rectal temperature, respiratory rate, heat tolerance response parameters and blood metabolites were measured. Weight loss due to water restriction was reduced by VC supplementation in treated groups. The attenuation effect of VC was significant ($P<0.05$) in responses to respiratory rate (RR), Na^{+}, K^{+}, Mg^{2+}, Cl^{-}, Ca^{2+} and urea. Supplementation of VC (either single or multiple) did not ($P>0.05$) improve the effect of water restriction on body condition scores (BCs), FAMACHA©, glucose, globulin, alanine aminotransferase (ALT) and high-density lipoprotein (HDL). In the second objective, intake, nutrient digestibility, nitrogen and mineral balance were evaluated. The reduced nutrient intake was similar ($P>0.05$) irrespective of water restrictions and VC supplementations. Water-restriction levels did not affect nutrient intake/metabolic weight. Retained nitrogen increased with water-restriction levels but not influenced by VC. NH$_{3}$-N decreased with the levels of water restriction while the pH was similar across the water restricted groups. Supplementation of VC had no effect ($P>0.05$) on apparent digestibility and digestible nutrients induced by water restriction levels. The amount of Ca, K, Mg retained increased ($P<0.05$) with water-restriction levels. In the third objective, stress indices, carcass characteristics and meat quality parameters were evaluated. The level of plasma cortisol concentration due to water stress was lessened ($P<0.05$) by VC. Follicle stimulating hormone (FSH), Luteinizing hormone (LH) and insulin were not affected

($P>0.05$) by levels of water restriction. The hot and carcass weights were lower ($P<0.05$) in $W70^+$ and $W50^+$ compared to W70 and W50 respectively. Vitamin C treated goats had higher redness (a*) compared to W70, W50 and W0. The shear force and cooking loss increased ($P<0.05$) with water restriction levels, while thaw loss, pH_{45min} and pH_{24h} were not affected by water restriction levels. In the final objective, the treatments did not affect ($P>0.05$) the oxidative stability of lipids (TBARS). The moisture and fat content in the water restricted groups were lower ($P<0.05$) than W0. The consumer meat sensory appearance decreased ($P<0.05$) with levels of water restrictions. Regardless of VC concentration and supplementation, vaccenic, and docosahexaenoic acid increased ($P<0.05$) while linolenic acid decreased as the water restriction levels increased. Nonetheless, treatment did not affect ($P>0.05$) the saturated and unsaturated fatty acids. These studies showed that suboptimal water intake negatively affects the growth performance and nutrient intake of Xhosa goats. However, the reduction in body weight loss and decreased plasma cortisol (chief stress hormone) concentration following vitamin C supplementation (especially at a daily single dose [3g/d]) may well reduce the effect of water stress in goats.

Keywords: water restriction, vitamin C, Xhosa goats, digestibility, performance, meat quality.

Chapter One

Introduction

1.1 Background of the study

Global demand for meat and animal products is highly expected to increase in the following decades. This supposed increase in the livestock market is attributed to increased population growth, increasing urbanization and rising income (FAO, 2016). Therefore, the growing populaces future concern will be centered on food security, among other things. Developing the livestock production sector (among other sectors) to cope with the world population growth, projected to grow by 34% from over 6.8 billion to 9.1 billion in the year 2050, is highly essential (United Nation, 2017). However, changes in the global climate in the form of global warming are expected to impact general agriculture either directly or indirectly (Nardone *et al.*, 2010). Based on the intergovernmental panel on climate change (IPCC, 2007), the earth's surface temperature is expected to increase from 2.4 to as high as 6.4 ^{o}C with frequent heat waves, warm spells, windstorms, and water scarcity, among other things. Such alteration in climatic variables over a long period is bound to affect the productivity of crop and livestock systems (Rojas-Downing *et al.*, 2017).

Small ruminants' contributions, especially goats, to the livestock industry's development have been emphasized (Kosgey *et al.*, 2008). This is because goats are imbued with good attributes such as hardiness, adaptability to harsh climates, good foraging abilities, quick reproduction and low production cost (Ngambi *et al.*, 2012). They are also critical to developing sustainable and environmentally sound production (Ben Salem, 2010) system, given the socio-economic role they play in communities living in arid and semi-arid regions. Goats are hardy and well-adapted to harsh climates. However, the challenge of water and poor quality feed are problems that affect the production of small ruminant production in the arid and semi-arid regions (Akinmoladun *et al.*, 2019).

Like many animal species, goat productivity is maximally expressed when reared in an environment that equates with their thermo-comfort or neutral zones [range; 38.7-40.7 ^{o}C] (Bengtsson and Whitaker, 1988). However, the climatic conditions in the long summer season of the Eastern Cape, South Africa, is plagued by unusually high ambient temperature, humidity, water scarcity, and highly lignified/fibrous fodders (Hashem *et al.*, 2015). In general, South Africa experiences limited water availability as it receives mean annual precipitation of about 500mm, where only 9% of this being converted to river run-off (Dennis and Dennis, 2012). In the Eastern Cape Province, the climate is arid to semi-arid, receiving 350-550mm rainfall per annum (Palmer, 2004). The region has a mean annual temperature of 17.6 ^{o}C (mean monthly range of 12.3-22.4 ^{o}C) with daily maximum temperatures in summer regularly reaching 40 ^{o}C (Mills et al., 2005). These harsh conditions induce stress and portend a major constraint to animal agriculture and productivity (Rojas-Downing *et al.*, 2017). Under conditions of heat stress, performance indices of animals such as feed intake, fertility and conception rate, milk production are affected negatively. Johnson (2018) affirmed the interruption of normal internal milieu/homeostasis resulting from imbalances in water metabolism, hormonal secretion, blood metabolites, energy, nitrogen, and reduced production in heat-stressed animals. During summer, adaptable breeds of sheep are forced to stay up for several days, trekking long distances with little or no water (Chedid *et al.*, 2014). The thermoregulatory demand for water is further exacerbated due to increased fermentative heat from fibrous forage (Nardone *et al.*, 2010) in ruminants. Despite the adaptability of indigenous breeds to water scarcity in hot arid and semi-arid regions, insufficient water intake affects animals' physiological homeostasis, resulting in lower feed intake, reduced body weight, decreased fertility, and immunity to diseases (Beede, 2012).

Several stress alleviations approaches have been worked upon by several researchers. Such approaches could be in the form of feed type and feed composition manipulation (Abdel-

Samee *et al.*, 2008), housing pattern, ventilation and cooling system (Lin *et al.*,1998; Armstrong *et al.*,1999) to selection for drought-resistant breeds, among others (Morrison *et al.*,1983). However, their availability and sustainability depend on the overall price or cost incurred, the amount of energy and water to be consumed, and the farming system adopted (Thorne, 2007; Renaudeau *et al.*, 2012). Besides, several drugs and antioxidant supplements for stress alleviation in domestic animals have been tested. Such drugs include xylazine and sodium betaine administered to sheep and goats (Ali *et al.*, 2005), electrolyte therapy in cattle (Schaefer *et al.*, 1997) and aspirin (Acetyl-salicylic acid) in layers, pigs and rats (Galil, 2004; Fields *et al.*, 2001; Drew *et al.*, 2005). Attention is currently focused on ascorbic acid (vitamin C) or its salts in ruminants' stress management. Studies have documented its use in stress management in Red Sokoto goats undergoing road transportation (Minka and Ayo, 2012) and water-restricted Awassi sheep (Ghanem *et al.*, 2008)

L-ascorbic acid or vitamin C is an essential water-soluble antioxidant (Pehlivan, 2017). It is regarded as the most critical extracellular antioxidants, acting as the first line of defence against free radicals. Under normal conditions, ruminants, including goats, can synthesize vitamin C endogenously within their bodies by bio-synthesizing L-ascorbic acid from glucose through the glucuronic acid pathway in the liver and kidney. However, a decrease in vitamin C levels in the blood is commonly observed, especially during stress and disease conditions (Ranjan *et al.*, 2005; Rejeb *et al.*, 2016). Unlike vitamin C, small ruminants' requirements were defined only for A, D and E (NRC, 2007). Supplementation of ascorbic acid to ewes orally, increases their ability to withstand water stress (Ghanem *et al.*, 2008) and with notable improvements in fertility and weight of pregnant ewes and their lambs (Halilo˘ and Serpek, 2000). During periods of harsh environmental conditions, animal productivity also improves when fed diets supplemented with vitamins (Marai *et al.*, 2008). However, supplementation of these nutritional supplements, especially vitamin C on small ruminants during stress management, have been very short (Ghanem *et al.*, 2008; Minka and Ayo, 2012), and the results obtained are sometimes conflicting (Ghanem *et al.*, 2008; Hashem *et al.*, 2016).

The Xhosa lob-eared is an indigenous and adaptable breed, raised primarily for meat and milk. It is mainly distributed in the Eastern Cape's arid region, the Southern part of South Africa. It is a well-muscled, medium to large-framed goat breed and usually spotted by various coat colours patterns (Saymore *et al.*, 2010). Being an ecotype of the indigenous Veld goats, they are resilient to local diseases, parasites and climatic changes with an ability to live in a 'free range' natural environment. Although water insufficiency has been a persistent and challenging problem in arid and semi-arid regions, studies on quantitative data for adaptable breeds during long periods of water scarcity or deprivation are very few.

Several studies have been conducted on water stress in livestock (Chedid *et al.*, 2014; Casamassima *et al.*, 2016). However, the capability to withstand intermittent watering and the coping mechanisms may vary between breeds, and these will determine their worthiness for selection and improvement. Jaber *et al.* (2013) reported that different breeds responded differently to water stress. This necessitates investigations on indigenous breeds' ability to regulate their intake and body fluid homeostasis during the period of water restriction. Besides, no research has documented the use of vitamin C in water stress management of indigenous Xhosa goats in the Eastern Cape of South Africa. With the above research gap, the study is conducted to evaluate the effect of supplementation of vitamin C to the diet of water restricted Xhosa goats during the summer season by assessing their performance, blood metabolites and meat quality.

1.2 Statement of Research Problem

The continuous shortfall in rainfall patterns as a result of climate change has reduced the amount of fresh water available to a major part of the earth surface. This threat to water availability has not only affected humans but also livestock production. The implication of this scarcity could be enormous for the arid and water limited regions of the world. Although small ruminants seem to have the capacity to tolerate limited water intake compared to other specie of livestock, suboptimum water intake compromises the integrity of the animal's total wellbeing and makes livestock production increasingly challenging. Small ruminants respond to water stress by decreasing their feed intake, resulting in weight reduction due to water and body mass loss. Also, the stress effect in ruminants induced by suboptimal water intake and elevated temperature, has the potential to compromise blood metabolites as well as the meat quality parameters if sustained for a long period of time. Given the growing concern of future global water scarcity, including competition between humans and livestock, new research plans in the form of management strategies need to be formulated in semi-arid and arid environments for rural farmers.

Studies examining the effect of water stress in small ruminants, especially goats, have not gained much attention. This is because goats are assumed to be more adaptable and contribute minimally to meat or milk production. The Xhosa ear-loped goats are very predominant among resource-limited farmers in the Eastern Cape of South Africa as a source of income and livelihood, and knowledge of their water tolerance capacities and vitamin C's role in stress management will help to sustain production.

Vitamins play an essential role in the diets of livestock species as their supplementation during the period of stress have shown to have positive effects on dry matter intake, reproduction, immune status, and disease resistance. The research will help farmers ensure efficient feed utilization and improved production performance using vitamin C in water stress management.

1.3 Aims and Objectives

1.3.1 Research Aim

This study aims to evaluate the effects of vitamin C supplementation in water-restricted Xhosa ear-loped goats on the growth performance, blood biochemical profile, nutrient utilization, carcass characteristics and meat quality in the summer period.

1.3.2 Research Objectives

The specific objectives are to determine the effects of water restriction and vitamin C supplementation on:

1. the growth performance (total feed intake, total weight gain), heat tolerance response (temperature-humidity index, rectal temperature, respiration rate) and blood metabolites (osmolality, AST, ALT, Total protein, Total glucose, creatinine, urea) of Xhosa goats.
2. the nutrient intake, nutrient digestibility, nitrogen and mineral balance of Xhosa goats
3. on stress indices (cortisol, Tri-iodothyronine T_3, Thyroxine T_4), carcass traits and meat quality of Xhosa goats.
4. the fatty acid composition, lipid oxidation and consumer sensory attributes of Xhosa goats.

1.4 Hypothesis

This study is premised on the null hypothesis that water restriction and vitamin C supplementations to Xhosa goats during summer will not affect their;

1. growth performance (total feed intake, total weight gain), heat tolerance response (temperature-humidity index, rectal temperature, respiration rate) and blood metabolites (osmolality, AST, ALT, Total protein, Total glucose, creatinine, urea)
2. intake, nutrient digestibility, nitrogen and mineral balance.
3. stress indices (cortisol, Tri-iodothyronine T3, Thyroxine T4), carcass traits and meat quality
4. fatty acid composition, lipid oxidation and consumer sensory attributes

1.5 Reference

Abdel-Samee, A.M., Abd-Alla, O.A.M. and EL-Adawy, S.A.I. (2008). Nutritional treatments for alleviation of heat stress in Awassi sheep using acacia and olive pulp in subtropics. *Egypt. J. Comp. Path. Clinic. Path*. **21**(1), 466-477.

Akinmoladun, O.F., Muchenje, V., Fon, F.N. and Mpendulo, C.T. (2019). Small ruminants: farmers' hope in a world threatened by water scarcity. *Animals* **9**(7), 456.

Ali, B.H., Al-Qarawi, A.A. and Mousa, H.M. (2005). Stress associated with road transportation in desert sheep and goats, and the effect of pretreatment with xylazine or sodium betaine. *Res. Vet. Sci.* **80**(3), 343-348.

Beede, D.K. (2012). What will our ruminants drink? *Anim. Front*. **2**, 36–43.

Bengtsson, L.P. and Whitaker, J.H. (1988). Farm structures in tropical climates: a textbook for structural engineering and design. FAO/SIDA Cooperative Programme. Rural structures in east and south-east Africa. Rome: Food and Agriculture Organization of the United Nations

Ben Salem, H. (2010). Nutritional management to improve sheep and goat performances in semi-arid regions. *R. Bras. Zootec*. **39** (supl. especial), 337-347.

Casamassima, D., Vizzarri, F., Nardola, M. and Palazzo, M. (2016). Effect of water restriction on various physiological variables on intensively reared Lacauna ewes. *Vet. Med*. **61**, 623–634.

Chedid, M., Jaber, L.S., Giger-Reverdin, S., Duvaux-Ponter, C. and Hamadeh, S.K. (2014). Review: Water stress in sheep raised under arid conditions. *Can. J. Anim. Sci*. **94**, 243–257.

Dennis, I. and Dennis, R. (2012). Climate change vulnerability index for South African aquifers. Potchefstroom. Unit for Environmental Sciences and Management, North-West University. Retrieved from www.wrc.org.za

Doreau, M., Corson, M.S. and Wiedemann, S.G. (2012). Water use by livestock: A global perspective for a regional issue. *Anim. Front*. **2**, 9–16.

Drew, J., Arthur, J., Farquharson, A, Russel, W., Morrice, P. and Duthie, G. (2005). Salicylic acid modulates oxidative stress and glutathione peroxidase activity in the rat colon. *Biochem. Pharmacol*. **70**(6), 888-893.

Fields, M., Lewis, C. and Bureau, I. (2001). Aspirin reduces blood cholesterol in copper deficient rats: A potential antioxidant agent? *Metabolism* **50**(5), 558-561.

Food and Agriculture Organization of the United Nations [FAO]. (2016). Statistical Year Book; Food and Agriculture Organization of the United Nations: Rome, Italy, Volume 1.

Galil, M A. (2004). Effect of using some anti-heat stress compounds on the performance of some local breeds of chicken under hot climatic condition. *Egypt. Poult. Sci. J.* **24**(2), 417-427.

Ghanem, A.M., Jaber, L.S., Abi Said, M., Barbour, E.K. and Hamadeh, S.K. (2008). Physiological and chemical responses in water-deprived Awassi ewes treated with vitamin C. *J. Arid Environ*. **72**, 141–149.

Haliloglu, S. and Serpek, B. (2000). The effects of plasma vitamin C and ceruplasmin levels and exogenous vitamin C supplementation on reproduction in sheep. *Turk. J. Vet. Anim. Sci.* **24**, 403–412.

Hashem, N.M., El-Azrak, K.M., Nour El-Din, A.N.M., Taha, T.A. and Salem, M.H. (2015). Effect of GnRH treatment on ovarian activity and reproductive performance of low-prolific Rahmani ewes. *Theriogenology* **83**, 192–198.

Hashem, N.M., Abd-Elrazek, D., Abo-Elezz, Z.R. and Latiff, M.G.A. (2016). Effect of vitamin A or C on physiological and reproductive response of Rahmani ewes during subtropical summer breeding season. *Small Rumin. Res*. 144, 313-319.

IPCC (Intergovernmental Panel on Climate Change) (2007). Synthesis Report. In Contribution of Working Groups I, II and II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change; Pachauri, R.K., Reisinger, A., Eds.; IPCC: Geneva, Switzerland, p. 104.

Johnson, J.S. (2018). Impact of heat stress on livestock and mitigation strategies to improve productivity and well-being. *J. Anim. Sci.* **96**(suppl. 2), 6.

Kosgey, I.S., Rowlands, G.J., van Arendonk, J.A.M. and Baker, R.L., (2008). Small ruminant production in smallholder and pastoral/extensive farmingsystem in Kenya. *Small Rumin. Res.* **77**, 11–24

Marai, I.F.M., El- Darawany, A.A., Fadiel, A. and Abdel-Hafez, M.A.M. (2008). Reproductive performance traits as affected by heat stress and its alleviation in sheep. *Trop. Subtrop. Agroecosyst*. **8**, 209–234.

McGrath, J., Duval, S.M., Tamassia, L.F.M., Kindermann, M., Stemmler, R.T., de Gouvea, V.N. *et al.* (2018). Nutritional strategies in ruminants: A lifetime approach. *Res. Vet. Sci.* **116**, 28-39.

Mills, A. J., Cowling, R. M., Fey, M. V., Kerley, G. I. H., Donaldson, J. S., Lechmere-Oertel, R. G., ... Rundel, P. (2005). Effects of goat pastoralism on ecosystem carbon storage in semiarid thicket, Eastern Cape, South Africa. *Austral Eco*. **30**, 797–804

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

Morrison, S.R. (1983). Ruminant heat stress: effect on production and means of alleviation. *J. Anim. Sci.* **57** (6), 1594-1600.

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

National Research Council (NRC). (2007). Nutrient Requirements' of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids; NRC, Washington, DC, USA, 384p.

Ngambi, J.W., Alabi, O.J. and Norris, D. (2013). Role of goats in food security, poverty alleviation and prosperity with special reference to Sub-Saharan Africa: A Review. *Indian J. Anim. Res*. **47**(1), 1-19.

Palmer, T. (2004). Vegetation of Makana. Makana LEAP: Comprehensive environmental audit. Grahamstown: ARC-Range & Forage Institute

Pehlivan, F.E. (2017). Vitamin C: An antioxidant agent, Vitamin C, Amal H. Hamza, IntechOpen.

Ranjan, R., Swarup, D., Naresh, R. and Patra, R.C. (2005). Enhanced erythrocytic lipid peroxides and reduced plasma ascorbic acid, and alteration in blood trace elements level in dairy cows with mastitis. *Vet. Res. Commun*. **29**, 27–34.

Rejeb, M., Sadraoui, R. and Najar, T. (2016). Role of Vitamin C on Immune Function under Heat Proc. Natl. Acad. Sci. USA 94:13816– 13819.

Renaudeau, D., Collin, A., Yahav, S., de Aasilio, V., Gourdine, J.L. and Collier, R.J. (2012). Adaptation to hot climate and strategies to alleviate heat stress in livestock production. *Animal* **65**, 707-728.

Rojas-Downing, M., Nejadhashemi, A.P., Harrigan, T. and Woznicki, S.A. (2017). Climate change and livestock: Impacts adaptation and mitigation. *Clim. Risk Manag.* **16,** 145-163.

Schaefer, A.L., Jones, S.D.M. and Stanley, R.W. (1997). The use of electrolyte solutions for reducing transport stress. *J. Anim. Sci.* **75**, 258-265.

United Nation. (2017). Sustainable Development Goals. Revision of World Population Prospects; United Nations:San Francisco, CA, USA.

Chapter Two

Literature Review One

Small ruminants: Farmers' hope in a world threatened by water scarcity

(Published in *Animals* Journal)

Abstract: Availability and sustainability of portable drinking water is a global concern. Such uncertainties threaten livestock production with an attendant ripple effect on food security. Small ruminants, including sheep and goats, appear promising to smallholder farmers in solving this problem because of their ability to survive in water-limited areas and harsh environments compared with large ruminants. Their small body size is also seen as an advantage because less water will be required for proper digestion and feed utilization. Therefore, this review will provide information on the adaptive responses of small ruminants on thermoregulation, blood biochemical profile, immune status, drug pharmacokinetics, reproduction, and hormonal indices during the period of water stress. Adaptable and indigenous ruminant breeds are known to be more tolerant to water stress than selected breeds. However, decreased feed intake and weight, reduced respiratory rate, and increased concentration of blood metabolites are the general effects and observations encountered by small ruminants during the period of water stress. The concept of water tolerance, either as deprivation and/or restriction of indigenous and adaptable breeds of small ruminants, is gaining ground in research studies worldwide. However, more research seeking to explore adaptable breeds' water tolerance capacity, especially in arid and water limiting areas, is still needed.

Keywords: sheep, goat, water restriction, adaptation, thermoregulation, water scarcity.

2.1 Introduction

Over 40 per cent of the people on the earth's surface is already affected by the scarcity of freshwater, and more than 1.7 billion people are currently living in a highly water-limited basin (United Nation, 2015). Water demand will continue to rise across the globe due to climate change, increased demand from the growing population, polluted supplies, land use and economic change (Naqvi *et al.*, 2015). The Fourth Assessment Report, which emanates from the Intergovernmental Panel on Climate Change (IPCC, 2007), affirms that stress on future water resources will continue to increase if mitigating strategies are not implemented. Areas most vulnerable and with very limited freshwater resources are North Africa, Northeast China, Pakistan, India, North America, and Middle East (Schlosser *et al.*, 2014; Damerau *et al.*, 2015). Presently, the inability to satisfy the demand for good quality water has reached a critical stage in many areas. Such an imbalance creates concern in meeting up with the water-requirements for food production for the world's growing population

Water is an essential nutrient and should be readily available for animals in the required amounts (Nejad, 2014). However, livestock is the worst hit, with suitable drinking water unavailability becoming an enormous concern for farmers. In addition to being worst hit is the transhumance conflict often generated when livestock are moved seasonally across geographical regions in search of feed and water. Low rainfall affects drinking water and reduces feeds' availability for small ruminants, especially for those grazing in semi-arid zones (Abdelatif *et al.*, 2010). Insufficient amount of drinking water for livestock negatively impacts all performance indices and may result in food insecurity, especially for smallholder farmers in developing countries.

The way forward under this shifting condition is probably to decide the animal species (non-ruminants, large or small ruminants) to rear and produce. In this regard, small ruminants appear more promising because of their low production cost, short generation interval, suitability to small-holdings, multipurpose (meat, milk and fibre) use, ability to utilize crop residues effectively and most importantly, their tolerance to harsh climatic variables (such as low rainfall and heat stress) than cattle and other monogastric. The shifting trend of keeping more small ruminants than cattle by pastoralists (the Samburu) in Maasai, Kenya and Afar, Ethiopia was partly because of their drought-tolerant ability (Peacock, 2005). Peacock (2005) argued that increased frequencies of drought have resulted in pastoral families' inability to re-establish larger stock because of the constant struggle to 'recover' from the last drought. For this reason, they increasingly depend on small ruminants, particularly goats. In addition, the preponderance of small ruminants, mainly sheep and goats, over cattle and other animal species in Asia and the Middle East (low rainfall areas) currently faced with desertification and high temperature (Ben Salem and Smith, 2008) is indicative of their drought-tolerant abilities. In a graphical illustration on the choice of livestock species with respect to drought-induced temperature, Seo and Mendelsohn (2006) estimated that the probability of choosing beef and dairy cattle decreases rapidly at elevated temperature as compared to a higher likelihood of selecting goats and sheep. However, the choice of raising chicken is estimated to assume a normal distribution curve (bell-shaped) with a maximum peak at a mean temperature of 22^{o}C.

Many research studies have shown goats' capability (Silanikove, 2000) and sheep (Jaber *et al.*, 2004; Casamassima *et al.*, 2008) to tolerate dehydration. Small ruminants are imbued with water-saving mechanisms (e.g. reduced panting and respiration rate) that help them minimize water loss and enhance their ability to withstand water shortage (Doreau *et al.*, 2012; Jaber *et al.*, 2013). They have evolved an efficient water economy, which has enabled them to maintain their dry matter and production intake during times of water shortfall or scarcity, even at elevated temperature. This is because, during dehydration, dry matter digestibility is improved and coupled with a reduced metabolic rate. In addition, the rumen also performs the function of a water reservoir (15% of animal body weight) for use when water is scarce (Silanikove, 2000). Such adaptation ensures that they continue to live despite extended periods of water scarcity while grazing several kilometres away from points of water and exploiting the sparsely distributed pastures efficiently. During water stress, small ruminants respond by reducing their feed intake culminating in weight reduction because of body mass and water loss (Chedid *et al.*, 2014). Water losses amounting to 18% of body weight can be tolerated by cattle; sheep and goat, 20%; camel, 25% and even more than 40% in some desert Bedouin and Barmer goats because the fore-stomach can accumulate water to be used when supply is low. However, a body mass loss of 15% and above due to reduced water intake in other animals is harmful and can be fatal (Shkolnik *et al.*, 1980). Though such an imbalance in water and energy metabolism negatively impacts general health and productivity, small ruminants, including sheep and goat, have evolved adaptive mechanisms that enable them to thrive and breed successfully in water-limited and arid lands. Therefore, the present review seeks to discuss water resources and use in a future perspective, current small ruminants' distribution, the impact of water scarcity on these animals, and the adaptive mechanisms evolved by them.

2.2 Global water resources and use

The percentage of freshwater from the total water volume on Earth is estimated to be about 2.50%. However, about two-thirds of this is locked in glaciers and ice caps (Shiklomanov *et al.*, 1993). Just 0.77% of all water (~10,665,000km^3) is held in aquifers, soil pores, lakes, swamps, rivers, plant life, and the atmosphere (Shiklomanov *et al.*, 1993). The mean annual surface and subsurface (groundwater/shallow aquifer) runoff accumulated as river discharge is assumed to constitute the sustainable water supply accessible to local human populations (Postel *et al.*, 1996). According to Kundzewicz and Döll (2009), about 3 billion people source their drinking water from groundwater. Unfortunately, most of the fresh and groundwater resources accessible to the population have been largely depleted, resulting in a sharp drop in agricultural production and inflated prices. In arid and water-limited regions, the expected precipitation over the next century will decrease by 20% or more, and the situation may be even worse with an unprecedented increase in population. Unfortunately, these regions (Asia, Arab, Middle East, Northeast China, India, and some Africa countries) largely depend on natural resources and climate-driven activities (Misra *et al.*, 2014). Fresh water-limited areas will experience the largest percentage and absolute increase in demand-driven water stress, with agriculture remaining the most prominent user. This is based on the conventionally developed scenario (CDS) of projected water use developed from conjectures about probable alterations in demand components (Schlosser *et al.*, 2014; Damerau *et al.*, 2015). It was estimated that by the year 2025, over 30 countries would be found to be water-stressed as compared to seven countries in 1955, and by the year 2050, two-thirds of the world population may be already water-stressed simply due to a rise in population, industrialization, global climate change and water use (Gossain *et al.*, 2006). Future water resource use will be influenced by climatic changes, population growth and clustering, irrigation expansion, revolutionary industrial changes, water use efficiency, and demand management (Fujimori *et al.*, 2017). Fig. 2.1 is a projection of global future water resource use (1999-2050).

As estimated recently (Schlink, 2010), animal agriculture utilizes about 8% of the available global water supply. About 1% is available for animals' nourishment, servicing on-farm activities and processing animal products into food. The remaining 7% is used in irrigating feed crops in the intensive production of livestock in developed countries. Given the above projections of high-water demand and use in agriculture, all livestock sector procedures involving water use during production and management must be reviewed. Information about distribution patterns and feeding habits, adaptation to climatic changes, especially to limited water, must be critically reviewed and updated for their proper management and sustainability of the livestock industry.

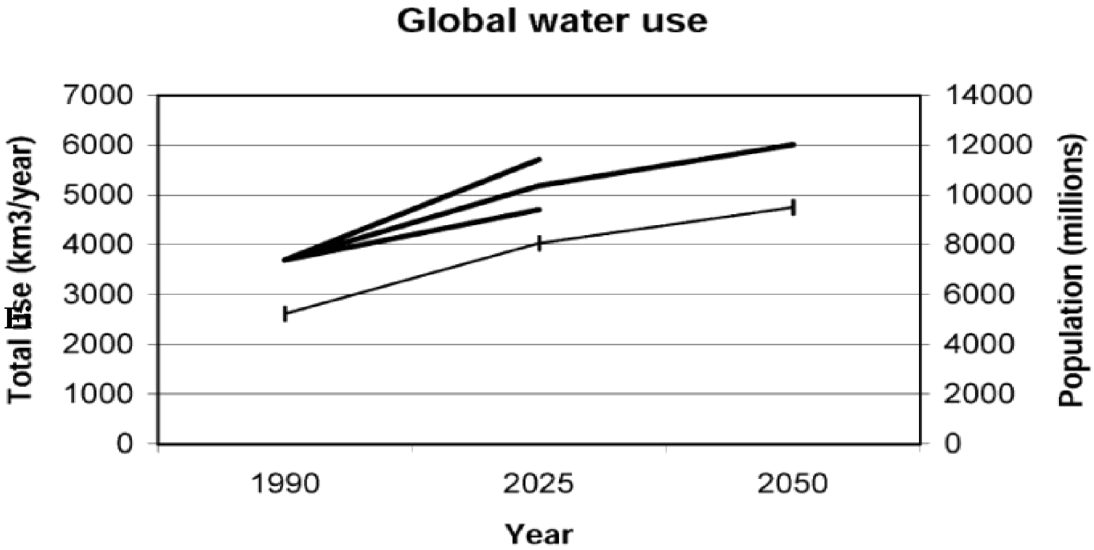


Figure 2. 1 Global water resource withdrawals, 1990-2050**.** The thick lines show total withrawals, under the mid-range, low and high (to 2025 only) Conventional Development Projections. The thin line shows the total population. By 2025, global water use would have increased under the mid-range scenario by 35% over 1995 and by 67% by 2050. The mid-range, high and low case projections were based on the assumption that countries will have the same rate of population but operates at different economic growth rate and technological improvements. Withrawals means fresh water use channels (i.e. irrigation, industrial uses etc.)

Source: Arnell, (1999)

2.3 Small ruminant distribution

Small ruminant agriculture plays a vital role in social and economic development, especially for developing countries. They contribute to the management and development of landscapes, ecosystems maintenance, biodiversity conservation, and job opportunities from their products (meat, skin, milk, etc.) and by-products in the market (Marino *et al.*, 2016). The sector is so important such that 56 % of the world ruminant domestic populations (3,872 million heads) are sheep (1178 million) and goats (1000 million) (FAOSTAT, 2013; FAO, 2016) and are distributed all over different types of ecology. Over 56 % of the world's small ruminants is located in water-limiting and dry zones in developing countries (Table 2.1), whereas temperate and humid zones account for 27 % and 21 %, respectively (FAOSTAT, 2013; Marino *et al.*, 2016). As earlier affirmed, this high small ruminant population in the dry zones is largely as a result of their preference, owing to their efficient water use abilities, and continuous breeding by dwellers of that area. Though less than sheep and cattle, the goat population of the world has constantly increased since the 60s, especially in the countries of low income or less-favoured regions of the world (Skapetas and Bampidis, 2016), and a 60% increase in global sheep number is expected by 2050 (Foresight, 2011). According to the working document submitted to the Food and Agricultural Organization (FAO) on domestic animal biodiversity (Scherf 2000), sheep and goats, clustered in 1314 and 570 breeds, are distributed across various geographical and agro-ecological zones around the world. The population size and distribution of buffalo, cattle, sheep, and goat worldwide, excluding extinct breed, are shown in Table 2.1. Market demand and shifting to more profitable agricultural activities seem to be the main determinants for changes in the small ruminant population (Iniguez, 2011). More than 28 million tons of milk and 13 million tons of meat are produced from sheep and goats worldwide, respectively. This represents a 1.3 and 1.7 per cent increase per year, respectively, during the past two decades (FAOSTAT, 2013).

Table 2. 1 Population size and distribution of buffalo, cattle, sheep, and goat

Ruminant type/distribution	Population size (x 10^3)	Breed number	Population share (%)	Breed share (%)	Breeds per million
Buffalo					
Africa	0	3	0	3	0
Asia+Pacific	152404	61	96	71	0.4
Europe	412	3	0	3	7.3
LatinAmerica+ Carribean	1711	9	1	10	5.3
Near East	3998	10	1	12	2.5
NorthAmerica	0	0	0	0	0
Total	158525	86			
Cattle					
Africa	174556	251	13	21	1.4
Asia+Pacific	461197	236	35	19	0.5
Europe	162119	482	12	39	3.0
LatinAmerica+ Carribean	356069	107	27	9	0.3
Near East	71913	86	5	7	1.2
NorthAmerica	111481	62	8	5	0.6
Total	1337335	1224			
Goat					
Africa	137104	89	19	16	0.6
Asia+Pacific	390433	146	55	26	0.4
Europe	26092	187	4	33	7.2
LatinAmerica+ Carribean	40752	34	6	6	0.8
Near East	114572	94	16	16	0.8
NorthAmerica	1428	20	0	4	14.0
Total	710381	570			
Sheep					
Africa	127440	147	12	11	1.2
Asia+Pacific	408098	233	39	18	0.6
Europe	185035	629	18	48	3.4
LatinAmerica+ Carribean	89372	42	8	3	0.5
Near East	242770	201	23	15	0.8
NorthAmerica	7891	62	1	5	7.9
Total	1060606	1314			

NB: Population share is the contribution of each region for a particular type of ruminant to the total world population; Breed share is the total number of breeds per fraction of world total breeds of a given ruminant inhabitants of a particular region in a particular region; Breed per million is the average number of breeds in one million population number of a particular ruminant type in a given geographical area.

Most of the grazing lands worldwide are found in seasonal environments with marked variations in resource abundance, the arid and semi-arid zones of the tropical belt being characteristics examples. Small ruminants' socio-economic importance (mainly sheep and goat) to smallholder and commercial farmers in the entire world cannot be overemphasized. This is because the demand for their product and by-product' keeps increasing with population increase. Therefore, there is a need to continue investigating different methods of sustaining the increase in demand with limited population resources. With the limitation of freshwater, as observed in the previous section, there is a need to understand these animals' water requirements so that their production can be sustained and effectively managed.

2.4 Water requirements and metabolism

The amount of water required by small ruminants is a function of their body metabolism, ambient temperature, body size and weight, restriction patterns, dry matter intake, feed composition and energy consumed, water quality, species, physiological status, production stages, breed and wool covering (NRC, 2007). The amount of water consumed by ruminants voluntarily is two times that of dry matter consumption. Water consumption tends to increase when diets rich in protein or salt are fed to animals. However, the expression of water requirement per animal is usually the total of the needed amount for all physiological stages (i.e. maintenance, growth, pregnancy, and lactation) (Meyer *et al.*, 2004; NRC, 2007). According to Beede (2012), satisfying the daily requirement is fulfilled when net water intake to water loss is zero [i.e. (free drinking water + water in or on feeds consumed) = (water excreted in urine and faeces + water secreted in milk, sweat and respiratory pore)]. In the view of Esminger *et al.* (1990), the actual total water required by ruminants is a complex process, and a balance must be struck to ensure that total water intake (TWI) equals water loss (WL) and water retained (WR). TWI accessible to animals includes water sources from metabolic (nutrient catabolism), drinking and feed water. Nutrient catabolism often generates some metabolic water as part of the end product, which is also available as a water source to the animal. Based on that assumption, 1g of metabolized carbohydrates, fat and protein will yield 0.56, 1.07 and 0.42 g of water, respectively (Maynard *et al.*, 1981).

However, higher water losses by the lungs due to an increase in breathing in the case of fat oxidation usually result in the production of less metabolic water during fat hydrolysis when compared to carbohydrate hydrolysis (Esminger *et al.*, 1990). In addition, water concentrations in succulent feeds like fresh legumes, grasses and silage are usually very high, constituting an essential source of water for sheep and goats reared in arid and water-limited areas (Araujo *et al.*, 2010). Water intake per animal depends on the degree of dehydration or restriction, drinking time allowances, and the number of animals drinking together at a watering point (Beede, 2012). Mammals were therefore grouped based on their ability to replenish lost water rapidly or gradually.

Sheep and goat can drink and adequately replace 18-40% of their body mass within 3-10 minutes at first drinking (Silanikove, 2000). During heat stress, water intake increases while feed consumed decreases and weight gain decline, a situation that is parallel to feed consumption and nutrient balance (Ratnakaran *et al.*, 2017). In addition, the attempt to balance body temperature by an animal during elevated temperature often results in raising their energy (Kerr, 2015). The way drinking troughs are arranged and the ease of accessibility of animals to water supply points also affect water intake (Araujo *et al.*, 2010). This is a common observation during the dry season, especially in arid and water-limited areas when water supply points get limited and accessibility decreases. As pointed out, more than half of the small ruminant population in the world is found in water limiting and arid regions. There exist, therefore, a possibility of an adaptive response that favours their survival, growth, and reproduction.

2.5 Adaptive responses of small ruminants to water shortages and deprivation

Severe water shortages for herds are widespread in the arid and semi-arid regions around the world. This is further compounded by the poor forage quality and low humidity level that usually accompany the dry season. An animal's water intake is generally restricted to once per day during access to a water source (Doreau *et al.*, 2012). Research studies on small ruminants simulating water scarcity conditions or limitation in arid or water limiting areas in the form of water deprivation or restriction to assess their adaptive responses or changes at all levels of production and physiology are gaining global attention.

2.5.1 Effect on metabolic, rectal temperature, respiratory and pulse rate

Evidence abounds that metabolic rate in animal decreases during water restriction, a process that is suggestive of an energy conservation response (Choshniak *et al.*, 1995). Such imbalance in water intake can precipitate an increase in body heat and rectal temperature (Rahardja *et al.*, 2011; Ghassemi *et al.*, 2017). According to Davis and DeNardo (2007), adjustment to a lower metabolic rate and slower water loss during dehydration enhanced an animal's survival value because the duration that an animal can survive without eating under dehydration is extended. Evaporative cooling via sweating which constitutes a primary cooling avenue during heat stress for goats adapted to hot arid zone (Ratnakaran *et al.*, 2017), is usually reduced in dehydrated animals (Mckinley *et al.*, 2007). Dehydration-induced hyperthermia may be adaptive in conserving water as it increases the temperature at which animals switch from thermoregulation via convection and radiation to evaporative cooling Mitchell *et al.*, 2002). The rectal temperature (RT) of Nubian goats increased significantly in the third day of water deprivation, despite the marked decrease in feed intake that could have influenced the energy budget. The daily average rectal temperature in dehydrated goats was 0.5°C to 0.9°C higher than hydrated ones, a pointer to reduce evaporative heat loss (Jessen *et al.*, 1998). RT increases as water deprivation continues in the three local Saudi Arabia goat breeds. However, in lactating and dry Awassi ewes watered once every three days or daily, no changes were observed in their RT (Hamadeh *et al.*, 2006). A similar result was observed in water restricted (W80 and W60% of *ad lib* water), Lacauna ewes (Casamassima *et al.*, 2016) and Aardi goat (Alamer, 2010). Sheep are noted to be thermo-stable even during periods of dehydration (Fuller *et al.*, 2007). When water-deprived, there is a rise in small ruminants' body temperature because of reduced thermoregulatory evaporation (Alamer, 2009). This depression of cutaneous evaporation presumably triggers the water conservation mechanism (Mckinley *et al.*, 2007) with an attendant rise in RT during the water deprivation period. Respiratory activity was noted to be reduced during water deprivation periods in goats (Alamer, 2006) and sheep (Ismail *et al.*, 1996). Outcomes from some experimental studies on changes in rectal temperature following intermittent watering regimen are summarized in Table 2.2. Changes in respiratory rate (RR) may sometimes seem undetectable in goats (Das *et al.*, 1994) due to the combined effect of heat stress and water deprivation. Water restricted Lacauna ewes (W80 and W60% of *ad lib* water) had their respiratory acts per minute reduced from Day 0 to Day 14. As a defence mechanism, small ruminants reduce respiratory activities during water deprivation to prevent water loss and dehydration via pulmonary evaporation (Casamassima *et al.*, 2016). The water conservation mechanism in small ruminants ensures that water losses via respiration are managed effectively during shortages.

Panting rate (breathe/min) decrease in water restricted and dehydrated sheep at elevated temperature (Ghassemi *et al.*, 2017).

Table 2. 2 Effect of water restriction regimen on rectal temperature in small ruminants

		Average RT (°C)					
Water restriction	Specie	Water restriction	Control	Age (yrs)	Ambient temp(°C)	RH (%)	Ref
3 days	Nubian goat	37.90	39.90^{s}	2-3	34.8	25.5	(Abdelatif *et al.*, 2010)
4 days	Sheep	38.86	38.99^{ns}	Mature	24.8	NG	(Li *et al.*, 2000)
4 days	Sheep	39.78	39.37^{ns}	Mature	0.4	NG	(Li *et al.*, (2000)
20%, 40% less *ad lib* and alternate day	Malpura ewes	38.68, 38.80 and 38.74 respectively	38.80^{ns}	2-4	39.86	26.03	(Kumar *et al.*, 2016)
20%, 40% less *ad lib* and alternate day *ad lib*	Malpura ewes	38.55, 38.46, and 38.47 respectively	38.53^{ns}	2-4	32.37	36.67	(Kumar *et al.*, 2016
Once every 3 days	Lactating Awassi ewes	39.53	39.51^{ns}	Mature	27-31	61-85	(Hamadeh *et al.*, 2006)
Once every 3 days	Dry Awassi ewes	39.47	39.67^{ns}	Mature	27-31	61-85	(Hamadeh *et al.*, 2006)
3-15h per day	German black-head mutton sheep	38.7	39.0^{ns}	1.8	12.7	73.5	(Al-Ramamneh *et al.*, 2012)
3h per day	German black-head mutton sheep	38.6	38.7^{ns}	1.8	12.7	73.5	(Al-Ramamneh *et al.*, 2012)
6h on the second day	German black-head mutton sheep	38.3	38.8^{ns}	1.8	12.7	73.5	(Al-Ramamneh *et al.*, 2012)
3-15h per day	Boer goats	37.8	38.1^{ns}	4.3	8.9	71.0	Al-Ramamneh *et al.*, 2012)
3h per day	Boer goats	37.8	38.1^{ns}	4.3	8.9	71.0	Al-Ramamneh *et al.*, 2012)
6h on the second day	Boer goats	37.3	37.7^{ns}	4.3	8.9	71.0	Al-Ramamneh *et al.*, 2012)
50% of *ad lib*	Baladi goat	38.98	38.93^{ns}	1.5-2	NG	NG	(El Khashab *et al.*, 2018)

ns=not significant; s=significant; RH=relative humidity; RT=rectal temperature; NG= not given; ref=reference; temp=temperature

Table 2. 3 Effect of water restriction regimen on respiratory rate in small ruminants

		Average RR (breath/min)					
Water restriction	Specie	Water restriction	Control	Age (yrs)	Ambient Temp (°C)	RH (%)	Ref
3-15h per day	German black-head mutton sheep	35.9	36.7^{s}	1.8	12.7	73.5	Al-Ramamneh *et al.*, 2012)
3h per day	German black-head mutton sheep	34.1	36.1^{s}	1.8	12.7	73.5	Al-Ramamneh *et al.*, 2012)
6h on the second day	German black-head mutton sheep	32.0	25.1^{s}	1.8	12.7	73.5	Al-Ramamneh *et al.*, 2012)
3-15h per day	Boer Goats	20.5	20.1^{s}	4.3	8.9	71.0	(El-hadi, 1986)
3h per day	Boer Goats	19.1	18.4^{s}	4.3	8.9	71.0	Al-Ramamneh *et al.*, 2012)
6h on the second day	Boer Goats	18.7	17.6^{s}	4.3	8.9	71.0	Al-Ramamneh *et al.*, 2012)
50% of *ad lib*	Baladi goat	34.77	36.97^{s}	1.5-2	NG	NG	(El-hadi, 1986
80% and 60% *ad lib*	Lacauna ewes	22.60 and 20.20 respectively	26.20^{s}	Mature	NG	NG	(Casamassima *et al.*, 2016)
20% and 40% *ad lib* and alternate day	Malpura ewes	38.23, 40.40 and 37.03 respectively	46.23^{s}	2-4	32.37	36.67	(Kumar *et al.*, 2016)
20%, 40% *ad lib* and alternate day *ad lib*	Malpura ewes	59.43, 62.87 and 60.69	67.26^{ns}	2-4	39.86	26.03	(Kumar *et al.*, 2016)

ns=not significant; s=significant; NG=not given; RR= respiratory rate; Temp = temperature; RH = relative humidity; Ref = reference

However, RR could increase during periods of water shortages and high temperature. The outcome of the different experiments following intermittent watering on the respiratory rate is shown in Table 2.3.

2.5.2 Drinking behaviour, body weight and feed intake

Tolerance to water scarcity or dehydration can be judged based on body weight changes during water deprivation. Giving the close relationship between body water and body weight, water-tolerant animals are those who possess the ability to conserve water more (Ismail *et al.*, 1996). Small ruminants tend to recoup as much water as possible in the rumen during rehydration, an adaptation mechanism that allows them to endure severe dehydration. This high rumen volume usually exceeds the extracellular fluid volume causing a sudden drop in rumen osmolality and a huge osmotic gradient (200-300mOsm/kg) between the rumen and systemic fluid (Silanikove, 2000). Ethiopian Somali water-restricted goats drank 1.34 (watered every second day), 2.01 (watered every third day) and 2.51 (watered every fourth day) times as much as the water *ad lib* group (Mengistu *et al.*, 2007). During rehydration, Bedouin goats were also reported to consume large water volumes (Choshniak *et al.*, 1984). However, average daily water intake decreases when water deprivation exceeds 48 h. Rams watered at an interval of 24, 48, and 72 h had reduced water intake compared with those with free access to water (Abdelatif and Ahmed, 1994). When Tswana goats were water-deprived for 48 and 72 h, their free water intake dropped as compared to goats watered every 24 h (Adogla-Bessa and Aganga, 2000). Water deprivation usually results in reduced body weight and becomes more pronounced when the ambient temperature is high, especially during summer (Silanikove, 2000). Sheep watered only in the evening (20:00) had their body weight reduced by 7.00% (winter) and 11.00% (summer) (Li *et al.*, 2000). In a study conducted by Alamer and Al-hozab (2004) on the effect of water deprivation and season on body weight changes in Awassi and Najdi sheep, it was recorded that in spring, body weight decreased by 13.30% and 15.00% in Awassi and Nadji sheep, respectively, whereas 18.00% and 21.50% decreases were observed during summer. In a 3-days water deprivation study at elevated temperature using three local Saudi Arabia goat breeds (Hipsi, Aardi and Zimri), their body weights were reduced by 21.00% (Alamer, 2006), as opposed to Sudanese male goats, that under the same period of water deprivation showed 18.00% of body weight loss at a lower ambient temperature (El-hadi, 1986). Such higher body loss at elevated temperatures is attributed to water losses via respiratory and cutaneous routes. Loss of body weight connected with water shortages, can be attributed to feed and water intake reductions, coupled with body water loss (Parker *et al.*, 2003).

The need to compensate for a decrease in dietary intake, leading to the mobilization of fat (and possibly muscle) for energy metabolism (Jaber *et al.*, 2004), produces weight loss. However, such weight loss is quickly regained at the next watering point. A 16.30% loss in body weights of Dorper sheep was replenished in just a few minutes of water availability (Degen and Kam, 1992). Replenishment of all water losses by the black Bedouin goats was accomplished in a couple of rehydration minutes (Choshniak and Shkolnik, 1997). However, the speed with which body weight can be replenished is affected by breed, species, rumen capacity, and weight loss immensity. Marked responses to feed intake during water deficiency under various water restriction regimens were detected in Yankasa sheep and native goats (Ikhatua *et al.*, 1992; Abdelatif and Ahmed, 1994). In a water restriction experiments conducted at an ambient temperature of 22°C, Maloiy *et al.* (2008) reported an increasing and significant reduction in feed intake from Turkana goats (-58.30%) to fat-tailed sheep (-48.00%) and Zebu (-50.00%). Some studies, however, reported that feed intake is not significantly affected by water restriction (Mengistu *et al.*, 2004) because ruminants can store up water for use during scarcity or shortfalls. According to Hadjigeorgiou *et al.* (2000), dry matter voluntary intake in sheep water-restricted for one hour per day or given 65 % of the *ad libitum* intake of water was not affected. In contrast, others have observed a significant decline in feed intake following water deprivation. For example, when sheep was water-deprived for 3-days, their feed intake was significantly reduced (Alamer and Al-hozab, 2004), and Nubian goats reduced their feed intake by about 60.00% during the first day of water deprivation and consumed only 5.00% of the control intake by the third day of water deprivation (Abdelatif *et al.*, 2010). After 3 days of water deprivation, Egyptian Baladi goats maintained only about 35.00% of the control feed intake (Hassan, 1989), while Aardi goats deprived of water for 2-days almost stopped eating altogether (El Nouty *et al.*, 1990). However, a comparative study between camels and goats revealed a higher and significant decrease in roughage feed intake for camel (-54.60%) compared with goats (-27.80%) following a 72 h of water deprivation (Ayoub and Saleh, 1998). In a similar comparative study, Mousa *et al.* (1983) observed that water restriction in sheep, goats and camels for five days caused a decrease in dry matter intake in the three species, but the reduction was higher in camels than in the other two species. This further explains their preference for rearing sheep and goats over other animal types during periods of drought and water scarcity.

The type of feed offered to an animal during water restriction also affects feed intake. Goat fed legume hay had its feed intake reduced by 18.80% compared to a reduction of 21.20% when a low protein content diet (meadow hay) was fed (Muna and Ammar, 2001). Similarly, Osman and Fadlalla (1974) used eight adult water-restricted rams in five successive trials that were provided different feeds (*Medicago sativa* hay, *Doclichos lablab* hay, *Zea mais* hay, concentrate mixture and a mixture of dry desert grasses) and observed that animals fed the desert grass mixture had significantly lower dry matter intake (34.17%) than those provided Lucerne hays (8.00%). It has also been shown that Bedouin goats consumed more lucerne hay than wheat straw during dehydration (Silanikove, 1994).

Drinking is shown to be positively correlated with feed intake in ruminants, and therefore, the adaptive mechanism during the period of water shortage, which leads to reduced dry matter intake would help to reduce further the water loss linked with feed metabolism and heat dissipation (Thang *et al.*, 2012). Small ruminants seem to be more resilient to dehydration than cattle. In fact, sheep acclimatized to arid tropical weather conditions may be expected to survive for 6-10 days without drinking water, while cattle under range management in desert areas died following 3-5 days without water (Macfarlane and Howard, 1972). Compared with sheep, cattle had a shorter survival time during water deprivation, which was linked to a greater water loss rate than sheep. In addition, the ability to effectively concentrate urine by sheep and goats makes them superior to cattle in terms of adaptability to water-limited areas and arid environments (Chedid *et al.*, 2014). Reduction in feed intake during water restriction could be induced by decreased ruminal fluid's postprandial hyperosmolality (Langharns *et al.*, 1991). The decrease in the volume of the circulating blood (hypovolemia) and high blood solute concentration (hyperosmolality) may occur after feed intake in the animal because of the secretion of saliva and gastric juices, a mechanism forcing them to drink while eating, or on the other hand reduce feed intake during water restriction (Jaber *et al.*, 2013).

2.5.3 Effect on nitrogen balance and digestibility

Water restriction improves the digestibility of nutrients by increasing digesta retention time to allow more time for degradation by microbes and microbial synthesis (Osman and Fadlalla, 1974; Casamassima *et al.*, 2008). Corriedale ewes that were water restricted for 2 and 3h after feeding had greater digestibility than those with access to water *ad libitum* after feeding

(Nejad, 2014). Although both rumination and digestion require water, the rumen's digestive capacity is enhanced during water restriction, resulting in improved feed digestibility. Positive nitrogen retention in water-restricted sheep increased crude protein digestibility compared with the negative nitrogen retention in sheep with access to *ad libitum* water after feeding (Nejad, 2014). A decrease in urine output and nitrogen losses is associated with water deprivation or restriction, and this could probably be the result of reduced filtration in the kidney glomeruli produced by water deprivation (Wittemberg *et al.*, 1986; Nejad, 2014).

2.5.4 Effect on blood metabolites

Kumar *et al.* (2016) restricted the water of Malpura ewes at alternate day, 20% and 40% of *ad libitum* water and reported that the 40% treatment group had higher haemoglobin (Hb) and Packed cell volume (PCV), with the 'alternate day' group being significantly lower. A similar result of high haemoglobin concentration was reported in sheep that were water restricted for four days (Li *et al.*, 2000). Increased Hb and PCV values following water restriction have been attributed to severe haemoconcentration due to reduced water intake. However, local goats were less affected, as shown in three days comparative study with water deprivation, as reflected in their low PCV (19.33%) and Hb (7.43%) compared to 34.00% and 15.97% in camel's PCV and Hb, respectively (Ayoub and Saleh, 1998). Due to low feed intake following water restriction, plasma glucose in sheep dropped (li *et al.*, 2000) below the normal range of 48-75mg/dL (Kramer, 2000). Plasma glucose concentration in response to water restriction in goats remains unchanged, as reported by some authors (Alamer, 2006). However, other studies reported a decline of 13.00% in plasma glucose level in Sudanese desert sheep watered every 72 h (Abdelatif and Ahmed, 1994). Insufficient water intake increases creatinine, cholesterol, total protein and plasma glucose concentrations in Yankasa and Awassi sheep (Igbokwe, 1993; Jaber *et al.*, 2004). There were changes in cholesterol, creatinine, total protein, albumin, urea, sodium, chloride and triglycerides, in Comisana ewes restricted of water at 60% and 80% of *ad libitum* water intake for 40 days (Casamassima *et al.*, 2008) as a result of haemoconcentration phenomena caused by lower blood water level. The increase in the concentration of blood metabolites (cholesterol, urea, creatinine, total proteins and electrolytes) was also confirmed in several studies in different sheep (Jaber *et al.*, 2004; Nejad, 2014) and goat (Alamer, 2006) breeds subjected to water restriction. High blood urea concentration following water restriction resulted from the kidney recycling much water and reduced blood flow towards the urinary apparatus (Casamassima *et al.*, 2016), while elevated creatinine was attributed to changes in the clearance rate of endogenous creatinine (Baxmann *et al.*, 2008). An increase in sodium concentration in water restricted animal is due to a greater aldosterone activity that increases the kidney's electrolyte level and gradually declines (Ashour and Benlemilh, 2000). Chloride ion concentration follows the same pattern as Na^+ since Cl^- is distributed passively according to electrical gradients established by the active transport of Na^+ (Tasker, 1971). Studies are divided on concentrations of Ca^{++} and K^+ under water restriction. Some reported increasing levels (Casamassima *et al.*, 2008, 2016), while others said no difference in blood K^+ following water deprivation (Igbokwe, 1993; Hamadeh *et al.*, 2006). Plasma potassium concentration in water restricted Awassi sheep (watered once every four days) was reported to decrease (Jaber *et al.*, 2004). However, pregnant Yankassa sheep watered once every 48 or 72 h had increased potassium concentration, but the concentration remains unchanged in non-pregnant Yankasa sheep (Igbokwe, 1993). Plasma osmolality generally increases with an increase in plasma sodium concentration following water deprivation and drops to low values the day after drinking. This is because sodium and its associated ions determine osmolality and extracellular fluid volume (Mengistu et al., 2007) majorly.

2.5.5 Effect on physiological status

2.5.5.1 Effect on reproductive traits and hormones

Adequate supply and availability of sufficient water resources are required both for survival and reproduction. Hence, any imbalance or shortfall of water resources below the optimal level can compromise an animal's health and general vigour (Moeller *et al.*, 2013). Dehydration at elevated ambient temperature decreases plasma volume because of the uptake of water by the tissue, and an attempt by the animal to maintain fluid balance usually results in an increased secretion of aldosterone and cortisol (Schneider, 1990). Usually, the endocrine glands are stimulated following water restriction with the sole purpose of modifying metabolic activities depending on the ambient temperature (Afsal *et al.*, 2008). Alteration in follicular growth and reduced oestrus cycle have been linked to negative energy balance and reduced feed intake resulting from water deprivation (Naqvi *et al.*, 2011). Oestrus duration in Kivircik sheep in the semi-arid region was reduced when feed intake drops (30.00% of *ad libitum* intake) (Koyuncu and Canbolat, 2009). However, when Malpura ewes treated with progesterone impregnated with intravaginal sponges for estrus synchronization were water restricted (20%, 40% less *ad lib*itum water and alternate day), the oestrus per cent and oestrus duration were not affected (Kumar *et al.*, 2016). Nutritional insufficiency in small ruminants is usually implicated during water scarcity periods, evidently by their reduced feed intake and body weight. The effect is a decreased estrus response and estrus duration attributed to the delayed ovarian follicular maturation and impaired reproductive endocrinology (Liker *et al.*, 2010; Sejian *et al.*, 2011). Ovarian follicular development diminished during periods of stress because of repressed peripheral gonadotropins levels (Roth *et al.*, 2000). This became very noticeable in water restricted groups of Malpura ewes (Kumar *et al.*, 2016) and undernourished ewes (Kiyma *et al.*, 2004), having decreased plasma estradiol levels. Generally, and during stress, productive functions such as growth and reproduction are suppressed by endocrine hormones favouring survival and maintenance (Narayan and Parisella, 2017). During periods of water scarcity or deprivation, hormones are mobilized, giving their critical roles to ensure that energy needs are satisfied, and water losses minimized. Due to a decrease in feed intake in sheep, fat mobilization is conjoined and modulated by reducing insulin levels (Deck *et al.*, 2017). Likewise, leptin levels are decreased to guard against excessive mobilization in underfed ruminants, which, if not curtailed, could result in a high build-up of harmful circulating fatty acids (Chilliard *et al.*, 2000). This is because severe dehydration leads to reduced tissue perfusion and can predispose animals to increased lactic acid production and the development of lactic acid acidosis (Kraut and Madias, 2014). However, feed intake and plasma progesterone level are inversely related, and this might be due to differences in progesterone's metabolic clearance rate rather than differences in secretion levels (Lozano *et al.*, 1998). When ewes were water restricted, their plasma progesterone levels reportedly increase, a condition linked to reduced feed intake (Kumar *et al.*, 2016). Plasma vasopressin (anti-diuretic hormone) concentration in water restricted animals usually increases above the maximum concentration and decrease following rehydration (Mengistu *et al.*, 2007). Kaliber *et al.* (2015) water restricted (56, 73 and 87% of *ad lib*) 20 cross-bred and 3-year-old female goats and reported an increasing vasopressin trend with increasing restriction. Such higher vasopressin levels during reduced body fluids balance help maintain body water alongside extracellular fluid concentrations of sodium ion (Roumelioti *et al.*, 2018).

2.5.5.2 Lactation

When lactating sheep and goat are water-deprived for 72h, milk production is affected in 50% of the sheep, while that of goat remain unaffected, leading to an increase in the viscidity of milk as well as lactose, fat, protein, fat and mineral salts (Aganga, 1992). On the contrary, Casamassima *et al.* (2008) water restricted Comisana ewes (100%, 80% and 60% of *ad libitum* water intake) for 40 days and reported that the quantity of milk was not affected. The author observed that the low water requirement precipitated by the low ambient temperature (6.1^{o}C) was the cause of the loss in milk production generally. At low temperature (5^{o}C), blood flow towards the udder decreases, leading to a decrease in prolactin secretion and milk production (Alamer, 2011). The black Bedouin goat is a desert-adapted breed and has developed a high resistance to water scarcity, as evidenced by its ability to sustain milk production for 2-d without water (Choshniak and Shkolnik, 1997). When water-deprived for four days followed by two days of rehydration, total milk yield and milk solids were about 70.00% of normal yields, and the progeny's normal growth was not compromised (Hossaini-Hilali *et al.*, 1994). In the water restriction study of Alamer (2009), the rate of drop in milk yield with 25% restriction was slightly higher than that with 50% restriction (20.00% vs 18.00%). This indicates that water was effectively used in the group with 50% water restriction. The author attributed the drop in daily hay intake as the cause for the reported decrease in milk solid and fat in goats with a 25% restriction (compared to a 50% restriction). A decline in feed intake following water deprivation/restriction is partly responsible for a decline in milk yield. During stressful conditions (e.g. water deprivation), milk production was proposed to be downregulated due to a reduction in mammary blood flow following a sustained period of dehydration (Alamer, 2011). Increased plasmin activity (milk synthesis inhibitor) during water restriction is correlated with the fall in milk production. This modification in milk production enhances the survival potential in response to intense heat or water stress, and the drop in yield is recovered following rehydration at the next watering point (Silanikove, 2000). The osmo-active milk component (urea, sodium, and chloride) also rises with water deprivation intensity (Jaber *et al.*, 2013). During a sustained period of water deprivation, lactating animals readjust to minimize losses in weight. In a 6-d water deprivation study (50 and 25% water intake), live weight losses of lactating Aardi goats were stabilized not until after 4-d of water deprivation and thereafter (Alamer, 2009). This adjustment to sustained water restriction has been linked to the activation of and continuous increase in vasopressin (a water-saving mechanism), which reduces renal water secretion following a prolonged period of water dehydration in lactating goats (Mengistu *et al.*, 2007). When lactating Moroccan goats were water-deprived for 2-d, feed consumption dropped by 10.00% (Hossaini-Hilali *et al.*, 1994). However, feed consumption in Black Bedouin goats was maintained unchanged for 48 h during complete water deprivation (Maltz and Shklonik, 1984). The feed intake of lactating sheep receiving 60 % of their normal water intake was also not affected during lower ambient temperature periods (Casamassima *et al.*, 2016). This ability to maintain feed intake during the period of water shortages during lactation has been reported to be an adaptive mechanism to sustain an adequate supply of milk to nourish the newly born animals (Alamer 2009).

Unlike the usual haemoconcentration of blood metabolites in dry animals following water deprivation or restriction (Li *et al.*, 2000; Kumar *et al.*, 2016), lactating ewes had significantly reduced PCV and Hb concentration (Hamadeh *et al.*, 2006). Similarly, haemoglobin formation was reduced in Barki ewes lactating for four weeks (El-Sherif and Assad, 2001). This is not surprising since the higher body water content and plasma volumes of lactating ewes due to increased water mobilization to the mammary glands could be responsible for the haemodilution (Abdelrahman and Aljumaah, 2012). A similar result was obtained for total protein concentration, globulin, creatinine, and urea in lactating ewes by others (El-Sherif and Assad, 2001; Hamadeh *et al.*, 2006).

2.5.6 Immune response and drug pharmacokinetics

When an animal is deprived access to sufficient drinking water with respect to time, various biological functions are affected. Water imbalance (restriction/deprivation) imposes stressful conditions on animal and negatively impact on their productivity, blood metabolites (Hamadeh *et al.*, 2006) including perturbations in behaviour and physiology (Dupoue *et al.*, 2014). Despite the negative correlations between stress and immunity, a detailed analysis of the inter-relationship of deprivation, immune function, and stress is yet to be conducted within a single species. Water imbalance is a physiological stressor capable of eliciting various endocrine responses (Maresh *et al.*, 2004). During dehydration, plasma glucocorticoids level, the principal hormone involved in stress response, is raised (Busch and Hayward, 2009). Such elevations may initially be beneficial on immunity, but if prolonged, they have more depressing effects, although the response may also depend on stress hormone concentration. This is due to its depressive effect on the synthesis or release of immune-promoting molecules and its potential to stimulate or depress B- and T-cells' proliferation, depending on physiological conditions (Sapolsky and Munck, 2000). However, a glucocorticoid response to water deprivation and dehydration is lacking in Awassi sheep (Jaber *et al.*, 2004; Hamadeh *et al.*, 2006). Similar stressors, either environmental or temperature-related, have the potential to alter immune parameters. Immunoglobulins (e.g. IgG), white blood cells, including the differential counts, are often used as indices of immune status and animals' stress levels. A high ratio of heterophils or neutrophils to lymphocytes in blood samples indicates high-stress levels (Davis *et al.*, 2008). However, plasma immunoglobulin G (IgG) levels and white blood cells of Corriedale ewes deprived of water 2 and 3h after feeding were not altered. The humoral antibody response of Awassi ewes to *Salmonella enteritidis* following water restriction was found to be significantly lower and decreased by 38.50% than the control watered *ad libitum* (Nejad, 2014).

Administrations of veterinary drugs or antimicrobials (e.g. gentamicin) to treat several gram-negative and gram-positive bacteria infections are common in animal production. Water deprivation of small ruminants found in water-limited areas could result in dehydration, or conditions can also occur when animals are sick and are off feed and water, thereby requiring treatment. However, changes in water compartments, kidney, and liver functions (Roumelioti *et al.*, 2018) following water deprivation and restriction can predispose animals to modifications in the disposition kinetics or alter the processes determining the fate of drugs in the animal. For example, gentamicin, a broad-spectrum aminoglycoside veterinary antibiotic, accumulates in renal proximal tubular cells following administration, and its uptake is concentration-dependent (Ramirez and Tolmasky, 2010). In water-deprived animals, there is a possibility that uptake is enhanced by increasing the time/concentration of the drug in the plasma, thus leading to proximal tubular necrosis or nephrotoxicity (Sastrasinh *et al.*, 1982). Six water-deprived young male Nubian goats (12-14months) and weighing 10-12kg were used to study the pharmacokinetics of ampicillin trihydrate administered intravenously (Elsheikh and Intisar, 1998). Intravenous administration (10mg/kg body weight) in the goats was studied in four stages and conditions; when (i) watered *ad lib* (ii) body weight loss averaged 7.50% following 2-days water deprivation (iii) body weight loss averaged 9.80% following 3-days water deprivation and (iv) body weight loss averaged 12.60% following 4-days water deprivation. Each of these stages was interspersed by washout periods of free access to feed and drinking water for 2, 3 and 4 weeks in stages ii, iii and iv, respectively. At 7.50 % body weight loss, the pharmacokinetics of the drugs (i.e. elimination half-life, volume disappearance of steady-state) had a minimal effect when compared to the period of hydration. However, the drug's total body clearance was slower and progressed significantly as the body weight loss increases. At 12.60 % body weight loss, the central compartment (Vc) volume and volume of distribution at steady state (Vdss) significantly dropped. However, the author reported that the ‘elimination half-life time’ of the drug ($t_{1/2\beta}$) across the different water deprivation periods was not significant. This describes the water-deprived goats' ability to effectively cope by ensuring that the drugs progressively ‘decay’ and are eliminated during water deprivation. In another study in which gentamicin was administered intravenously (3mg/kg body weight) to water-deprived Nubian goats (Elsheikh *et al.*, 1999) and with watering regime and body weight loss during injections similar to the study of (Elsheikh and Intisar, 1998), the half-life of distribution ($t_{1/2\alpha}$) and elimination ($t_{1/2\beta}$) was not significantly affected at 7.20, 10.40 and 12.80% body weight loss. The mean plasma concentrations significantly increase as body weight continues to drop. However, total body clearance (Cl_{total}) and volume of distribution of steady state (Vdss) significantly decrease as body weight loss increases. Oukessou and Toutain (1992) reported limited effects on the distribution and elimination kinetics of ampicillin following a period of 72 h water deprivation. These small ruminants' abilities might account for adaptive clearance mechanisms in animals, though to a certain dehydration level. During dehydration, body water loss is mainly from the extracellular fluid, especially the blood (Hooper *et al.*, 2016). Therefore, it is expected that water compartments changes could influence the elimination of drugs, especially those with a small volume of distribution. In addition, haemoconcentration leading to a reduction in the blood flow to the kidney in dehydrated animals and the reduction of renal filtration could reduce the rate of body clearance and elimination of drugs. Impairment of renal function leading to reduced renal filtration and renal plasma flow has been confirmed in dehydrated sheep (Singh *et al.*, 2017). There is a need for more studies on adaptable breeds both in sheep and goats on the pharmacokinetics of drugs at different levels of water restriction and ambient temperature.

2.6 Modulating genes in small ruminants found in dry areas and future research gap

Drinking behaviour, maintenance of fluid, electrolyte, and homeostasis in dehydrated animal are partly controlled by several cascades of activities and hormonal interplay with known water balance effects. Among them are the water-retaining hormone systems, including the renin-angiotensin system, aldosterone, and anti-diuretic hormone. Blocking of angiotensin II AT1 receptors with losartan during a 20 days' water deprivation study with camels significantly enhanced the effect of dehydration on body weight reduction and increased serum metabolites (Ali *et al.*, 2012). This substantiates the role of the renin-angiotensin system during deprivation. Adaptation of animals to dry and water-limited areas is mediated by a complex network of genes (Mwacharo *et al.*, 2016). Selection for these genes using appropriate genomic tools could be the way to select livestock to be adapted to water stress and still reach an adequate level of production to support agricultural industries, especially in arid and water-limited areas. Unfortunately, selection using conventional breeding strategies is ineffective as adaptation traits are usually of low heritability ($h^2 \leq 0.25$), difficult and expensive to measure accurately. In the study of Elbeltagy *et al.* (2016), several genetic approaches, including signature of selection (SS) analyses (identifying long conserved stretches of chromosomal regions associated with stress tolerance traits), genome-wide association (GWAS) analysis (detect genomic regions associated with stress affected physiological parameters) and 50k Illumina SNP Beadchips were deployed to identify genes involved in desert stress tolerance and also investigate and compare tolerance to grazing stress [GS] (poor pasture and limited water intake) in the Egyptian desert and non-desert sheep and goats. According to the authors, the genes in goat's chromosome 7 were identified to influence respiration rate as well as heat generation, detection of temperature stimulus and homesotasis. In sheep, however, genes present on chromosome 25 regulate cellular amide metabolic process, playing a role in single organism cell adhesion and small nuclear RNA, as well as changes in rectal temperature. Yang *et al.* (2016) sequence the whole-genome of native sheep and identified four pathways and the associated genes responsible for the successful adaptation of Taklimakan desert sheep; arachidonic acid metabolism pathway [ANXA6] (Annexin VI), GPX3 (Glutathione Peroxidase 3), GPX7 (Glutathione Peroxidase 7), and PTGS2 (Prostaglandin-endoperoxide synthase)), renin-angiotensin system pathway [CPA3] (Carboxypeptidase A3), CPVL (Carboxypeptidase Vitellogenic Like), and ECE1 (Endothelial Converting Enzyme 1)), oxytocin signalling pathway (CALM2 (Calmodulin 2), CACNA2D1 (Calcium Voltage-Gated Channel Auxiliary Subunit Alpha2delta1), KCNJ5 (Potassium voltage-gated channel subfamily J member 5), and COX2 (cyclooxygenase2)) and pancreatic secretion pathway (RAP1A (member of RAS Oncogene Family), SLC4A4 (solute carrier family 4 member 4)). The three systems regulate water retention and re-absorption in renal cells and blood vessels in the kidney, while the last one is responsible for protein and carbohydrate digestion and absorption functions. Other desert-adaptation functions mediated by the pathway genes include renal vasodilation, salt-water metabolism, ion trans-membrane transport and bicarbonate absorption.

2.7 Conclusions

As a result of the continuous shortfall in rainfall patterns arising from climate change, small ruminants have gained attention from scientists and communal farmers because of their ability to tolerate intermittent watering during periods of water scarcity without seriously compromising production indices. There exist, however, differences in the level of adaptation to intermittent watering across different breeds of sheep and goats. Isolation of such candidate genes using appropriate genetic tools or approaches, especially among adaptable breeds that have undergone natural selection for breeding and selection purposes, is a key research gap. Given the high number of sheep and goat breeds globally, studies investigating the potential of adaptable breed to low water intake are still few. There is a need to fully explore water tolerance capacity in adaptable breeds in the form of water restriction or deprivation and across all physiological stages.

2.8 References

Abdelatif, A.M. and Ahmed, M.M.M. (1994). Water restriction, thermoregulation, blood constituents and endocrine responses in Sudanese desert sheep. *J. Arid Environ.* **26**, 171–180.

Abdelatif, A.M., Elsayed, S.A. and Hassan, Y.M. (2010). Effect of state of hydration on body weight, blood constituents and urine excretion in Nubian Goats (*Capra hircus*). *World J. Agric. Sci.* **6** (2), 178-188.

Abdelrahman, M.M. and Aljumaah, R.S. (2012). Metabolic profiles and milk composition of periparturient and early lactation periods in sheep. *Asian J. Anim. Vet. Adv.* **7**(11), 1130-1138.

Adogla-Bessa, T. and Aganga, A.A. (2000). Responses of Tswana goats to various lengths of water deprivation. *S. Afr. J. Anim. Sci*. **30**, 87–91.

Afsal, A., Sejian, V., Bagath, M., Krishnan, G., Devaraj, C. and Bhatta, R. (2008). Heat Stress and Livestock Adaptation: Neuro-endocrine Regulation. *Int. J. Vet. Anim. Med*. **1(**2), 108.

Aganga, A.A. (1992). Water utilization by sheep and goats in Northern Nigeria. World Anim. Rev. FAO. **73**, 9-14.

Alamer, M. (2011). The role of prolactin in thermoregulation and water balance during heat stress in domestic ruminants. *Asian J. Anim. Vet. Adv.* **6**(12), 1153-1169.

Alamer, M. (2010). Effect of water restriction on thermoregulation and some biochemical constituents in lactating Aardi goats during hot weather conditions. *J. Basic Appl. Sci. Res.* **11**, 189–205.

Alamer, M. (2009). Effect of water restriction on lactation performance of Aardi goats under heat stress conditions. *Small Rumin. Res.* **84**, 76-81.

Alamer, M. (2006). Physiological responses of Saudi Arabia indigenous goats to water deprivation. *Small Rumin. Res*. **63**,100-109.

Alamer, M. and Al-hozab, A. (2004). Effect of water deprivation and season on feed intake, body weight and thermoregulation in Awassi and Nadji sheep breeds in Saudi Arabia. *J. Arid Environ.* **59**, 71-84.

Ali, M.A., Adem, A., Chandranath, I.S., Benedict, S., Pathan, J.Y., Nagelkerke, N. *et al.* (2012). Response to dehydration in the one-humped camel and effects of blocking the renin-angiotensinn system. *PLoS ONE* **7**(5), e37299.

Al-Ramamneh, D., Riek, A. and Gerken, M. (2012). Effect of water restriction on drinking behaviour and water intake in German black-head mutton sheep and Boer goats. *Animal* **6**, 173–178.

Araujo, G.G.L., Voltolini, T.V., Chizzotti, M.L., Turco, S.H.N. and de Carvalho, F.F.R. (2010). Water and small ruminant production*. R. Bras. Zootec*, **39**, 326-336.

Arnell, N.W. (1999). Climate change and global water resources. *Global Environ. Chang*. **9**, 31–49.

Ashour, G. and Benlemilh, S. (2000). Adaptation of Mediterranean breeds to heat stress and water deprivation. In: Guessous, F., Rihani, N., Ilham, A. (Eds.), Livestock Production and Climatic Uncertainty in the Mediterranean, Proceedings of the Joint ANPA-EAAPCIHEAM-FAO Symposium, EAAP Publication No. 94. Agadir, Morocco, pp. 63–74.

Ayoub, M.A. and Saleh, A.A.A. (1998). Comparative physiological study between camels and goats during water deprivation. *Proceedings of the Third Annual Meeting for Animal Production under Arid Conditions* **1**, 71-87.

Baxmann, A.C., Ahmed, M.S., Marques, N.C., Menon, V.B., Pereira, A.B., Kirsztajn, G.M. and Heilberg, I.P. (2008). Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. *Clin. J. Am. Soc. Nephro.* **3(2**), 348-355.

Beede, D.K. (2012). What will our ruminants drink? *Anim. Front***. 2(2),** 36-43.

Ben Salem, H. and Smith, T. (2008). Feeding strategies to increase small ruminant production in dry environment. *Small Rumin. Res.* **77**(2), 174-194.

Busch, D.S. and Hayward, L.S. (2009). Stress in a conservation context: a discussion of glucocorticoid actions and how levels change with conservation-relevant variables. *Biol. Conserv.* **142**, 2844-2853.

Casamassima, D., Pizzo, R., Palazzo, M., Alessandro, A.G.D. and Martemucci, G. (2008). Effect of water restriction on productive performance and blood parameters in Comisana sheep reared under intensive condition. *Small Rumin. Res.* **78,** 169–175.

Casamassima, D., Vizzarri, F., Nardola, M. and Palazzo, M. (2016). Effect of water restriction on various physiological variables on intensively reared Lacauna ewes. *Vet. Med.* **61**(11), 623-634.

Chedid, M., Jaber, L.S., Giger-Reverdin, S., Duvaux-Ponter, C. and Hamadeh, S.K. (2014). Review: water stress in sheep raised under arid conditions. *Can. J. Anim. Sci*. **94**, 243–257.

Chilliard, Y., Ferlay, A., Faulconnier, Y., Bonnet, M., Rouel, J. and Bocquier, F. (2000). Adipose tissue metabolism and its role in adaptations to under-nutrition in ruminants. *Proc. Nutr. Soc*. **59**, 127-134.

Choshniak, A. and Shkolnik A. (1997). Rapid rehydration in the black Bedouin goat and role of rumen red blood cells fragility. *Comp. Biochem. Physiol.* **56A**, 581-583.

Choshniak, I., Ben-Kohav, N., Taylor, C.R., Robertshaw, D., Barnes, R.J., Dobson, A., Belkin, V. and Shkolnik, A. (1995). Metabolic adaptations for desert survival in the Bedouin goat. *Am. J. Physiol*. **268**(5), 1101–1110.

Choshniak, I., Wittenberg, C., Rosenfeld, J. and Shkolnik. A. (1984). Rapid rehydration and kidney function in the black Bedouin goat. *Physiol. Zool.* **57**, 573-579.

Damerau, K., van Vliet, O.P.R. and Patt, A.G. (2015). Direct impacts of alternative energy scenarios on water demand in the Middle East and North Africa. *Clim. Change* **130,** 171–83.

Das, S.K., Kumar, P., Singh, D. and Singh, K. (1994). Effect of water restriction on thermoregulatory responses in bucks. *Indian J. Anim. Sci*. **64**, 899–901.

Davis, A.K., Maney, D.L. and Maerz, J.C. (2008). The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. *Rev. Funct. Ecol*. **22**, 760-772.

Davis, J.R. and DeNardo, D.F. (2007). The urinary bladder as a physiological reservoir that moderates dehydration in a large desert lizard, the Gila monster Helodermasuspectum. *J. Exp. Biol.* **210**, 1472-1480.

Deck, C.A., Honeycutt, J.L., Cheung, E., Reynolds, H.M. and Borski, R.J. (2017). Assessment of the functional role of leptin in energy homeostasis and the stress response in vertebrates. *Front. Endocrinol*. (Lausanne). **8**, 63.

Degen, A.A. and Kam, M. (1992). Body mass loss and body fluid shifts during dehydration in Dorper sheep. *J. Agric. Sci.* (Camb.) **119**, 419-422.

Doreau, M., Corson, M.S. and Wiedemann, S.G. (2012). Water use by livestock: A global perspective for a regional issue. *Anim. Front.* **2**, 9-16.

Dupoué, A., Angelier, F., Lourdais, O., Bonnet, X. and Brischoux, F. (2014). Effect of water deprivation on baseline and stress-induced corticosterone levels in the Children's python (Antaresiachildreni). *Comp. Biochem. Physiol. - A Mol. Integr. Physiol*. **168**, 11-16.

El Khashab, M.A., Semaida, A.I. and Abd El-Ghany, M.A. (2018). Water restriction and its effect on blood hormones, minerals and metabolite in Baladii goats. *Int. J. Curr. Microbiol. App. Sci.* **7**(3), 747-755.

Elbeltagy, A.R., Kim, E., Rischkowsky, B., Aboul-naga, A.M., Mwacharo, J.M. and Rothschild, M.F. (2016). "Genome-wide Analysis of Small Ruminant Tolerance to Grazing Stress Under Arid Desert," Animal Industry Report: AS 662, ASL R3106.

El-Hadi, H.M. (1986). The effect of dehydration on Sudanese desert sheep and goats. *J. Agric. Sci.* **106**, 17- 20.

El-Nouty, F.D., Al-Haiderary, A.A. and Basmaeil, S.M. (1990). Physiological responses, feed intake, urine volume and serum osmolality of Aardi goats deprived of water during spring and summer. *Aust. J. Anim. Sci*. **3**, 331–336.

Elsheikh, H.A. and Intisar O.M. (1998). Pharmacokinetics of ampicillin trihydrate following intravenous administratio no water-deprivedd Nubian goats. *Res. Vet. Sci.* **65**, 111-114.

Elsheikh, H.A., Intisar O.M. and Abdullah, A. (1999). Pharmacokinetics of gentamicin administered intravenously to water-deprived goats. *Small Rumin. Res.* **34**, 15-20.

El-Sherif, M.M.A., and Assad, F. (2001). Changes in some blood constituents in Barki ewes during pregnancy and lactation under semi-arid conditions. *Small Rumin. Res.* **40**, 269-277.

Esminger, M.E., Oldfield, J.L. and Heinemann, J.J. (1990). Feeds and nutrition 2nd ed. Clovis: Esminger Publishing, 1552p.

FAO, (Food and Agriculture Organization of the United Nations). (2016). *Statistical Yearbook* (Vol. 1). Rome, Italy: Food and Agriculture Organization of the United Nations.

FAOSTAT. (2013). Food and Agriculture Organization of the United Nations, http://faostat3fao.org/home/index.html. (accessed 23rd February, 2019).

Foresight. (2011). The Future of Food and Farming. Final Project Report. The Government Office for Science, London, UK <http://www.bis.gov.uk/assets/foresight/docs/food-and-farming/11-546-future-of-food-and-farmingreport.Pdf.</doc.ref>.**2011** (accessed 20th, February 2019).

Fujimori, S., Hanasaki, N. and Masui, T. (2017). Projections of industrial water withdrawal under shared socioeconomic pathways and climate mitigation scenarios *Sustain. Sci*. **12,** 275–92.

Fuller. A., Meyer, L.C.R., Mitchell, D. and Maloney, S.K. (2007). Dehydration increases the magnitude of selective brain cooling independently of core temperature in sheep. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **293**, R438–R446.

Gosain, A.K., Sandhya, R. and Debajit, B. (2006). Climate change impact assessment on the hydrology of Indian river basins. *Curr. Sci.* **90**(3), 346–353.

Hadjigeorgiou, I., Dardamani, K., Goulas, C. and Zervas, G. (2000). Effect of water availability on feed intake and digestibility in sheep. *Small Rumin. Res.* **37**, 147-150.

Hamadeh, S.K., Rawda, N., Jaber, L.S., Habre, A., Abi Said, M. and Barbour, E.K. (2006). Physiological responses to water restriction in dry and lactating Awassi ewes. *Livest. Sci*. **101,** 101-109.

Hassan, G.E. (1989). Physiological responses of Anglo-Nubian and Baladi goats and their crossbreeds to water deprivation under subtropical conditions. *Livest. Prod. Sci*. **22**, 295–304.

Hooper, L., Bunn, D.K., Abdelhamid, A. *et al.* (2016). Water-loss (intracellular) dehydration assessed using urinary tests: how well do they work? Diagnostic accuracy in older people. *Am. J. Clin. Nutr.* **104** (1), 121-131.

Hossaini-Hilali, J., Benlamlih, S. and Dahlborn, K. (1994). Effects of dehydration, rehydration, and hyperhydration in the lactating and non-lactating black Moroccan goat. *Comp. Biochem. Physiol.* **109**(A), 1017-1026.

Igbokwe, I.O. (1993). Haemoconcentration in Yankasa sheep exposed to prolonged water deprivation. *Small Rumin. Res*. **12**, 99-105.

Ikhatua, U.J., Dede, T.I. and Apumami, A.S. (1992). Water and feed utilisation relationship in West African Dwarf (forest) goats of Southern Nigeria. *Nig. J. Anim. Prod.* **19,** 120–124.

Iniguez, L. (2011). The challenges of research and development of small ruminant production in dry areas. *Small Rumin. Res*. **98**, 12-20.

IPCC (Intergovernmental Panel on Climate Change), (2007). Synthesis Report. In: Pachauri, R.K.; Reisinger, A.(eds.), Contribution of Working Groups I, II and II to the Fourth assessment report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland, p. 104.

Ismail, E., Hassan, G.A., Abo-Elezz, Z. and Abdellatiff, H. (1996). Physiological responses of Barki and Suffolk sheep and their crossbreeds to dehydration. *Egypt. J. Anim. Prod.* **33**, 89–101.

Jaber, L., Chedid, M. and Hamadeh, S. (2013). Water stress in small ruminants. In: Akinci, S. (Ed.), Responses of Organisms to Water Stress. InTech, JanezaTrdine 9, 51000 Rijeka, Croatia, pp. 115-149.

Jaber, L.S., Habre, A., Rawda, N., AbiSaid, M., Barbour, E.K. and Hamadeh, S.K. (2004). The effect of water restriction on certain physiological parameters in Awassi sheep. *Small Rumin. Res.* **54**, 115-120.

Jessen, C., Dmi'el, R., Choshniak, I., Ezra, D. and Kuhnen, G. (1998). Effects of dehydration and rehydration on body temperatures in the black Bedouin goat. *Pflüg. Arch. Eur. J. Phy.* **436**, 659–666.

Kaliber, M., Koluman, N. and Silanikove, N. (2015). Physiological andbehaviourall basis for the successful adaptation of goats to severe water restriction under hot environmental conditions. *Animal* **10**, 82-88.

Kerr, S.R. (2015). Livestock heat stress: recognition, response, and prevention. Washington State University. Extension Fact Sheet FS157E, Washington, US Pullman.

Kiyma, Z., Alexander, B.M., Van Kirk, E.A., Murdoch, W.J., Hallford, D.M. and Moss, G.E. (2004). Effect of feed restriction on reproductive and metabolic hormones in ewes. *J. Anim. Sci*. **82**, 2548-2557.

Koyuncu, M. and Canbolat, O. (2009). Effect of different dietary energy levels on the reproductive performance of Kivircik sheep under a semi-intensive system in the South-Marmara region of Turkey. *J. Anim. Feed Sci*. **18,** 620-627.

Kramer, J.W. (2000). Normal haematology of cattle, sheep and goats. In: Feldman, B.F., Zinkl, J.G., Jain, N.C. (Eds.), Schalm's Veterinary Haematology, 5th edn. Lippincott Williams and Wilkins, Philadelphia, pp. 1075-1084.

Kraut, J.A and Madias, N.E. (2014). Lactic acidosis. *N. Engl. J. Med.* **371**, 2309–2319.

Kumar, D., De, K., Singh, A.K., Kumar, K., Sahoo, A. and Naqvi, S.M.K. (2016). Effect of water restriction on physiological responses and certain reproductive traits of Malpura ewes in a semiarid tropical environment. *J. Vet. Behav.* **12**:54-59.

Kundzewicz, Z.W. and Döll, P. (2009). Will groundwater ease freshwater stress under climate change? *Hydrol. Sci. J. –J. Des. Sci. Hydrol*. **54**, 665–675.

Langhans, W., Scharre, E. and Meyer, A.H. (1991). Changes in feeding behaviour and plasma vasopressin concentration during water deprivation in goats. *J. Vet. Med.* **38**, 11-20.

Li, B.T., Christopherson, R.J. and Cosgrove, S.J. (2000). Effect of water restriction and environmental temperatures on metabolic rate and physiological parameters in sheep. *Can. J.Anim. Sci.* **80**, 97-104.

Liker, S., Gunes, S., Murat, Y., Funda, K. and Ahmed, C. (2010). The effects of body weight, body condition score, age, lactation, serum triglyceride, cholesterol and paraoxonase levels on pregnancy rate of Saanen goats in the breeding season. *J. Anim. Vet. Adv.* **9**, 1848-1851.

Lozano, J.M., Abecia, J.A., Forcada, F., Zarazaga, L. and Alfaro, B. (1998). Effect of undernutrition on the distribution of progesterone in the uterus of ewes during the luteal phase of the oestrous cycle. *Theriogenology* **49**, 539-546.

Macfarlane, W.V. and Howard, B. (1972). Comparative water and energy economy of wild and domestic mammals. Symposium of the Zoological Society, London. 31: 261-296.

Maloiy, G.M.O., Kanui, T.I., Towett, P.K., Wambugu, S.N., Miaron, J.O. and Wanyoike, M.M. (2008). Effects of dehydration and heat stress on food intake and dry matter digestibility in East African ruminants. *Comp. Biochem. Physiol.* **151,** 185–190.

Maltz, E. and Shkolnik, A. (1984). Milk composition and yield of black Bedouin goat during dehydration and rehydration. *J. Dairy Res*. **51**, 23-27.

Maresh, C.M., Gabaree-Boulant, C.L., Armstrong, L.E., Judelson, D.A., Hoffman, J.R., Castellani, J.W., Kenefick, R.W., Bergeron, M.F. and Casa, D.J. (2004). Effect of hydration status on thirst, drinking, and related hormonal responses during low-intensity exercise in the heat. *J. Appl. Physiol*. **97**, 39-44.

Marino, R., Atzori, A.S., D'Andrea, M., Iovane, G. Trabalza-Marinucci, M. and Rinaldi, L. (2016). Climate change: Production performance, health issues, greenhouse gas emissions and mitigation strategies in sheep and goat farming. *Small Rumin. Res.* **135**, 50-59.

Maynard, L.A., Loosli, J.K., Hintz, H.F. and Warner, R.G. (1981). Animal nutrition. Tata McGraw-Hill Publishing, New Delhi, India.

Mckinley, M.J., Martelli, D., Pennington, G.L., Trevaks D. and McAllen, R.M. (2007). Integrating Competing Demands of Osmoregulatory and Thermoregulatory Homeostasis. *Am. Phys. Soc.* **33**(3), 170-181.

Mengistu, U., Dahlborn, K. and Olsson, K. (2004). Effect of intermittent watering on dry matter intake and body weight of male Ethiopian Somali goats. *J. Anim. Feed Sci*. **13**, 647-650.

Mengistu, U., Dahlborn, K. and Olsson, K. (2007). Effects of intermittent watering on water balance and feed intake in male Ethiopian Somali goats. *Small Rumin. Res.* **67**, 45-54.

Meyer, U., Everinghoff, M., Gadeken, D. and Flachowsky, G. (2004). Investigations on water intake of lactating dairy cows. *Livest. Prod. Sci*. **90**, 117–121.

Misra, A.K. (2014). Climate change and challenges of water and food scarcity. *Int. J. Sustain.* **3**,153-165.

Mitchell, D., Maloney, S.K., Jessen, C., Laburn, H.P., Kamerman, P.R., Mitchell, G. and Fuller, A. (2002). Adaptive heterothermy and selective brain cooling in arid zone mammals. *Comp. Biochem. Physiol. Part B.* **131**(4), 571-585.

Moeller, K.T., Butler, M.W. and Denardo, D.F. (2013). The effect of hydration state and energy balance on innate immunity of a desert reptile. *Front. Zool.* **10**, 23.

Mousa, H.M., Ali, K.E. and Hume, I.D. (1983). of water deprivation on urea metabolism in camels, desert sheep and desert goats fed dry desert grass. *Comp. Biochem. Physiol. Part A: Physiol.***74**, 715-720.

Muna, M.M.A. and Ammar, I.E.S. (2001). Effects of water and feed restriction on body weight change and nitrogen balance in desert goats fed high and low quality forages. *Small Rumin. Res.* 41, 19–27.

Mwacharo, J.M., Kim, E., Elbeltagy, A., Aboul Naga, M., Rischkowsky, B. and Rothschild, M.F. (2016). Genome-Widee Scans Reveal Multiple Selection Sweep Regions in Indigenous Sheep (Ovis Aries) from a Hot Arid Tropical Environment. In Proceedings of the Plant and Animal Genome Conference XXIV, San Diego, CA, USA, 9–13 January 2016; International Centre for Agricultural Research in the Dry Areas: Addis Ababa, Ethiopia.

Naqvi, S.M.K., Kumar, D., Kalyan, D. and Sejian, V. (2015). Climate change and water availability for livestock: impact on both quality and quantity. In: Climate change Impact on livestock: adaptation and mitigation. Sejian, V., Gaughan, J., Baumgard, L., Prasad, C. S. (Eds), Springer-VerlagGMbH Publisher, New Delhi, India, pp 81-96.

Naqvi, S.M.K., Soren, N.M. and Karim, S.A. (2011). Effect of concentrate supplementation on performance, ovarian response, and some biochemical profile of Malpura ewes. *Trop. Anim. Health Prod.* **43,** 905-913.

Narayan, E. and Parisella, S. (2017). Influences of the stress endocrine system on the reproductive endocrine axis in sheep (*Ovisaries*). *Ital. J. Anim. Sci.* **16**(4), 640-651.

National Research Council (NRC). (2007). Nutrient requirements'of small ruminants: sheep, goats, cervids, and new world camelids. Washington, D.C., 384p.

Ghassemi, N.L., Lohakare, J.D., West, J.W. and Sung, K.I. (2014). Effects of water restriction after feeding during heat stress on nutrient digestibility, nitrogen balance, blood profile and characteristics in Corriedale ewes. *Anim. Feed Sci. Technol*. **193**, 1-8.

Ghassemi, N.L. and Sung, K. (2017). Behavioral and physiological changes during heat stress in Corriedale ewes exposed to water deprivation. *J. Anim. Sci. Tech.* **59,**13.

Osman, H.E. and Fadlalla, B. (1974). The effect of level of water intake on some aspects of digestion and nitrogen metabolism of desert sheep of the Sudan. *J. Agric. Camb*. **82**, 61-69.

Oukessou, M. and Toutain, P.L. (1992). Effect of water deprivation on absorption (oral, intramuscular) and disposition of ampicillin in sheep. *J. Vet. Pharmacol. Ther.* **15**, 421-432.

Parker, A.J., Hamlin, G.P., Coleman, C.J. and Fitzpatrick, L.A. (2003). Dehydration in stressed ruminants may be the result of a cortisol induced diuresis. *J. Anim. Sci.* **81**, 512–519.

Peacock, C. (2005). Goats-A pathway to poverty. *Small Rumin. Res.* **20,** 179-186.

Postel, S.L., Daily, G.C. and Ehrlich, P.R. (1996). Human appropriation of renewable fresh water. *Science* **271,** 785-788.

Rahardja, D.P., Toleng, A.L. and Lestari, V.S. (2011). Thermoregulation and water balance in fat-tailed sheep and Kacang goat under sunlight exposure and water restriction in a hot and dry area. *Animal* **5,** 1587–93.

Ramirez, M.S. and Tolmasky, M.E. (2010). Aminoglycoside modifying enzymes. *Drug Resist. Updat.* **13,** 151–171.

Ratnakaran, A.P., Sejian, V., Jose, V.S., Vaswani, S., Bagath, M., Krishnan, G. *et al.* (2017). Behavioral Responses to Livestock Adaptation to Heat Stress Challenges. *Asian J. Anim. Sci.* **11,** 1-13.

Roth, Z., Meidan, R., Braw-Tal, R. and Wolfenson, D. (2000). Immediate and delayed effects of heat stress on follicular development and its association with plasma FSH and inhibin concentration in cows. *J. Reprod. Fertil.* **120(**1), 83–90.

Roumelioti, M.E., Glew, R.H., Khitan, Z.J. *et al.* (2018). Fluid balance concepts in medicine: Principles and practice. *World J. Nephrol*. **7**(1), 1–28.

Sapolsky, R.M., Romero, L.M. and Munck, A.U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory and preparative actions. *Endocr. Rev*. **21**, 55-89.

Sastrasinh, M., Knauss, T.C., Weinberg, J.M. and Humes, H.D. (1982). Identification of the aminoglycoside binding site in rat renal brus hborde rmembranes. *J. Pharmacol. Exp. Ther.* **222,** 350-358.

Scherf, B.D. (Ed.). (2000). World Watch List of Domestic Animal Diversity, third ed. Food and Agriculture Organization of the United Nations, Rome, Italy, p. 726.

Schlink, A.C., Nguyen, M.L. and Viljoen. G.J. (2010). Water requirements for livestock production: A global perspective. *Rev. Sci. Tech. Off. Int. Epiz*. **29,** 603–619.

Schlosser, C. A., Strzepek, K., Gao, X., Fant, C., Blanc, É., Paltsev, S., Jacoby, H., Reilly, J. and Gueneau, A. (2014). The future of global water stress: an integrated assessment. *Earth's Future* **2**, 341–61.

Schneider, E.G. (1990). In water deprivation osmolality become an important determination of aldosterone secretions. *News Physiol. Sci*. **5,** 197–201.

Sejian, V., Maurya, V.P. and Naqvi, S.M.K. (2011). Effect of thermal, nutritional and combined (thermal and nutritional) stresses on growth and reproductive performance of Malpura ewes under semi-arid tropical environment. *J. Anim. Physiol. Anim. Nutr.* **95**, 252-258.

Seo, S.N. and Mendelsohn, R. (2006). Climate change adaptation in Africa: A microeconomic analysis of livestock choice. CEEPA Discussion Paper, No. 19, Centre for Environmental Economics and Policy in Africa, University of Pretoria, South Africa.

Shiklomanov, I.A. (1993). In Water in Crisis: A Guide to the World's Fresh Water Resources, P. H. Gleick, Ed. (Oxford Univ. Press, New York, 1993), pp. 13-24.

Shkolnik, A., Maltz, E. and Choshniak, I. (1980). The role of the ruminants digestive tract as a water reservoir. In Digestive physiology and metabolism in ruminants (ed. Y Rockebusch and P Thiven), pp. 731–741. MTP Press, Lancaster, UK.

Silanikove, N. (1994). The struggle to maintain hydration and osmoregulation in animals experiencing severe dehydration and rapid rehydration: The story of ruminants. *Exp. Physiol.* 79, 281-300.

Silanikove, N. (2000). The physiological basis of adaptation in goats to harsh environments. *Small Rumin. Res*. **35**, 181-193.

Silanikove, N., Shamy, A., Shinder, D. and Moran, A. (2000). Stress down regulates milk yield in cows by plasmin induced beta-casein product that blocks K+ channels on the apical membranes. *Life Sci.* **67**, 2201–2212.

Singh, R.R., Sajeesh, V., Booth, L.C., McArdle, Z., May, C.N., Head, G.A., Moritz, K.M., Schlaich, M.P. and Denton, K.M. (2017). Cather-Based Renal Denervation Exacerbates Blood Pressure Fall During Hemorrhage. *J. Am. Coll. Cardiol.* **69** (8), 951-964.

Skapetas, B. and Bampidis, V. (2016). Goat production in the World: present situation and trends. *Livest. Res. Rural* **28**, 200.

Tasker, J.B. (1971). Fluids, electrolytes, and acid-base balance. In: Kaneko, J.J., Cornelius, C.E. (Eds.), Clinical Biochemistry of Domestic Animals, 2nd edition. Academic Press Inc., London, pp. 65– 75.

Thang, T.V., Sunagawa, K., Nagamine, I., Kishi, T. and Ogura, G.A. (2012). Physiological stimulating factor of water intake during and after forage feeding in large-type goats. *Asian-Austral. J. Anim.* **25**(4):502-514.

United Nations (2015). Sustainable Development Goals, clean water and sanitation: why it matters.

Wittemberg, C., Choshniak, I., Shkolnik, A., Thurau, K. and Rosenfeld, J. (1986). Effect of dehydration and rapid rehydration on renal function and plasma renin and aldosterone levels in the black Bedouin goat. *Pflugers Arch*. **406**, 405–408.

Yang, J., Li, W., Lv, F., He, S. and Tian, S. (2016). Whole-Genome Sequencing of Native Sheep Provides Insights into Rapid Adaptations to Extreme Environments. *Mol. Biol. Evol*. **33**, 2576–2592.

Literature Review Two

Modulatory effect of ascorbic acid in ruminants during stress: A review

Under review: Tropical Animal Health and Production

Abstract: Ruminants, as well as other livestock, can synthesize vitamin C (VC) in their liver and therefore, dietary requirements from exogenous supplementation are often ignored. However, metabolic demand may be exceeded, leading to a decreased endogenous synthetic capacity of VC following exposure to stressful conditions. This includes high thermal load, drought or limited water intake, physiological status, and infectious diseases. The attendant effect resulting from such stressful exposure are decreased performance, susceptibility to infections and increased mortality. This review discusses the potential role of vitamin C in ruminants' stress management and summarizes the *in vitro* and *in vivo* research to date. The different administration routes, comparative advantages and supplementation outcomes on growth, production parameters and physiological status were also identified. In addition, areas where there were lack of evidence or controversial, including important literature research gaps, were identified while the mechanism of VC's actions on significant outcomes explained.

Keywords: vitamin C; stress; ruminant; bioavailability; immunomodulation

---

2.9 Introduction

The livestock sector, especially ruminants, is a pillar of the global food system and a contributor to poverty reduction, food security and agricultural development (Reynold *et al.*, 2015). According to FAO (2020), ruminants (buffalo, cattle, goat, and sheep) play a dominant role in food security by supplying 51% of all protein from the livestock, of which 67% and 33% are from milk and meat, respectively. Apart from meeting the food needs, animal agriculture, in general, provides job opportunities either directly or indirectly to a vast majority of the human populace. Thornton (2010) evinced an estimated population number of about 1.3 billion people globally that are directly employed by the livestock sector in a long-organized market chain. Apart from the continuous rise in the demand for meat and its product due to a surge in human population and increasing urbanization and income, the high unemployment rate around the globe means that more will be expected from the animal agriculture industry. Since the United Nation (2017) projected that human populations could reach 9.8 billion in 2050, it means that to meet their meat needs, the livestock production activities, including their welfare, must be effectively improved for sustainable food production, livelihood, and employment opportunities.

Domestic animals have been continuously exposed to various unfavourable physical and/or psychological stressors, and the welfare of domestic animals during such periods is of significant interest (Kumar *et al.*, 2012). This continuous exposure to such stressful conditions, including heat, cold, handling, transportation, diseases and introduction to a new flock, disrupt the normal homeostasis, leading to a new adaptation that is perceived to be damaging to the animal. Stress is responsible for various physiological alterations in the body that indirectly alter the immune system through the generation of metabolic radicals (reactive oxygen species [ROS]), which impair productivity, fertility as well as low immunity and economic losses to the farmer (Kumar *et al.*, 2012). It is believed that during stress, the body's natural antioxidants are overwhelmed, and their capacity to cope with the elimination of excess free radicals or ROS reduces, thus exposing the cell to harmful effects of ROS (Kurutas, 2016).

Animals undergo various kinds of stressors; environmental (extreme heat and cold), physical (handling or transportation), nutritional (feed or water shortages), chemical (toxin, pathogen or disease), psychological (fear or restrain) and physiological (pregnancy or lactation). Heat stress, when combined with limited water intake (water stress) in livestock, appears to be an essential stressor, especially in the tropical, subtropical (Nardone *et al.*, 2010), arid and semiarid (Al-Dawood, 2015, Akinmoladun *et al.*, 2019) regions of the world. The coping mechanisms to stressors vary with animal species, nature of stressful stimuli, the genotype in the same species, duration and time of exposure, and the animal's physiological stage (Koolhaas *et al.*, 2007; Novais *et al.*, 2017). Increased respiration rate, sweating, vasodilatation, reduced metabolic rate, decreased feed intake and utilization and alteration of water balance are some of the observed responses in livestock under stress (Akinmoladun *et al.*, 2019). Stress compromises the ovarian function and development of an embryo and redistributes body resources, including energy and protein, thus decreasing fertility, performance, and animals' wellbeing (Gupta *et al.*, 2013; Kandemir *et al.*, 2013). Given the preceding, what is most required is an antioxidant therapy that may retard cortisol production (the primary stress hormone), detoxify ROS, and enhance the animal's immunity. Such antioxidant may be incorporated as a reliable management practice option for mitigating the adverse effects of stress in animals (Balz, 2003; Urban-Chmiel *et al.*, 2009).

L-ascorbic acid or vitamin C (VC) (compounds showing the biological activity of ascorbic acid) are essential nutrients for only a few species (e.g. humans and guinea pigs) without a synthetic ability for the vitamin due to an enzyme deficiency. This enzyme (L-gluconolactone oxidase) is responsible and necessary for transforming the glucose into vitamin C during the last metabolic phase. However, mammals (ruminants, swine, dogs, horses, cats), including marsupials, can synthesize AA from glucose in the liver or the kidney (Comb, 2008). Supplementation of VC was, therefore, not a dietary requirement in animals that can synthesize it. Unfortunately, plasma ascorbic acid concentration reportedly decreases during stress and disease conditions in animals (Sivakumar *et al.*, 2010; Kim *et al.*, 2012). In such situations, it is possible that the endogenous synthesis of the vitamin has ended or could be due to increased demand or a combination of both. The significant improvements usually recorded following supplementations suggest VC deficiency during episodes of stress and compromised health status.

The ameliorative potentials of ascorbic acid on stressful stimuli are considered to be non-depressive, safer, more practicable and devoid of the inherent shortcomings that preclude the use of central nervous system (CNS) depressant drugs (e.g. xylazine, morphine, propionyl and pentobarbitone) in food animals (Reinheim *et al.*, 2000; Ali *et al.*, 2001; Ali and Al-Qarawi, 2002). In addition to being depressive and counterproductive, the accessibility and the manner of applying these drugs by farmers (e.g. underdosing) are somewhat tricky, while the effects produced are not consistent and less effective (Ali *et al.*, 2006; Ferguson and Warner, 2008). VC is cheap, readily available, non-toxic, administered and absorbed quickly, devoid of the withdrawal period and without consequential effect at very high doses *in vivo* (Seifi *et al.*, 2010). This stress alleviating potential of VC owes its mechanism of action not only to the scavenging of ROS and other oxidative radicals but also its ability to potentiate α-aminobutyric acid (GABA) (Brikas, 1994), which helps to retard the release of prolactin and cortisol usually implicated in splenic contraction and cellular damage (Power and Jackson, 2008; Minka and Ayo, 2010). Some studies on stress-simulated oxidative damage with respect to vitamin C have been shrouded with controversy and sometimes inconclusive. However, there is reasonable evidence and body of knowledge supporting VC's use as an anti-stress (Minka and Ayo, 2007; Power and Jackson, 2008; Akinmoladun *et al.*, 2020a,b). This review discusses the various physiological and performance indices in stressed ruminants modulated by vitamin C, route of administration differences, its mechanism of action, and future research gap.

2.10 Antioxidant potentials of vitamin C

L-ascorbic acid is a non-enzymatic and water-soluble antioxidant in plasma and tissues. Coupled with reduced glutathione, tocopherols and other antioxidants, VC protects cells, supports the sparing of vitamin E, recycles α-tocopherol, and promotes non-heme iron utilization (Chambial *et al.*, 2013). It also ensures that appropriate oxidation states of enzyme-bound metals are maintained in the enzymatic biosynthesis of carnitine, collagen, and non-epinephrine (Tauler *et al.*, 2003). Through its antioxidant ability to donate free electrons (molecules of hydrogen), VC ensures membrane integrity stability, thus limiting its susceptibility to lipid peroxidation (Bernabucci *et al.*, 2002). In heat-stressed animals, VC regulates oxygen consumption by increasing loss through a more efficient thermal exchange between the environment and the body or reducing heat load generated from metabolic activities within the body (Minka and Ayo, 2010). L-ascorbic acid is thought to improve the humoral and cellular immunity and thus, increase resistance to infection, defense mechanism and antioxidant status of the animal and reduce the detrimental effects of certain eicosanoids (Chambial *et al.*, 2013). Figure 2.2 is a summary of the antioxidant potentials of vitamin C.

Ruminants, especially young ones, are more vulnerable to cold stress and require increased plasma ascorbic acid concentration to confer immunity and protection (Carr and Maggini, 2017). Sivakumar *et al.* (2010) reported that ascorbic acid prevents neonatal calve diarrhoea and scours. In addition to cortisol-inhibition activities, VC is reported to actively participate in the restriction and prevention of free radical propagation, leading to the protection of blood cells (lymphocytes and monocytes) from oxidative damage (Powers and Jackson, 2008; Carr and Maggini, 2017). The antioxidant potentials of AA in the management of stressful stimuli in ruminants (Ghanem *et al.*, 2008; Minka and Ayo, 2012; Akinmoladun *et al.*, 2020a,b), monogastric (Minka and Ayo, 2010; Lechowski *et al.*, 2015) and even humans (Tauler *et al.*, 2003) are well documented.

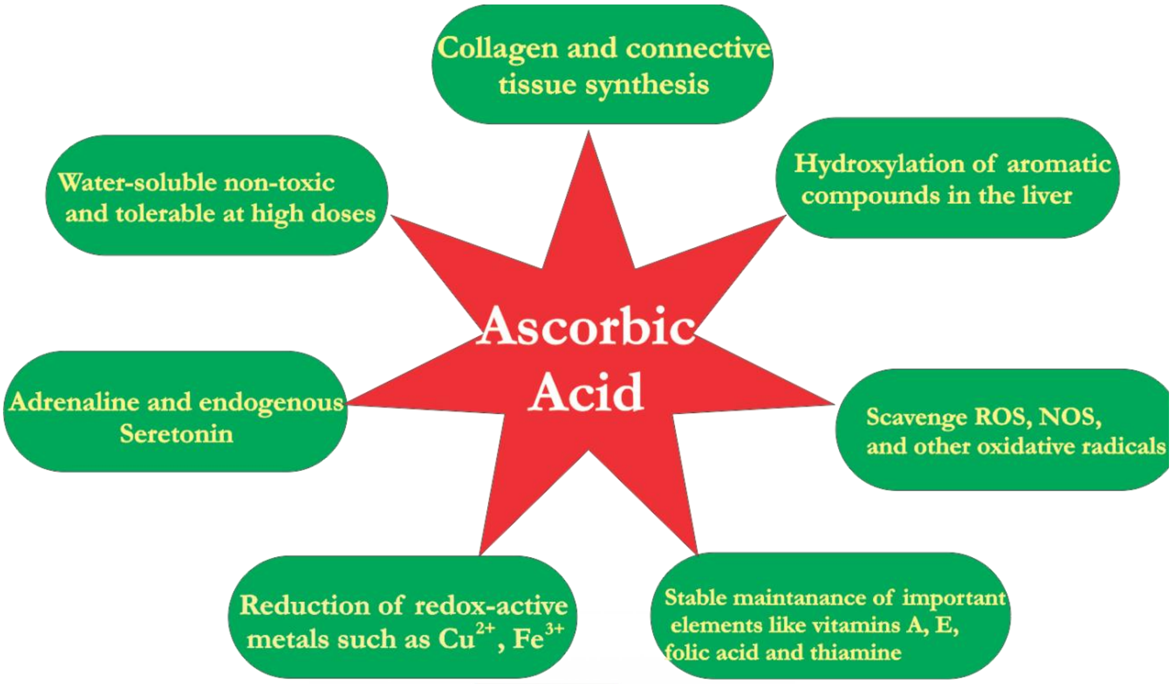


Figure 2. 2 Summary of antioxidant potentials of ascorbic acid

2.11 Biopotency, uptake and tissue distribution

Compounds showing the biological activity of ascorbic acid are also described as vitamin C. It is a six-carbon ketolactone structure whose biological activity depends on this 6-carbon lactone having a 2,3-enediol structure. Thus, it is an effective quencher of free radicals such as singlet oxygen (O_2). It reduces ferric (Fe^{3+}) to ferrous (Fe^{2+}) iron (and other metals analogously). As a strong reducing agent, ascorbic acid is oxidized to dehydroascorbic acid via the radical intermediate semi-hydroascorbic acid in a reversible redox system (Tu *et al.*, 2017). Although there are several synthetic analogues of vitamin C, their relative biopotencies and biological activity differ. For example, 6-deoxy-L-ascorbic acid and several esters of ascorbic acid (e.g. 6-deoxy-6chloro-L-ascorbic acid, ascorbyl-6-palmitate) have good biological activity, whereas (e.g. L-glucoascorbic acid) have little or no activity (Combs Jr. and McClung 2017).

Higher animals can synthesize vitamin C via the glucuronic pathway (Fig. 2.3). In lower animal groups, including egg-laying mammals, reptiles and amphibians, the pathway's enzyme is found in the kidney. In contrast, in higher animal groups (mammals and passerine birds), the enzyme is located in the liver (Chaterjee *et al.*, 1975). The enzyme (gluconolactone oxidase) is absent (evolutionary loss) in primates, humans, bat and some species of fish and birds and therefore requires exogenous VC supplementation to meet dietary needs (Banhegyi *et al.*, 1997). For vitamin C (ascorbic acid, ascorbate) to function as a vitamin, its entry into cells is essential (Padayatty and Levine, 2016). Simple diffusion across membranes cannot distribute VC across cells because it is a large polar molecule, charged at physiological pH, requiring transporters for cell entry (Li and Schellhorn, 2007).

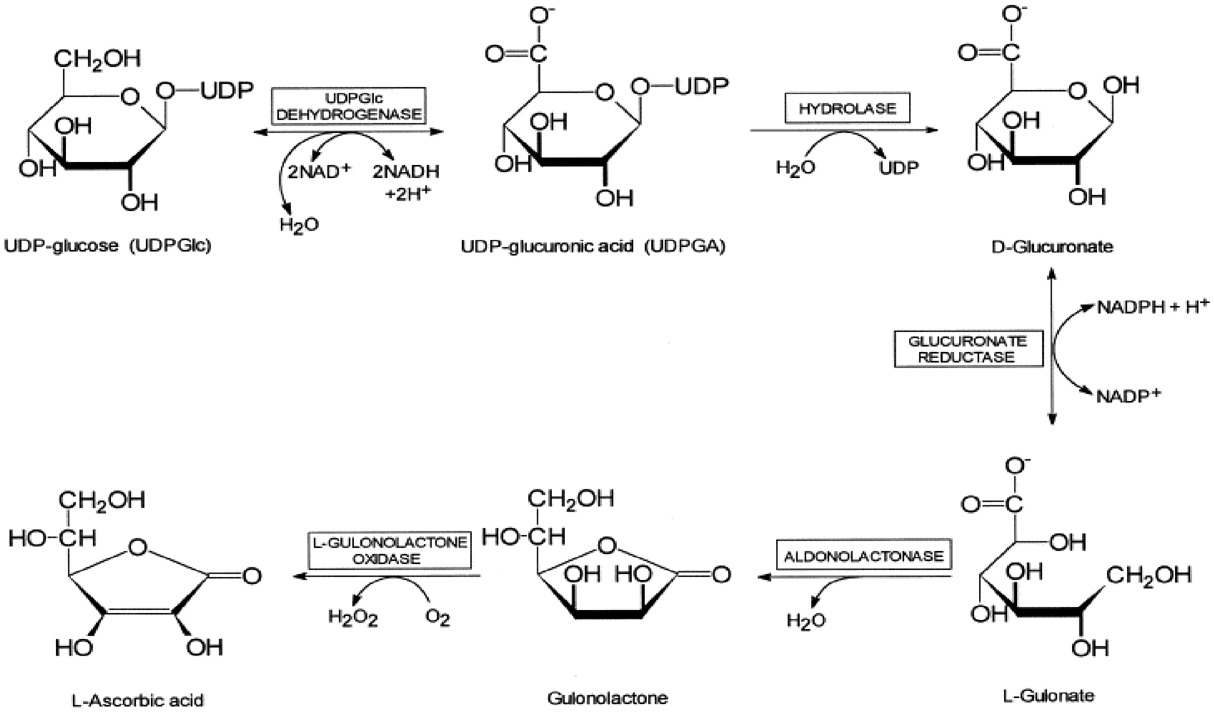


Figure 2. 3 Vitamin C biosynthesis

Source: (Banhegyi *et al.*, 1997)

Two distinct transport channels have been characterized; sodium-dependent vitamin C transporters (SVCT1 and SCVCT2) (Daruwala *et al.*, 1999) and hexose (glucose) transporters (GLUTs) (Corpe *et al.*, 2013). The GLUTs channel first oxidizes ascorbates to dehydroascorbic acid (DHA) and gets reduced back to ascorbate within the cell as a form of ascorbate recycling (May *et al.*, 1995). The adrenal, pituitary, liver, thymus, brain, and pancreas glands are very rich in VC within the body, and the concentration decrease in that order (Rucker *et al.*, 2008). In the adrenals, ascorbate concentration could be as high as 168 mg/100g in a cow. About one-third of the vitamin is found at the site of catecholamine formation, from where it is mobilized together with synthesized corticosteroids in response to stressful stimuli (Combs and McClung, 2017). The brain tissue concentration of ascorbate, especially catecholamine rich regions, tends to be high (5-28 mg/100g) and are the last to show deficiency symptoms following dietary deprivation in species that cannot bio-synthesize the vitamin. At vitamin C doses of 2 g/day, the ascorbate levels of white blood cells reach plateaus, while the ascorbate level of their differentials, lymphocytes, platelets, monocytes, and neutrophils decreases in that order (Combs Jr. and McClung, 2017).

Although there is no stable reserve of vitamin C as excesses are quickly excreted, the leukocyte ascorbate concentration provides an accurate measure of the vitamin's tissue levels (Mitmesser *et al.*, 2016). Ruminants, due to their biosynthetic ability, may not require dietary AA supplementation. However, the liver’s synthetic capacity may not accommodate VC's increased requirements in stressful situations, such as road transportation, exposure to high ambient temperature, sub-optimum water intake, diseases, and exercise (Sivarkumar, 2010). Therefore, supplementation of VC to ruminants under conditions perceived to be stressful may provide a potentially important, cheap, non-toxic alternative treatment. The range of plasma ascorbate concentrations in ruminants is shown in Table 2.4.

Table 2. 4 Plasma ascorbate concentrations range of healthy ruminants

Ruminant type	Range of plasma ascorbate concentrations (µg/L)	References
Cattle	2400-4700	Smith *et al.* (2009)
	1500-3300	Haiying *et al.* (2003)
	2670-4080	Padilla *et al.* (2007)
Buffalo	5300-5500	Chanda (1958)
Sheep	4670-4770	Mohamed *et al.* (2004)
	1288-1438	Kassab and Mohammed (2014)
Goat	1750-1920	Sivakumar *et al.* (2010)

2.12 Forms and routes of VC administration in ruminants and their comparative advantage

Several means of exogenous supplementation of VC for ruminants have been worked upon in experimental trials. A major challenge limiting the bioavailability of VC in tissues is the rapid destruction by ruminal microflora and urinary excretion losses (Padilla *et al.*, 2007). However, during low VC status, the amount excreted may be limited (McDowell, 2000). VC administration could be routed through the mouth (*per os* or oral) or parenteral (non-oral). Forms of oral administration could be through drinking water (after dissolving the powder in water) (Ghanem *et al.*, 2008; Akinmoladun *et al.*, 2020a,b), through feed as rumen-protected VC (coating of VC with ethylcellulose or hydrogenated soybean oil) (Hidiroglou, 1999; Padilla *et al.*, 2007) or fed directly (uncoated) as a dietary mixture (Kim *et al.*, 2012). Parenteral administration could either be intravenous (Liu *et al.*, 1994), intramuscular (Sonmez and Demirci, 2003), or subcutaneous (Fazeli *et al.*, 2010). Assessment of VC's bioavailability in ruminant is primarily determined by the change in plasma ascorbic acid concentrations after supplementation. Studies conducted have shown plasma ascorbic acid concentration to be higher in ruminants receiving coated VC or administered parenterally than ordinary powdered VC in drinking water or as a dietary mixture (Hidiroglou, 1999). Comparing the potency of different VC preparations such as powdered VC, VC coated with ethyl cellulose or silicon and ascorbyl-2-polyphosphate, Hidiroglou *et al.* (1997) observed a much-increased plasma VC concentration in the VC-silicon-coated preparation compared to others. Also, direct supplementation via the abomasum or intra-duodenum increased plasma VC compared to oral (mouth) (Hidiroglou, 1999). While study comparing the effectiveness of different routes and/forms of vitamin C supplementation in ruminants have been scanty, higher plasma ascorbate with respect to administration routes does not seem to translate into much-improved stress management. In a comparative study on the effectiveness of route of

VC administration, Biobaku *et al.* (2018) observed that, despite higher plasma ascorbic acid concentration, the responses (cortisol, anti-oxidants and erythrocyte biomarkers) of 2 h transportation-stressed Kalahari goats administered with VC (200 mg/kg) intramuscularly was similar to oral supplementation. There is a need for more studies on the safest and efficient route of administration that will produce the highest level of stress management.

2.13 Modulatory role of vitamin C in ruminants

2.13.1 Effect on vital signs and behavioural kinetics

The vital signs, especially rectal temperature (RT) and respiratory rate (RR), provides a quick measure of the degree of stress imposed on animals. Under high thermal load, RR can be used to assess heat stress, and the result is reported to correlate with corticoid concentration while pulse rate (PR) indicates the homeostasis of the animal body along with the general metabolic status (Kassab and Mohammed, 2014). Usually, these physiological variables or vitals are usually raised when ruminants are exposed to unfavourable stressors and may impair the animal's performance (Akinmoladun *et al.*, 2019). An elevation in the physiological variables (RT and PR) observed during road transportation decreased in goats (Minka and Ayo, 2012; Nwunuji *et al.*, 2014) and sheep (Kassab and Mohammed, 2014) following the administration of VC. During transportation, stress is usually imposed on the animal during handling, loading and the entire road transportation process, which include; the movement of vehicle, vibration, noise, water and food deprivation, change of environment and confinement (Minka and Ayo, 2012). There was an improvement in RR in water restricted Awassi ewes (Ghanem *et al.*, 2008) and Xhosa goats (Akinmoladun *et al.*, 2020a) with VC treatment. Based on documented reports, it is suggestive that vitamin C alleviates stress by decreasing prostaglandin output (which is usually elevated during stress) by directly altering thermal set points. This effect on prostaglandin output by these vitamins directly modulates the hypothalamic thermoregulatory activity and may explain the observed outcome in stressed ruminants (Sivakumar *et al.*, 2010). Small ruminants' adaptive nature and tolerance to stressful stimuli (e.g. heat stress) may sometimes cloud the VC supplementation impact. In the report of Hashem *et al.* (2016), the effect of VC supplementation on summer heat-stressed Rahmani ewes was not significant in the various heat tolerance parameters measured. Following prolonged exposure, small ruminants develop specific heat tolerance mechanisms to reduce metabolic heat production through feed intake reduction as well as stretching out the body to lose heat (Akinmoladun *et al.*, 2019).

Skin thickness, an important assessor of severe dehydration, especially in calves (Atkinson, 1992), could decrease following sustained exposure to stressors. This could result from the high thermal load, suboptimal water intake during water scarcity or when animals are deprived of water during transportation from one location to another. Also, the thickness of the skin is affected by cellular substances, the number of collagen fibres and interstitial fluid content. These intrinsic features significantly influence the skin's biophysical properties including, elasticity, turgor and distensibility (Weller *et al.*, 2008). Normal skin contains high concentrations of vitamin C, and an excessively oxidized environment, either from thermal load or otherwise, can deplete levels of VC below the threshold, thereby affecting its supporting functions like the stimulation of collagen synthesis, free radicals scavenging activities and modulation of cell signalling and epigenetic pathways (Pullar *et al.*, 2017). However, there is a possibility that the skin thickness will be improved with exogenous VC supplementation. According to Minka and Ayo (2012), the skin thickness of goats undergoing 12 hr transportation stress was raised from 1.8 mm in control to 2.1 mm in the vitamin C treated groups. In an *in vitro* study, the cell signalling pathway attributes of vitamin C are reported to enhance the differentiation of epidermal keratinocytes cells with the markedly improved ultrastructural organization of the stratum corneum (Pasonen-Seppanen *et al.*, 2001; Marks, 2004). This stratum corneum is one of the four major components (other three; stratum spinosum, stratum granulosum and stratum lucidum) of the upper layer of the epidermis responsible for skin thickness (Marks, 2004).

When ruminants are exposed to stressful stimuli, the frequency of urination and the egestion of gut content increases. This stress-induced bowel movement is attributed to increased intestinal motility and cortisol-induced diuresis (Karl *et al.*, 2018). Nwe *et al.* (1996) alluded to the increased elimination of urine and faeces from transportation stress to nervousness or excitation. This stressful condition can further be heightened by suboptimal water intake and adverse climatic conditions, leading to dehydration (Popkin *et al.*, 2010). Under elevated temperature, Nejad and Sung (2017) reported increased sitting duration and decreased standing time when sheep were water-deprived for 2 or 3 hrs after feeding. Minka and Ayo (2012) reported that the faecal water content and elimination behaviour, in general, were less in transported goats treated with VC. This indicates that VC can reduce the excitation of the nervous system associated with road transportation stressors. However, the behavioural dispositions during vehicular movements of goats, including fall, slip, kick aggression, jump and baulk, were not affected by vitamin C (Minka *et al.*, 2009). The impact of VC on the behavioural kinetics of ruminants undergoing heat and/or water stress is unavailable as at the time of writing this review.

2.13.2 Effect on body weight and feed intake

During stress, anabolic activity decreases while tissue catabolism increase, and both combine to affect growth performance. Decreased anabolism is a response to decreased voluntary feed intake and essential nutrients, including vitamins and minerals, consequently leading to a loss in the production per unit of feed (Akinmoladun *et al.*, 2019). In addition to reduced feed intake, attempts to sustain the increased demand for energy results in high induction of free fatty acids and cholesterol from body fat reserves. The mobilized body fats are usually the cause of weight loss due to the loss of body solids. The severity of the stress-induced depression on body weight and feed intake in ruminants is further heightened in regions where intense climatic stresses (high amibient temperature and humidity) is combined with feed limitations and insufficient water supply. Decreased performance and production efficiency during stress in an animal are the results of metabolic acclimatization. Temperature stress (largest single stressor) seemingly changes the metabolic and endocrine status of animals. Osmanabadi goats had their feed intake and body weight reduced when exposed to either restricted feed intake or heat stress plus restricted feed (Chaidanya *et al.*, 2017). Feed intake depression is a physiological response in animal during stress (Akinmoladun *et al.*, 2019). For example when an animal is exposed to heat stress, the nerve impulses to the appetite centre in the hypothalamus are suppressed following stimulation by the peripheral thermal receptors, thereby causing feed intake depression (Marai *et al.*, 2007). In addition, there is a heat stress-induced upregulation of leptin and adiponectin secretions as well as their receptors (Morera *et al.*, 2012). While adiponectin modulates feed intake behaviour by acting as starvation signal, leptin reduces feed intake by stimulating the hypothalamus axis (Hoyda *et al.*, 2012).

Stress, in general, usually result in the excessive generation of free radicals and proinflammatory molecules. Such induced oxidative injury and infalmmation are the bedrock of various metabolic disturbances, maldjustment, and even death that commonly reflect in livestock (Srivastava and Kumar 2015). A depleted VC level often accompanies increased concentration of markers of oxidative stress (e.g. lipid hydroperoxide) in ruminants. In an attempt to simulate water stress conditions usually experienced during the period of water scarcity and extreme drought, a 12-d water restriction study was conducted by Ghanem *et al.* (2008) on Awassi ewes, and they reported a drop in final weight from 70.25 kg (*ad libitum* control) to 48.75 kg (restricted group). According to the authors, supplementation of VC (2.5 g/d, dissolved in 12.5 mL of water) to each ewe raised the final weight to 55.5 kg. A similar reduction of weight loss in a 75-d water restriction study on Xhosa goats was reported following VC (3 g/d) supplementation (Akinmoladun *et al.*, 2020a). Though the difference was not significant, supplementation of VC (125 mg/kg) to Farafra sheep before transportation reduced the weight loss from 1.22 kg to 0.47 kg. Similarly, the final body weight and total feed intake of heat-stressed ram lambs increased by 19.5% and 15.7%, respectively, following a daily dose of vitamin C [45 mg/kg body weight] (Abd-Allah and Zanouny, 2014). Deters and Hansen (2020) intramuscularly injected ascorbic acid (6g sodium ascorbate per steer) to some steers shortly before an 18 h (1675 km) transit drive. According to the authors, after 75-d post transit, the final body weight, average daily gain and dry matter intake increased from 429 – 436 kg, 1.67-1.84 kg and 9.0-9.5 kg, respectively. The stress-mediating mechanism of VC in animal leading to improved performance, is an indirect one. Apart from its antioxidant role, VC is involved in collagen biosynthesis and remodelling and these are processes that support skeletal muscle hypertrophy by facilitating the migration of satellite cells (Archile-Contreras, 2011). Another potential explanation of growth response to supplemental VC, though subject to further research, may be due to its role as a cofactor in the enzymes involved in carnitine biosynthesis. During stress, there is increased lipolysis due to feed intake reduction and cortisol elevation. Carnitine, however, is essential for transporting fatty acids into the mitochondria for catabolism. Hence a possibility exists of an increased need for carnitine to utilize the much-released fatty acid for growth and energy production (Deters and Hansen, 2020).

2.13.3 Physiological response

2.13.3.1 Blood metabolites and immunomodulatory effect of vitamin C

Empirical observations have suggested an alteration in the host resistance following sustained exposure to stressful conditions. The consequence of prolonged exposure to stress is a decline in immune cells' reactivity as well as a higher incidence of infections, especially during summer (Dahl *et al.*, 2020). According to Brown and Vosloo (2017), the elevated plasma concentrations of cortisol usually induced during heat stress may have an immunosuppressant effect followed by a reduced response of lymphocytes to mitogens. The impact of stressors, including physiological status (pregnancy/lactation), thermal load or otherwise, usually results in superoxide radicals' production above threshold concentrations.

Interestingly, such poor immune status is usually co-related with a depleted plasma VC concentration. Rejeb *et al.* (2016) reported a compromised immune system in dairy cows alongside a considerable reduction in plasma VC concentration when exposed to a high environmental temperature for a long time. However, supplementation effects of VC on immune response and blood metabolites have not been consistent. Administration of ascorbic acid (2 g/h/d) to heat-stressed ram lambs during the summer season did not affect plasma total protein (TP), albumin (Alb), total cholesterol (TC) and calcium (Ca) concentrations. However, the significant increase in TP of water restricted Awassi ewes was lowered following VC (2.5 g/d) supplementation (Ghanem *et al.*, 2008). Similarly, concentrations of the liver enzymes (aspartate aminotransferase, AST and alanine aminotransferase, ALT) were lowered following VC supplementation (Abd-Allah and Zanouny, 2014). In addition, elevated serum electrolytes (chloride, sodium, potassium, calcium, and magnesium) in goats transported by road for 12 hrs (Ayo *et al.*, 2009) or subjected to water restrictions (Akinmoladun *et al.*, 2020a) were reduced following administration of VC. Despite these significant outcomes, Kim *et al.* (2012) reported increased levels of albumin, creatinine and glucose in heat-stressed growing calves supplemented with VC. Aside from the elevated plasma cholesterol, VC failed to induce any significant change in biochemical blood indices in the summer heat-stressed Rahmani ewes [(Hashem *et al.*, 2016). The role of VC in energy balance, particularly during water stress, is still shrouded in controversy. Due to its role in norepinephrine and carnitine formations, VC is reported to increase free fatty acid concentration and its transport across the mitochondrial membrane (Steffens and de Boer, 1999; Mahan *et al.*, 2004). However, other authors have reported ascorbic acid to have a hypocholesterolemic effect when supplemented (Sahin *et al.*, 2002; Yousef, 2004).

Psychological and physiological stress in ruminants constitutes a serious welfare concern. Usually, catecholamines are released during the alarm phase of stress, and this is presumed to be the primary cause of lymphopenia and neutrophilia (Stanger *et al.*, 2005). The combined effects of high relative humidity and ambient temperature, together with the presumed release of elevated corticosteroids, decrease cytokines IL-2 and reduce lymphocytes' proliferation. Besides, lymphocytes cells become more vulnerable to apoptosis, contributing further to neutrophilia and lymphopenia (Dahl *et al.*, 2020). Dietary supplementation of AA to heat-stressed swampy buffaloes significantly increased the mean concentration values of packed cell volume (PCV), haemoglobin (Hb) and lymphocytes while the neutrophil remained the same compared to the control (Konwar *et al.*, 2017). The administration of VC (100 mg/kg body weight) ameliorated the impact of stress from loading and transportation on the neutrophil, lymphocyte counts and neutrophil/lymphocyte ratio in goats (Minka and Ayo, 2011). Similar enhancement of hematopoiesis in heat-stressed Rahmani ewes (Hashem *et al.*, 2016) and Brown Swiss bulls (Ecu *et al.*, 2000) with VC have been reported. This significant outcome is attributable to VC's ability to facilitate increased iron absorption and enhance the immune system. The combined effect of physical and emotional stress can elicit eosinopenia, and it is attributable to an increased surge of plasma adrenaline and cortisol. The mechanism of decrease (where applicable) is unclear, but it is suspected to be caused by decreased release from bone marrow, intravascular lysis (steroid-induced apoptosis of eosinophils), organs (spleen and liver), secludedness and increased tissue migration (Carter, 2018). The depression in the eosinophils values in goats undergoing transportation stress remains unaffected in groups treated with VC (Minka and Ayo, 2011).

2.13.3.2 Effect on plasma hormones and oxidative metabolites

Exposure to stress before slaughter may induce an excessive oxidative environment, with significant effects on feedlot performance, thus translating to producers' losses (Duff and Galyean, 2007; Akinmoladun *et al.*, 2020b). The magnitude of the stressors and the oxidizing environment in vivo are a function of the type, intensity and duration of stressors and the animal's vulnerability to them (Ferguson *et al.*, 2001). The pre-slaughter phase includes all activities and management practices that apply during growth until the animal is transported to the abattoir. During this period, there is a tendency to expose the animals to a range of challenging stimuli, including transportation stress and changing climatic variables (Minka and Ayo, 2007; Ibironke *et al.*, 2010). Chirase *et al.* (2004) reported decreased serum antioxidant capacity, increased serum malondialdehyde (MOD) and higher susceptibility to bovine respiratory disease as well as mortality in beef calves transported (over 3,500 km) to the feedlot. However, the administration of VC (100 mg/kg, i.m) to goats transported for 3.5 h reduced the MOD and superoxide dismutase (SOD) activities (Nwunuji *et al.*, 2014). Also, supplementation of VC (10 g/animal/d) to pregnant and heat-stressed buffaloes during thermal stress reduced the mean superoxide dismutase (SOD) and catalase (CAT) activities (Kumar *et al.*, 2010; Ganaie *et al.*, 2012). VC protects the body defence system and stabilizes animals' health status by scavenging the excessive production of free radicals generated during stress (Sivakumar *et al.*, 2010). According to Belge *et al.* (2003), VC modulates the decrease in MDA concentration by removing the singlet oxygen, hydroperoxyl, superoxide, lipid peroxyl and lipid-free radicals in animals subjected to stress.

During stress, ascorbic acid exerts its inhibitory effect on cortisol. In addition, it plays an essential role in thermoregulation by its activities as an inhibitory vitaminergic neurotransmitter in the hypothalamus (Karanth *et al.*, 2000). Also, the high induction of serum prolactin concentration during heat stress is attributed to hypothalamic-inhibitory stress response on peptides, which control prolactin secretion (Bernard *et al.*, 2019). According to Civen *et al.* (1980), an increase of 1% in the rate of ascorbic acid intake would result in a 27% and 22% decrease of both plasma corticosterone and adrenal, respectively. Studies indicated that the cortisol levels decreased significantly while the thyroid hormones (thyroxine (T_4) and triiodothyronine (T_3)) increased in heat-stressed swampy buffaloes (Konwar *et al.*, 2017) and goats (Sivakumar *et al.*, 2010, Akinmoladun *et al.*, 2020b) following supplementation with VC. Contrarily, reported studies in water-stressed animals did not indicate a significant change in cortisol concentrations with VC supplementation (Ghanem *et al.*, 2008; Parker *et al.*, 2003). Also, VC failed to induce any significant change in the cortisol of summer heat-stressed growing calves (Kim *et al.*, 2012). The variations in the response of plasma cortisol content have been attributed to possible differences in the amount of VC supplemented (Kim *et al.*, 2012). Free radicals (H_2O_2) generated during stress inhibit the catalytic synthesis of thyroid hormones through its scavenging role on the enzymes, thyroperoxidase and 5′ mono-deiodinase, mediating the synthesis and conversion of T_4 to T_3 (Mancini *et al.*, 2016). However, VC modulates the stress-induced reduction of these hormones by protecting the above enzymes from free radicals.

Table 2. 5 Studies investigating the effect of vitamin C supplementation on plasma cortisol response in stressed animal

Factor	Treatment condition	Species	Route	Rate	Response	References
Summer heat stress	150-day exposure to heat stress (av. THI, 78)	Swamp buffaloes	Diet	0, 10 and 15 g/d (mixed with 100 g of feed)	S	Konwar *et al.* (2017)
Heat stress	21-day exposure to heat stress (40ºC, 30%)	Black Bengal goats	Oral	2 mg/d (dissolved in 10 ml of water)	S	Sivakumar *et al.* (2010)
Water stress	12-day water restriction (1L of water per ewe on the 4th day and 3L of water per ewe on the 8th day	Awassi ewes	Oral	2.5 g/d (dissolved in 12.5 ml of water)	Ns	Ghanem *et al.* (2008)
Water stress	75-d water restriction (70% and 50% of *ad libitum* WI)	Xhosa goats	Oral	3 g/d/ or 3 g/d + extra 5g every 8th day (dissolved in 50 mL of water)	S	Akinmoladun *et al.* (2020b)
Heat stress	60-day exposure to summer heat stress (THI, >70)	Growing calves	Diet	20 g/d (mixed with concentrate)	Ns	Kim *et al.* (2012)
Transportation stress	3 h (225 km) transportation	Farafra Sheep	Oral	125 mg/kg BW (dissolved in 10 mL of sterile water)	S	Kassab and Mohammed (2014)
Transportation stress	10 h transportation	Crossbred (Alpine x Beetle) goats	Oral	180 mg/kg (dissolved in water)	Return to normal value after 6 h post transportation compared to 10 h in control	Mir *et al.* (2019)

ns: non-significant; s: significant

Table 2.5 describes studies investigating the effect of vitamin C supplementation on plasma cortisol response in stressed animals.

2.13.3.3 Effect on reproduction

Due to its presence in ovaries, corpus luteum (CL) and follicular fluid (Das *et al.*, 1993), AA hinders the apoptosis in murine cumulus-oocyte and bovine granulosa cells (Eppig *et al.*, 2000; Murray *et al.*, 2001), reduce heat shock protein 70 (HSPA1A) and the levels of reactive oxygen species intracellularly (Castillo-Martin *et al.*, 2014). However, plasma concentrations of antioxidants, especially VC, in ruminants are reported to decrease during stressful periods and at the beginning of ovarian cyclic activity and gestation (Salinas *et al.*, 2017). This may explain the increased oxidative stress, usually resulting in gestation disorders, embryopathies, abortions, pre-eclampsia and low birth weights (Al-Gubory *et al.*, 2010; Rong-Zhen and Dao-Wei, 2013) that are sometimes encountered in ruminants. The induced oxidative stress implicated by the depletion of antioxidant vitamins is explained by the vigorous steroidogenesis and cyclic activities of the ovaries. These findings have suggested that *in vivo* production of ascorbic acid alone in ruminants may not be sufficient to sustain and effectively modulate the various physiological processes.

Giving the established correlation between VC concentrations in the corpus luteum and plasma progesterone levels as well as weights/diameters of corpora lutea (Serpek *et al.*, 2001), it suffices to say VC affects reproduction functions and steroid hormone synthesis rate. Supplementation of VC to summer heat-stressed Rahmani ewes (Hashem *et al.*, 2016) and hypoxic pregnant sheep (Parraguez *et al.*, 2011) increased the number of ovulatory follicles lambing rate, lamb weight and fecundity. Also, the quality of bovine follicles and their survival *in vitro* improves with ascorbic acid supplementation (Thomas *et al.*, 2001). Pernes *et al.* (2016) demonstrated that VC supplementation could enhance nuclear maturation and the developmental competence of oocytes matured *in vitro*. Supplementation of ascorbic acid (100 µM) to heat-stressed caprine cumulus oocytes complexes (incubated, 41 °C) for 24 hrs *in vitro* had an increased cumulus expansion (42.98% to 66.67%) and nuclear maturation (metaphase II stage; 40% to 60%) (Khanday *et al.*, 2019). Similar improvement in nuclear maturation status and expansion of cumulus cells with VC inducing increased meiotic resumption of oocytes and higher proportions of oocytes reached at metaphase I and II stages have been reported in camel (Elsayed *et al.*, 2015) and other caprines (Hammami *et al.*, 2013). Though the number of does, retention rate, oestrous response and duration were not affected, intramuscular administration of ascorbic acid (50 mg) enhanced conception rate in Red Sokoto goats (Omontese *et al.*, 2014). This improvement in reproductive efficiency with VC, especially *in vivo*, has been attributed to increased survival and quality of the embryo by mechanisms such as: (i) increased production of progesterone during the early stage of gestation; (ii) enhanced function and maturation of the placenta, uterus and oviduct; (iii) improved blastocyte development; and (iv) circumvention of fetal resorption (Clagett-Dame and DeLuca, 2002; Hashem 2015). However, the concentration of ascorbic acid that is of physiological significance during supplementation is a crucial research area. This is because higher concentrations of ascorbic acid *in vitro* can inhibit physiological processes in the ovary, resulting in follicular degeneration (Murray *et al.*, 2001). In a study conducted by Andrade *et al.* (2012) on dose-dependent ascorbic acid supplementation to an *in vitro* culture of bovine preantral follicles, granulosa cells' proliferation was stimulated following ascorbic acid supplementation and reached a maximum (63.4 % granulosa cells) at 50 µg/ml of ascorbic acid. According to the authors, the maintenance of follicular viability during *in vitro* culture was not effective at higher (>100 µg/ml) or lower doses (<10 µg/ml) of ascorbic acid.

Apart from lacking significant cytoplasmic antioxidant's component, sperm cells have an abundance of polyunsaturated fatty acids in their membranes and are therefore susceptible to lipid peroxidation from O_2^- and H_2O_2 (Bansal and Bilaspuri, 2011). Ascorbic acid plays a role in protecting sperm from ROS and maintaining the genetic integrity of sperm cells by protecting sperm DNA from oxidative damage (Eidan, 2016). Because of these vital functions, deficient or reduced ascorbate level in male animals have been correlated with an increased number of abnormal sperm, low sperm counts, agglutination, and reduced fertility (Dawson *et al.*, 1990). Administration of ascorbic acid (20 mg/kg; i.m) for 30 days reportedly increases sperm concentration, semen volume and motility in rams (Sonmez and Demirci, 2003). Also, subcutaneous injection of VC (20 mg and 40 mg/live weight) in Makhoz goats for 90 days linearly increased total sperm ejaculate and sperm volume (Fazeli *et al.*, 2010). Modulation of ascorbic acid in ruminants' reproductive efficiency is hinged on maintaining lipids, DNA, proteins, enzymes, and other antioxidants at the required physiological range and homeostasis sufficient for optimum reproductive interactions (Sonmez and Demirci 2003; Minka *et al.*, 2009). However, the level of response and/or improvements recorded varies with concentrations of ascorbic acid used. The optimum concentration of VC required for maximum improvement is still a research gap.

2.14 Conclusion

While ruminants possess the ability to synthesize VC in the liver, the decreased plasma ascorbate concentration during stressful periods may compromise their immune system as well as production and performance indices. Unlike vitamins A, D and E, daily vitamin C requirements are not documented in the literature for ruminants. Research evaluating vitamin C requirements in ruminants and the attendant responses during periods deemed stressful and the amount that will be supplemented to produce maximum improvement is currently advocated. Differences in ruminant types, breed, nature of stressful stimuli, physiological status, route, and form of VC administration may influence and cause variations in response following supplementation. There is, therefore, a need to explore these areas to establish the most effective supplementation rate of VC as well as route and/or form.

2.15 Reference

Abd-Allah, M. and Zanouny, A.I. (2014). Ameliorative effect of ascorbic acid administration and chilled drinking water on ram lambs exposed to heat stress during summer season. *Egypt. J. Sheep & Goat Sci*. **9(3**), 17-28.

Akinmoladun, O.F., Fon, F.N., Mpendulo, C.T. and Okoh, O. (2020a). Performance, heat tolerance response and blood metabolites of water restricted Xhosa goats supplemented with vitamin C. *Transl. Anim. Sci*. **4(2)**, 1-15.

Akinmoladun, O.F., Fon, F.N. and Mpendulo, C.T. (2020b). Stress indicators, carcass characteristics and meat quality of Xhosa goats subjected to different watering regimen and vitamin C supplementation. *Livest. Sci*. **238**, 104083.

Akinmoladun, O.F., Muchenje, V., Fon, F.N. and Mpendulo, C.T. (2019). Small ruminants: farmers' hope in a world threatened by water scarcity. *Animals* **9(7**), 456.

Al-Gubory, K.H., Fowler, P.A. and Garrel, C. (2010). Roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. *Int. J. Biochem. Cell Biol*. **42,** 1634-1650.

Ali, B.H. and Al-Qarawi, A.A. (2002). An evaluation of drug used in control of stressful stimuli in domestic animals: A review. *Acta Vet. Brno.* **71(2**), 205-216.

Ali, B.H., Al-Qarawi, A.A. and Mousa, H.M. (2006). Stress associated with road transportation in desert sheep and goats, and the effect of pretreatment with xylazine or sodium betaine. *Res. Vet. Sci*. **80 (3**), 343– 348.

Ali, BH., Al-Qarawi, A.A., Mousa, H.M. and Mohamed, S.M. (2001). The effect of tyrosine on stress associated with transport in Desert sheep. *Vet. Res. Com.* **25,** 503-510.

Andrade, E.R., van den Hurk, R., Lisboa, L.A., Hertel, M.F., Melo-Sterza, F.A., Moreno, K. *et al.* (2012). Effect of ascorbic acid on in vitro culture of bovine preantral follicles. *Zygote* **20**, 379-388.

Atkinson, P.J. (1992). Investigation of the effects of transport and lairage on hydration state and resting behaviour of calves for export. *The Vet. Record* **130**, 413-416.

Archile-Contreras, A.C. and Purslow P.P. (2011). Oxidative stress may affect meat quality by interfering with collagen turnover by muscle fibroblasts. *Food Res. Int*. **44**, 582–588

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 J. Vet. Res.* **76**, 409-418.

Balz, F. (2003). Vitamin C intake. *Nutrient Disease* **14**, 1–18.

Banhegyi, G., Braun, L., Csala, M., Puskas, F. and Mandl, J. (1997). Ascorbic acid metabolism and its regulation in animals. *Free Radic. Biol. Med.* **23**(5), 793-803.

Bansal, A.K. and Bilaspuri, G.S. (2010). Impacts of oxidative stress and antioxidants on semen functions. *Vet. Med. Int.* 686137.

Belge, F., Cinar, A. and Selcuk, M. (2003). Effects of stress produced by adrenocorticotropinon lipid peroxidation and some antioxidants in vitamin C treated and non-treated chickens. *S Afr. J. Anim. Sci.* **33,** 201–205.

Bernabucci, U., Ronchi, B., Lacetera, N. and Nardone, A. (2002). Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. *J. Dairy Sci*. **85**, 2173–2179.

Bernard, V., Young, J. and Binart, N. (2019). Prolactin-a pleiotropic factor in health and disease. *Nat. Rev. Endocrinol.* **15**, 356-365.

Biobaku, K.T., Omobowale, T.O., Akeem, A.O., Aremu, A., Okwelum, N. and Adah, A.S. (2018). Use of goat interleukin-6, cortisol, and some biomarkers to evaluate clinical suitability of two routes of ascorbic acid administration in transportation stress. *Vet. World*, **11(5)**, 674-680.

Brikas, P. (1994). The role of GABA receptors in the control of the omAAl myoelectrical activity in sheep. *Res. Vet. Sci.* **56**, 69–74.

Brown, E.J. and Vosloo, A. (2017). The involvement of the hypothalamo-pituitary-adrenocortical axis in stress physiology and its significance in the assessment of animal welfare in cattle. *Onderstepoort J. Ves. Res.* **84** (1), 1398.

Carr, A.C. and Maggini, S. (2017). Vitamin C and immune function. *Nutrients* **9(11**), 1211.

Carter, C.M. (2018). Alterations in blood components. *Comprehensive Toxicol.* 249-293.

Castillo-Martin, M., Bonet, S., Morato, R.S.B. and Yeste, M. (2014). Supplementing culture and vitrification warming media with Lascorbic acid enhances survival rates and redox status of IVP porcine blastocysts via induction of GPX1 and SOD1 expression. *Cryobiology* **68(3)**, 451-458.

Chaidanya, K., Soren, N.M., Sejian, V., Bagath, M., Manjunathareddy, G B., Kurien, E.K. et al. (2017). Impact of heat stress, nutritional stress and combined (heat and nutritional) stresses on rumen associated fermentation characteristics, histophatology and HSP70 gene expression in goats. *J. Anim. Behav. Biometeorol*. **5**, 36-48

Chambial, S., Dwivedi, S., Shukla, K.K., John, P.J. and Sharma, P. (2013). Vitamin C in disease prevention and cure: An overview. *Indian J. Clin. Biochem.* **28(4)**, 314-328.

Chanda R. (1958). The effect of thyroxine on phosphatise, ascorbic acid and tocopherol content in the blood and milk of the cow and the buffalo. *Curr. Sci. India* **3,** 102–103.

Chatterjee, I.B., Majumder, A.K., Nandi, B.K. and Subramarian, N. (1975). Synthesis and some major functions of vitamin C in animals. *Ann. N.Y. Acad. Sci*. **258**, 24-47.

Chirase, N.K., Green, L.W., Purdy, C.W. *et al.* (2004). Effect of transport stress on respiratory disease, serum antioxidant status, and serum concentrations of lipid peroxidation biomarkers in beef cattle. *Am. J. Vet. Res*. **65 (6)**, 860-864.

Civen, M., Leeb, J.E., Wishnow, R.M. and Morin, R. (1980). Effect of dietary ascorbic acid and vitamin E deficiency on rat adrenal cholesterolester metabolism and corticosteroidogenesis. *Int. J. Vit. Nutr. Res.* **50**, 70–78.

Clagett-Dame, M. and DeLuca, H.F. (2002). The role of vitamin A in mammalian reproduction and embryonic development. *Annu. Rev. Nutr.* **22**, 347–381.

Collier, R.J., Renquist, B.J. and Xiao Y. (2017). A 100-year review: Stress physiology including heat stress. *J. Dairy Sci.* **100**, 10367-10380

Combs Jr, G.F. and McClung, J.P. (2017). The vitamins: Fundamental aspects in nutrition and health. Academic Press, 5th edition. Elsevier, San Diego, CA, USA.

Combs, G.F. (2008). Vitamin C. In: G.F. Combs (ed.). The Vitamins: Fundamental Aspects in Nutrition and Health. Third edition. Elsevier, San Diego, CA, USA.

Corpe, C.P., Eck, P., Wang, J., Al-Haani, H. and Levine, M. (2013). Intestinal dehydroascorbic acid (DHA) transport mediated by the facilitative sugar transporters, GLUT2 and GLUT8. *J. Biol. Chem***.** **288,** 9092–9101.

Dahl, G.E., Tao, S. and Laporta, J. (2020). Heat stress impacts immune status in cows across the life cycle. *Front. Vet. Sci*. **7**, 116.

Daruwala, R., Song, J., Koh, W.S., Rumsey, S.C. and Levine, M. (1999). Cloning and functional characterization of the human sodium-dependent vitamin C transporters hSVCT1 and hSVCT2. *FEBS Lett.* **460,** 480–484.

Das, P.C., Das, K.P., Bagchi, K. and Dey, C.D. (1993). Evaluation of tissue ascorbic acid status in different hormonal states of female rat. *Life Sci.* **52**, 1493-1498.

Dawood A. (2015). Adoption of agricultural innovations: Investigating current status and barriers to adoption of heat stress management in small ruminants in Jordan. *Amer.-Euras. J. Agric. Environ. Sci.* **15**, 388–398.

Deters E.L. and Hansen S.L. (2020). Pre-transit vitamin C injection improves post-transit performance of beef steers. *Animal*, **14**(10), 2083-2090

Duff, G.C. and Galyean, M.L. (2007). Board-Invited Review: Recent advances in management of highly stressed, newly received feedlot cattle. *J. Anim. Sci.* **85**, 823-840.

Ecü, T.K.E., Keskün, E. and Durgun, Z. (2000). The effects of environmental temperature and vitamin C on thyroid hormones of blood serum and some haematological values in Brown Swiss bulls. *Turk. J. Vet. Anim. Sci.* **24**, 353–359.

Eidan, S.M. (2016). Effect on post-cryopreserved semen characteristics of Holstein bulls of adding combinations of vitamin C and either catalase or reduced glutathione to Tris extender. *Anim. Reprod. Sci.* **167**, 1–7.

Elsayed, M.A., Taha, N.A., Hammam, A.M.M. and Sawiress, F.A.R. (2015). Effect of antioxidant supplementation on *in vitro* maturation of *Camelus dromedaries* oocytes. *J. Nat. Sci.* **13**(2), 17-24.

Eppig, J.J., Hosoe, M., O'Brien, M.J., Pendola, F.M., Requena, A. and Watanabe, S. (2000). Conditions that affect acquisition of developmental competence by mouse oocytes *in vitro*: FSH, insulin, glucose and ascorbic acid. *Mol. Cell. Endocrinol.* **163,** 109-116.

FAO, (2020). Food and Agricultural Organization of the United Nations: Reducing enteric methane for improving food security and livelihoods.

Fazeli, P., Zamiri, M.J., Farshad, A. and Khalili, B. (2010). Effects of vitamin C on testicular and seminal characteristics of Markhoz goats. *Iranian J. Vet. Res*. **11**(3), 32.

Ferguson, D.M., Bruce, H.L., Thompson, J.M., Egan, A.F., Perry, D. and Shorthose, W.R. (2001). Factors affecting beef palatability–Farmgate to chilled carcass. *Australian J. Exp. Agric*. **41**, 879–891.

Ferguson, D.M. and Warner, R.D. (2008). Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants? *Meat Sci*. **80**, 12-19.

Ganaie, A.H., Hooda, O.K., Singh, S.V. and Upadhdyay, R.C. (2012). Response of vitamin C supplementation on biochemical, hormonal and physiological parameters of pregnant Murrah buffaloes during hot-humid conditions. *Indian J. Anim. Nutr.* **293**: 214-21.

Ghanem, A.M., Jaber, L.S., Abi Said, M., Barbour, E.K. and Hamadeh, S.K. (2008). Physiological and chemical responses in water-deprived Awassi ewes treated with vitamin C. *J. Arid Environ*. **72,** 141–149.

Gupta M., Kumar S., Dangi S.S. and Jangir B.L. (2013). Physiological, biochemical and molecular responses to thermal stress in goats. *Intern. J. Livest. Res*. **3**, 27–38.

Haiying, L., Padilla, L., Yoshimatsu, K., Matsui, T., Kitagawa, M. and Yano, H. (2003). Determination of plasma vitamin C concentration in fattening cattle. *Anim. Sci. J.* **74**, 7-10.

Hammami, S., Morató, R., Romaguera, R., Roura, M., Catalá, M.G., Paramio, M.T., Mogas, T. and Izquierdo, D. (2013). Developmental competence and embryo quality of small oocytes from pre-pubertal goats cultured in IVM medium supplemented with low level of hormones, insulin-transferrin-selenium and ascorbic acid. *Reprod. Domest. Anim.* **48**(2**)**, 339-44.

Hashem, N.M., Abd-Elrazek, D., Abo-Elezz, Z.R. and Latiff, M.G.A. (2016). Effect of vitamin A or C on physiological and reproductive response of Rahmani ewes during subtropical summer breeding season. *Small Rumin. Res*. 144, 313-319.

Hashem, N.M., El-Azrak, K.M., Nour El-Din, A.N.M., Taha, T.A. and Salem, M.H. (2015). Effect of GnRH treatment on ovarian activity and reproductive performance flow-prolific Rahmani ewes. *Theriogenology* **83**, 192–198.

Hidiroglou, M. (1999). Technical note: forms and route of vitamin C supplementation for cows. *J. Dairy Sci.* **82,** 1831-1833.

Hidiroglou, M., Batra, T.R. and Zhao, X. (1997). Comparison of vitamin C bioavailability after multiple or single oral dosing of different formulations in sheep. *Reprod. Nutr. Dev*. **37**, 443-448.

Hoyda T.D., Samson W.K. and Ferguson A.V. (2012). Central system roles for adiponectin in neuroendocrine and automic function. In: V.R. Preedy R. J. Hunter (eds), Adipokines, Science Publishers, CRC Press, Boca Raton, FL, USA, pp. 167– 184

Ibironke, A.A., McCrindle, C.M.E., Adejuwon, T.A. and Cadmus, S.I.B. (2010). Losses associated with mortality of cattle and camels during transportation to Oko- Oba abattoir, Lagos, Nigeria. *Eur J Translational Myology - Basic Appl Myology* **1**, 13-16.

Kandemir C., Kosum N. and Taskin T. (2013). The effects of heat stress on physiological traits in sheep. *Macedonian J. Anim. Sci*. **3,** 25–29.

Karanth, S., Yu, W.H., Walczewska, A., Mastronardi, C. and McCann, M. (2000). Ascorbic acid acts as an inhibitory transmitter in the hypothalamus to inhibit stimulated luteining hormone-releasing hormone release by scavenging nitric oxide. *PNAS* **97,** 1891–1896.

Karl, J.P., Hatch, A.M., Arcidiacono, S.M., Pearce, S.C., Pantoja-Feliciano, I.G., Doherty, L.A. and Soares, J.W. (2018). Effects of psychological, environmental and physical stressors on the gut microbiota. *Front. Microbiol*. **9,** 2013.

Kassab, A.Y. and Mohammed, A.A. (2014). Ascorbic acid administration as anti-stress before transportation of sheep. *Egyptian J. Anim. Prod.* **51** (1), 19-25.

Khanday, S.B., Ahmed, J.A., Nashiruddullah, N., Sharma, U. and Chakraborty, D. (2016). Effect of antioxidant ascorbic acid on in vitro maturation of Caprine oocytes under normal and elevated temperatures. *Indian J. Anim. Res*. **53**(8**)**, 1020-1024.

Kim J., Mamuad, L.L., Yang, C., Chul-Ju, K.S., Ha J.K., Lee, W., Cho, K. and Lee, S. (2012). Hemato-biochemical and cortisol profile of Holstein growing-calves supplemented with vitamin C during summer season. *Asian Austral J. Anim. Sci*. **25,** 361– 68.

Konwar, D., Amonge, T.K., Dutta, D.J., Gogoi, A.K., Borah, R.S., Ch. Das, G., Bhuyan, R. and Roychoudhury, R. (2017). Dietary supplementation of ascorbic acid on hemato-biochemical and hormonal parameters in swamp buffaloes. *J. Anim. Res.* **7**(1), 39-47.

Koolhaas, J.M., de Boer, S.F., Buwalda, B. and van Reenem, K. (2007). Individual variation in coping with stress: a multidimensional approach of ultimate and proximate mechanisms. *Brain Behav. Evol*. **70**(4), 218-26.

Kumar, B., Manuja, A. and Aich, P. (2012). Stress and its impact on farm animals. *Front. Biosci*. **4,** 1759-1767.

Kumar, S.B.V., Singh, G. and Meur, S.K. (2010). Effects of addition of electrolyte and ascorbic acid in feed during heat stress in buffaloes. *Asian-Austr. J. Anim. Sci.* **23**, 880-88.

Kurutas, E.B. (2016). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutr. J.* **15**, 17.

Lechowski, J., Kasprzyk, A., Tyra, M. and Trawinska, B. (2016). Effect of ascorbic acid as a feed additive on indicators of the reproductive performance of Pulawska breed gilts. *Med. Weter*. **72**(6), 378-382.

Li, Y. and Schellhorn, H.E. (2007). New developments and novel therapeutic perspectives for vitamin C. *J. Nutr.* **137**, 2171-2184.

Liu, L., Scheffer, K.K. and Shuefea, D.M. (1994). Jugular infusion of vitamin C and color stability of beef. *J. Anim. Sci.* **72**(Suppl. 1),372.

Mahan, D.C., Ching, S. and Dabrowski, K. (2004). Developmental aspects and factors influencing the synthesis and status of ascorbic acid in the pig. *Annual Rev. Nutr*. **24**, 79–103.

Mancini, A., Segni, C.D., Raimondo, S., Olivieri, G., Silvestrini, A., Meucci, E. and Curro, D. (2016). Thyroid hormones, oxidative stress and inflammation. *Mediat. Inflamm.* **2016,** 6757154.

Marks R. (2004). The stratum corneum barrier: The final frontier. *J. Nutr*. 134, 2017–2021.

May, J.M., Qu, Z.C. and Whitesell, R.R. (1995). Ascorbic acid recycling enhances the antioxidant reserve of human erythrocytes. *Biochemistry* **34**, 12721–12728.

McDowell, L.R. (2000). Vitamins in Animal and Human Nutrition. Iowa State University Press, Ames, IA.

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

Minka, N.S. and Ayo, J.O. (2010). Behavioural and rectal temperature responses of Black Harco pullets administered vitamin C and E and transported by road during the hot-dry season. *J. Vet. Behav. Clin. Appl. Res.* **5,** 134–144.

Minka, N.S. and Ayo, J.O. (2011). Modulating effect of ascorbic acid on transport-induced immunosuppression in goats. ISRN *Vet. Sci.* **2011,** 749753.

Minka, N.S. and Ayo, J.O. (2007). Physiological responses of transported goats treated with ascorbic acid during the hot-dry season. *Anim. Sci. J.* **78,** 164–172.

Minka, N.S., Ayo, J.O., Sackey, A.K.B. and Adelaiye, A.B. (2009). Assessment and scoring of stresses imposed on goats during handling, loading, road transportation and unloading, and the effect of pretreatment with ascorbic acid. *Livest. Sci.* **125,** 275-282.

Mir, N.A., Ashutosh, A., Shergojry, S.A., Wani, S.A. and Sheikh, F.A. (2019). Effect of induced transportation stress in goats supplemented with vitamin C and jaggery during hot dry season. *Biol Rhythm Res*. **2019,** 50(3), 389-399.

Mitmesser, S.H., Ye, Q., Evans, M. and Combs, M. (2016). Determination of plasma and leukocyte vitamin C concentrations in a randomized, double-blind, placebo-controlled trial with Ester-C. *Springer Plus*, **5,** 1161.

Mohamed, H.E., Mousa, H.M. and Beynen, A.C. (2004). Vitamin C status of Sudanese cattle and sheep. *J. Biol. Sci.* **4,** 778–779.

Morera, P., Basirico, L., Hosoda, K. and Bernabucci, U. (2012). Chronic heat stress up-regulates leptin and adiponectin secretion and expression and improves leptin, adiponectin and insulin sensitivity in mice. *J. Mol. Endocrinol*. **48,** 129– 138.

Murray, A.A., Molinek, M.D., Baker, S.J., Kojima, F.N., Smith, M.F., Hillier, S.G. and Spears, N. (2001). Role of ascorbic acid in promoting follicle integrity and survival in intact mouse ovarian follicles follicles *in vitro*. *Reproduction* **121(**1), 89-96.

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. *Livest. Sci*. **130**, 57–69.

Nejad, J.G. and Sung, K.I. (2017). Behavioral and physiological changes during heat stress in Corriedale ewes exposed to water deprivation. *J. Anim. Sci. Technol. **59***, 13.

Novais, A., Monteiro, S., Roque, S., Correia-Neves, M. and Sousa, N. (2017). How age, sex and genotype shape the stress response. *Neurobiol. Stress* **6,** 44-56.

Nwe, T.M., Hori, E., Manda, M. and Watanabe, S. (1996). Significance of catecholamines and cortisol levels in blood during transportation stress in goats. *Small Rumin. Res.* **20**(2), 129–135.

Nwunuji, T.P., Mayowa, O.O., Yusoff, S.M., Bejo, S., Salisi, S. and Mohd E.A. (2014). The ameliorative effect of ascorbic acid on the oxidative status, live weight and recovery rate in road transport stressed goats in a hot humid tropical environment. *Anim. Sci. J.* **85**(5), 611-616.

Omontese, B.O., Rekwot, P.I., Ate, I.U. and Rwuaan, J.S. (2014). Ascorbic acid enhances conception rates of Red Sokoto goats following progestin (FGA-30 ®, FGA-45® and CIDR®) treatment during rainy season, *Livest. Res. Rural* **26**, 130.

Padayatty, S.J. and Levine, M. (2016). Vitamin C physiology; the unknown and the unknown and Goldilocks. *Oral Dis*. **22**(6), 463-493.

Padilla, L., Matsui, T., Ikeda, S., Kitagawa, M. and Yano, H. (2007). The effect of vitamin C supplementation on plasma concentrations and urinary excretion of vitamin C in cattle. *J. Anim. Sci.* **85**, 3367-3370.

Parker, A.J., Hamlin, G.P., Coleman, C.J. and Fitzpatrick, L. (2003). Dehydration in stressed ruminants may be the result of a cortisol-induced diuresis. *J. Anim. Sci.* 81, 512–519.

Parraguez, V.H., Atlagich, M., Araneda, O., García, C., Mu˜noz, A., De Los Reyes, M. and Urquieta, B. (2011). Effects of antioxidant vitamins on newborn and placental traits in gestations at high altitude: comparative study in high and low altitude in native sheep. *Reprod. Fertil. Dev*. **23**, 285–296.

Pasonen-Seppanen, S., Suhonen, T.M., Kirjavainen, M., Suihko, E., Urtti, A., Miettinen, M. *et al.* (2001). Vitamin C enhances differentiation of a continuous keratinocyte cell line (REK) into epidermis with normal stratum corneum ultrastructure and functional permeability barrier. *Histochem. Cell. Biol.* **116**, 287–297.

Pernes, A., Miclea, I., Zahan, M., Miclea, V., Orlovschi, D. and Codea, A.R. (2016). The influence of ascorbic acid on *in vitro* maturation of canine oocytes. *Bulletin UASVM Anim. Sci. Biotechn.* **73**(2), 230-234.

Popkin, B.M., D'Anci, K.E. and Rosenberg, I.H. (2010). Water, hydration and Health. *Nutr. Rev*. **68(8)**, 439-458.

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

Pullar, J.M., Carr, A.C. and Vissers, M.C.M. (2017). The roles of vitamin C in skin health. *Nutrients* **9**(8), 866.

Reinheim, B., Horsberg, T.E., Soli, N.E., Ryeng, K.A. and Arnemo, J.M. (2000). The effect of medetomidine and its reversal with atipamezole on plasma cortisol and noradrenaline in cattle and sheep. *J. Vet. Pharmacol. Therap.* **23,** 379-387.

Rejeb, M., Sadraoui, R. and Najar, T. (2016). Role of Vitamin C on immune function under heat. *Proc. Natl. Acad. Sci. USA* **94**, 13816–13819.

Reynolds, L.P., Wulster-Radcliffe, M.C., Aaron, D.K. and Davis, T.A. (2015). Importance of animals in agriculture sustainability and food security. *J. Nutr*. **145**, 1377-1379.

Rong-Zhen, Z. and Dao-Wei Z. (2013). Oxidative stress and role of natural plant derived antioxidants in animal reproduction. *J. Integr. Agric*. **12,** 1826-1838.

Rucker, R.B., Morris, J. and Fascetti, A.J. (2008). Clinical Biochemistry of Domestic Animals: Vitamins, 6th Ed. Academic Press. Pg. 695-730.

Sahin, K., Kucuk, O., Sahin, N. and Sari, M. (2002). Effects of vitamin C and vitamin E on lipid peroxidation status, serum hormone, metabolite, and mineral concentrations of Japanese quails reared under heat stress (34 1C). *Inter. J. Vit. Nutr. Res*. **72,** 91–100.

Salinas, R.T., Sánchez, T.E.M.T., Díaz, C.A., Cordero, M.J.L., Guinzberg, P.R., Rabanales, M.J.L., Figueroa, V.J.L. and Hernández, B.J. (2017). Oxidative state of ewes with different number of parity during gestation y lactation. *Pesq. Vet. Bras*. **37**, 1405-1410.

Seifi, H.A., Mohri, M., Delaramy, M. and Harati, M. (2010). Effect of short-term over-supplementation of ascorbic acid on hematology, serum biochemistry and growth performance of neonata dairy calves. *Food Chem. Toxicol.* **48**, 2059-2052.

Serpek, B., Baspinar, N., Haliloglu, S. and Erdem, H. (2001). The relationship between ascorbic acid, oestradiol 17 and progesterone in plasma and in ovaries during the sexual cycle in cattle. Revue. *Méd. Vét.* **152,** 253–260.

Srivastava, K.K. and Kumar, R. (2015). Stress, oxidative injury and disease. *Indian J. Clin. Biochem*. **30**(1), 3-10.

Sivakumar, A.V.N., Singh, G. and Varshney, V.P. (2010). Antioxidants supplementation on acid base balance during heat stress in goats. *Asian-Austr. J. Anim. Sci*. **23**(11), 1462 – 68.

Smith, S.B., Kawachi, H., Choi, C.B., Choi, C.W., Wu, G. and Sawyer. J.E. (2009). Cellular regulation of bovine intramuscular adipose tissue development and composition. *J. Anim. Sci.* **87**(E. Suppl.), E72–E82.

Sonmez, M. and Demirci, E. (2003). The effect of intramuscular vitamin C administration on semen quality in rams. *Journal of Firat University, Health Vet. Sci.* **17,** 195-201.

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, *J. Anim. Sci.* **11**, 2632–2636.

Steffens, A.B. and de Boer, S.F. (1999). Impact of stress on animal intermediate metabolism. In: Balm, P.H.M. (Ed.), Stress Physiology in Animal. Sheffield Academic Press, pp. 108–129.

Tauler P., Aguilo, A., Gimeno, I., Fuentespis, E., Tur, J.A. and Pons, A. (2003). Influence of vitamin C supplementation on endogenous antioxidant defense during exhaustive exercise. *Eur. J. Phys.* **440,** 658– 664.

Thomas, F.H., Leask, R., Sršen, V., Riley, S.C., Spears, N. and Telfer, E.E. (2001). Effect of ascorbic acid on health and morphology of bovine preantral follicles during long-term culture. *Reprod*. **122**, 487-495.

Thornton P.K. (2010). Livestock production: Recent trends, future prospects. *Phil. Trans. R. Soc. B*. **365,** 2853–2867.

Tu, Y., Njus, D. and Schlegel, H.B. (2017). A theoretical study of ascorbic oxidation and HOO/O2 radical scavenging. *Org. Biomol.Chem*. **15,** 4417.

United Nation (2017). Sustainable Development Goals. Revision of World Population Prospects; United Nations, San Francisco, CA, USA.

Urban-Chmiel, R., Kankofer, M., Wernicki, A., Albera, E. and Puchalski, A. (2009). The influence of different doses of -tocopherol and ascorbic acid on selected oxidative stress parameters in in vitro culture of leucocytes isolated from transported calves. *Livest. Sci*. **127,** 365–370.

Weller R.H., John A., Savin J. and Dahl M. (2008). The Function and Structure of Skin. 5th ed. Wiley-Blackwell; Massachusetts, MA, USA.

Yousef, M.I. (2004). Aluminium-induced changes in hemato-biochemical parameters, lipid peroxidation and enzyme activities of male rabbits: protective role of ascorbic acid. *Toxicology* **199**, 47–57.

Chapter Three

Growth performance, heat tolerance response and blood biochemical profile of water restricted Xhosa goats supplemented with vitamin C

(Published in *Translational Animal Science* Journal)

Abstract: Water restriction in small ruminants is usually accompanied by a drop in feed intake, body weight and disturbances in the normal internal milieu. However, attempts to lessen the burden of water stress with vitamin C supplementation have been greeted with conflicting reports. Therefore, this experiment was conducted to evaluate the effect of single and multiple vitamin C (VC) supplementations in water restricted Xhosa goats by assessing their performance, heat tolerance and blood metabolites. In total, 42 does, 12 months old and with an average weight of 15.92±2.12 kg, were evaluated for 75 days. The does were distributed into seven groups of six comparable animals: without water restriction (control, W0); water restriction of 70% of *ad libitum* water intake WI (W70); water restriction of 50% *ad libitum* WI (W50); water restriction of 70% of *ad libitum* WI plus 3g VC daily ($W70^+$); water restriction 50% of *ad libitum* WI plus 3g VC daily ($W50^+$); water restriction of 70% of *ad libitum* WI plus 3g VC and extra 5gVC given every eight-day ($W70^{++}$); water restriction of 50% of *ad libitum* WI plus 3g VC and extra 5gVC given every eight-day ($W50^{++}$). Regardless of VC supplementation, the body condition scores (BCs) decreased ($P<0.05$) with levels of water restriction. Weight loss due to water restriction was reduced by VC supplementation in treated groups. Changes in body thermal gradient, rectal temperature (RcT), cholesterol and bilirubin were similar ($P>0.05$) across the various experimental groups. The attenuation effect of VC was significant ($P<0.05$) in responses to respiratory rate (RR), Na^+, K^+, Mg^{2+}, Cl^-, Ca^{2+} and urea. Supplementation of VC (either single or multiple) did not ($P>0.05$) improve the effect of water restriction on body condition scores (BCs), FAMACHA©, glucose, globulin, alanine aminotransferase (ALT) and high-density lipoprotein (HDL). The additive effect of multiple VC influenced ($P<0.05$) Na^+ and Mg^{2+}. Limited water intake affects growth and other physiological parameters in Xhosa goats. However, supplementation of vitamin C may be beneficial at modulating the stressful stimuli imposed by water stress.

Keywords: vitamin C, Xhosa goat, thermo-tolerance, water stress, blood metabolites

---

3.1 Introduction

Small livestock such as goats contributes immensely to reducing poverty and improving livelihoods, especially for resource-limited rural communities and marginalized families in dry and water-limited regions of the world. The pivotal role they play ranges from animal protein provision, income generation from sales either as live animals or their primal cuts in the markets, to religious and cultural purposes. However, the sustainable productions of livestock are under threat due to increasing water scarcity and fluctuating precipitation. Irregularities in rainfall patterns caused by global warming and vagaries in weather conditions have limited the amount of fresh water available to most regions worldwide (Kurylyk and MacQuarrie, 2013). South Africa is considered a water-scarce country (Donnenfeld *et al.*, 2018) and goat production systems in the Eastern Cape Province's semi-arid region range from nomadic to semi-sedentary or exclusive scavenging type. Under this traditional pastoral farming system, animals are forced to walk a long distance, searching for water and feed throughout the year. Usually, the watering points accessible to these animals cannot be relied upon because they quickly dry up during the dry summer season. Animals in the field are therefore faced with dehydration while grazing far from widely spread watering points to meet their nutrient requirements.

Goats, especially the indigenous ones, are more adaptable to harsh environmental conditions of drought and heat (Silanikove, 2000). They can efficiently utilize limited forage and are less susceptible to endemic diseases than exotic or non-adaptable breeds. The Xhosa ear-lobe breed, indigenous to the Eastern Cape region, is well adapted to the semi-arid environment characterized by temperature extremes and limited water availability, a trait acquired through natural selection over the years. However, thermo-tolerance and the ability to withstand sub-optimal water intake vary with animal types, breed, and extent of adaptability. Although adaptation can be enhanced following prolonged exposure by livestock, studies have documented considerable differences in response in the different breeds (Habibu *et al.*, 2017). For example, desert goats raised under traditional systems may be watered once every 3-6 days during water scarcity. In contrast, the Black Bedouin and Barmer goats, an adapted breed, can live on a once every four-day watering regime (Silanikove, 1994). A comprehensive review of the adaptability and tolerance of small ruminants and their breeds to different water deprivation levels or restriction has been documented (Akinmoladun *et al.*, 2019). Despite this efficient water use in adaptable breeds, water balance disturbances portend a stressful stimulus and have been shown to hamper feed intake and body weight, influence the body heat balance and the composition of the body fluids (Akinmoladun *et al.*, 2019).

Supplementation of vitamin C (VC) to ruminants' diets is not common because they can bio-synthesize L-ascorbic acid through the glucuronic acid pathway in the liver (McDowell, 2000). However, plasma vitamin C is usually depleted during stress and disease conditions (Ranjan *et al.*, 2005), hence a possibility of improvement if supplemented. In addition, the endogenous metabolic process leading to the production of L-ascorbic acid takes too long. Hence, the need for exogenous supplementation. Despite the positive outcome following VC supplementation, others have reported a non-significant effect. For example, oral vitamin C (ascorbic acid) supplementation lessens body weight loss in goats exposed to heat and transportation stress (Minka and Ayo, 2012). Also, ewes' ability to withstand limited water intake (Ghanem *et al.*, 2008), reproductive traits of pregnant ewes, and their newborn lambs' weight were enhanced following VC supplementation (Haliloglu and Serpek, 2000). However, VC supplementation was found to have little or no effect on summer heat-induced stress in Rahmani ewes (Hashem *et al.*, 2016).

The mechanisms that allow indigenous goat breed to thrive, despite the harsh and unfavourable environmental conditions, are yet to be investigated. Furthermore, information about Xhosa ear-lobe goats' response, following supplementation of different vitamin C doses, to the combined effect of water stress and high ambient temperature is scarce. It was hypothesized that vitamin C's bioavailability would be boosted following multiple vitamin C supplementations and ultimately reduce the burden of water stress imposed on the animals. Therefore, this study focused on growth performance, heat tolerance, and blood metabolites of water restricted Xhosa ear-lobe supplemented with vitamin C

3.2 Materials and Methods

All experimental procedures complied with the guidelines of the Research Ethics Committee of the University of Fort Hare, South Africa (Ref. No: MUC011SAKI01).

3.2.1 Study site description

The experiment was conducted at the Honeydale farm, University of Fort Hare. It is located 5km east of Alice, Eastern Cape, South Africa, and 520 m above sea level. It is situated in the False Thornveld of the Eastern Cape with geographical coordinates, 32° 46′S, 26° 52′E, and receives 480-490 mm annual rainfall. The study was conducted during the summer season.

3.2.2 Experimental animals and management

Forty-two female goats (Xhosa breed) with an average age of 12 months and body weight 15.92±2.12 kg were used for the 75-days trials. The goats were de-wormed using ivermectin and vaccinated against the foot-and-mouth disease before the experiment. They were kept in individual metabolic cages (1.33 x 0.58 m), provided with a feeder and a water trough. The animals were weighed at the beginning of the trial, and feed was offered as total mixed ration (TMR) based on 4% of their body weights in the ratio of 70:30 of Lucerne hay and concentrate on DM basis, respectively. The ingredient and nutrient composition of the experimental diet is shown in Table 3.1.

3.2.3 Dietary treatments and design

The animals were balanced for body weight and randomly assigned to the seven dietary treatment groups: W0, without water restriction (control); W70, water restriction of 70% of *ad libitum* water intake; W50, water restriction of 50% *ad libitum* water intake; W70^{+}, water restriction of 70% of *ad libitum* water intake plus 3 gVC daily; W50^{+}, water restriction 50% of *ad libitum* water intake plus 3 gVC daily; W70^{++}, water restriction of 70% of *ad libitum* water intake plus 3 gVC and extra 5 gVC given every eight-day; W50^{++}, water restriction of 50% of *ad libitum* water intake plus 3 gVC and extra 5 gVC given every eight-day. It was assumed that the control group (W0) would not be stressed due to its unhindered access to *ad libitum* water intake and thus exempted from VC supplementation. The L-ascorbic acid (VC) used as a supplement was sourced from Minema Chemical Stores, Gauteng, South Africa. All animals receiving VC supplementation were subjected to a 6-day preparation period (coinciding with the last 6-day during the adaptation period), during which they were orally supplemented with 10 g vitamin C/50 ml water/animal. This was based on Ghanem et al. (2008) 's previous work to make sure the animals were at the same vitamin C status. The 3 g/d vitamin C dose was selected based on earlier findings on the effectiveness and higher bioavailability at lower doses (Tyler and Cummins, 2003). A multiple dose of VC was supplemented as against single dose as recommended by Hidiroglou *et al.* (1997). The experimental trial started in January and lasted for 89 consecutive days. The trial was preceded by a 14-day preliminary period.

3.2.4 Feed and growth measurements

Feed was offered two times a day, at 9:00 and 16:00 in equal proportions. The weight of the total mixed ration offered and refused was recorded daily to derive feed intake. Animals were weighed every 7 days before morning feeding, and the average daily gain (ADG) calculated by dividing final body weight gain with days of trial. Water restriction percentages for experimental groups were calculated based on the daily *ad libitum* intake of the control (W0) group. Water was supplied in containers of known volume and was topped-up once a day. In the control (W0) group, does receive *ad libitum* water daily at two different times of the day, at 8:00 and 15:00 h, to determine the quantity of water ingested. Total water intake (WI) was calculated as the difference between the amounts offered and leftovers, rebating water loss due to evaporation. Water loss due to evaporation was calculated by putting buckets filled with water at focal points in the pen so that loss due to evaporation can be inputted when calculating for total water intake. W70 and W50 groups did receive drinking water daily at 70% and 50% of the total water intake recorded in the control group. The efficiency of water use was determined by finding the ratio of water intake to dry matter intake (WI: DMI).

3.2.5 FAMACHA and body condition scores

Body condition score (BCs) was determined by palpating the spinous processes of the lumbar vertebrae and assigning a score as follow: 1 (very lean, sharp prominent backbone and spinal processes, little flesh coverage); 2 (lean); 3 (medium, slight rounding of flesh over the spine); 4 (fat); 5 (very fat, cannot detect any backbone or spinal processes), at 0.5 increments (McGregor, 2011). Goats were also monitored for evidence of anaemia by checking the colour of the ocular mucous membranes using the FAMACHA eye chart and score assigned as follow: 1 (optimal; red colour non-anaemic); 2 (acceptable; red-pink colour non-anaemic); 3 (borderline; pink mildly anaemic); 4 (Dangerous; pink-white anaemic); 5 (fatal; porcelain-white; severely anaemic) (Kaplan *et al.* 2004).

3.2.6 Meteorological parameters and heat tolerance measurements

Ambient temperature and relative humidity were recorded hourly in the experimental station with a portable data logger (Model: MT669, Major Tech, South Africa) during the 75-day trial. The temperature-humidity index (THI) was calculated for the whole 75-days of the treatment. The 75 days was divided into 5 intervals of 15 days each, and temperature-humidity index (THI) was similarly calculated for each of these intervals. The equation described by Marai *et al.* (2007) in which; THI = db ^{o}C - [(0.31-0.31 RH %) (db ^{o}C-14.4)]. Where db ^{o}C = dry bulb thermometer in Celsius and RH = relative humidity (%) was used. The extent of heat stress was determined based on the THI values (i.e. THI <22.2= absence of heat stress; 22.2 to < 23.3 = moderate heat stress; 23.3 to <25.6 = severe heat stress and 25.6> =extreme severe heat stress) (Marai *et al.*, 2007). Rectal temperature (RcT, ^{o}C) and respiration rate (RR, breathes/min) were recorded between the hours of 0900 h and 1400 h on days 15, 30, 45, 60 and 75 of the treatment periods. Rectal temperature (˚C) was measured after inserting a clinical digital thermometer about 5 cm deep into the animals' rectum and making contact with the mucous membrane for 2 minutes. Respiration rate (breathes/min) was obtained by counting flank movements during 1 min, with each inward-outward flank movements counted as one complete respiration. Skin temperatures on the neck, belly and thurl regions were measured using an infrared thermometer (Nubee NUB8380 Temperature Gun, California, USA). The infrared thermometer was held at a 15 cm distance away from the animal without direct contact (Zhang *et al.,* 2019). Each goat's average skin temperature in the current study was calculated by averaging the neck, belly, and thurl region's temperature. According to Richards (1973), body thermal gradients were calculated using the formulas; Internal gradient = rectal temperature – skin temperature; External gradient = rectal temperature – ambient temperature; Total thermal gradient = skin temperature–ambient temperature.

3.2.7 Blood sampling

Blood samples were collected from the jugular vein of each goat (three animals per treatment) into EDTA_ coated and heparinized vacutainer tubes with BD hemogard^{TM} on days 30, 60 and 75. Blood samples in heparinized tubes were centrifuged for 10 min at 3500 rpm using a Model 5403 centrifuge (Geratebay Eppendorf GmbH, Engelsdorp, Germany). Thereafter, the centrifuged samples were stored at -20^{o}C until analysis was conducted. The obtained plasma samples were analysed using a Checks machine (Next/Vetex Alfa Wasserman Analyser) and commercially purchased kits (Siemens). The concentration of blood serum total protein, albumin, creatinine, and alkaline phosphate was spectrophotometrically analysed using colourimetric procedures. Globulin concentration was calculated by subtracting albumin values from the corresponding total protein values. Glucose, total cholesterol, and urea were analysed using enzymatic methods, while aspartate transaminase and alanine transaminase were determined using ultraviolet techniques (Soul *et al.*, 2019).

3.2.8 Statistical analysis

Analysis of variance was performed based on averages per treatment groups using the GLM procedure of SAS (2013) to determine the fixed effect of treatment, experimental period and interactions.

Models;

$Y_{ijk} = \mu + T_i + D_j + (TD)_{ij} + \varepsilon_{ijk}$ and $Y_{ij} = \mu + T_i + \varepsilon_{ij}$ for non- repeated measures, in which Y_{ijk} is the value of the dependent variable determined from an observation taken from each animal, μ is the overall mean, T_i is the fixed effect of the i_{th} treatment (i=1:7), D_j is the fixed effect of the j_{th} day of observation (j=1:5), $(TD)_{ij}$ is the interaction between treatment and day of observation and ε_{ijk} is the residual error of animals. Analyzed data were considered significant at $P<0.05$.

3.3 Results

3.3.1 Temperature humidity index, dry matter intake and body weight changes

Diurnal temperature (^{o}C) and humidity (RH %) pattern in the housing units and body weight changes are shown in Fig.3.1 and Table 3.2, respectively. The average THI in the experimental room was 25.57±0.15 throughout the experiment. After 75 days of water restriction across the various treatment groups, the final body weight did not differ ($P>0.05$). However, values recorded for animals subjected to water restriction in groups W70 and W50 were low compared to the control (W0) group. This decrease in final weight was attenuated in the treated groups ($W70^{+}$, $W50^{+}$, $W70^{++}$ and $W50^{++}$). The extra dose of VC given to the animals on the eight-day ($W70^{++}$ and $W50^{++}$) did not further lessen the weight loss compared to those on single dose ($W70^{+}$ and $W50^{+}$) groups. The final weight (FW) decreased [W0>W70>W50] ($P>0.05$) with levels of water restriction. The body weight gain and average daily gain (ADG) decreased ($P<0.05$) with levels of water restriction. Multiple VC increased ($P<0.05$) the ADG and body weight gain of $W50^{+}$ when compared to W50. DM intake was significantly higher ($P<0.05$) in control (W0) group compared to the entire water restricted groups. The water intake (WI) followed the watering regimen adopted and decreased ($P<0.05$) as the percentage of *ad libitum* water given decreased.

3.3.2 Body thermal gradient and physiological response

The effect of water restriction with or without VC on body thermal gradient and physiological responses (respiratory rate [RR], rectal temperature [RcT]) are shown in Tables 3.3 and 3.4.

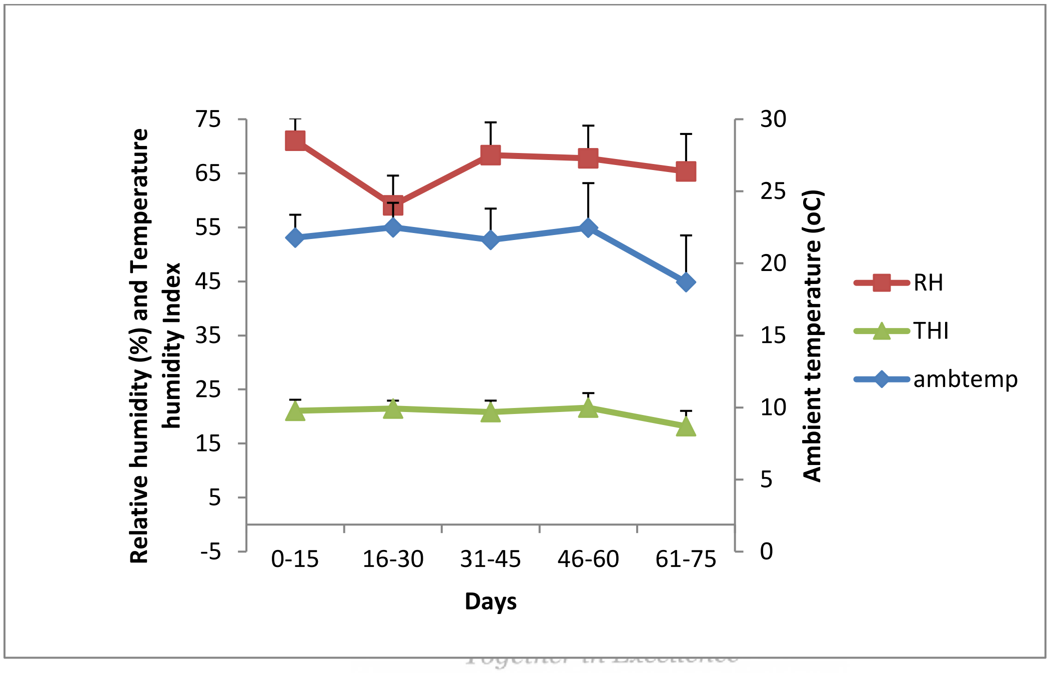


Figure 3. 1 Diurnal temperature and humidity pattern in the housing unit

s

Table 3. 1 Performance of water restricted Xhosa goats supplemented with VC

Parameter	W0	W70	W50	$W70^{+}$	$W50^{+}$	$W70^{++}$	$W50^{++}$	SEM	P-value
IW, kg	15.70	15.63	16.17	16.17	15.83	15.90	16.10	2.24	0.423
FW, kg	17.93	13.93	13.70	14.90	14.53	14.30	14.37	2.07	0.072
Gain, kg	2.23^{a}	-1.70^{bc}	-2.47^{c}	-1.27^{b}	-1.30^{b}	-1.60^{bc}	-1.73^{bc}	0.37	0.021
ADG, g/d	29.78^{a}	-22.67^{bc}	-32.89^{c}	-16.89^{b}	-17.33^{b}	-21.33^{bc}	-23.11^{bc}	4.87	0.015
BW^0.75	8.67	7.16	7.08	7.56	7.43	7.34	7.37	0.78	0.124
DMI, g/d	614.91^{a}	369.09^{b}	324.54^{c}	376.21^{b}	332.27^{c}	376.03^{b}	330.60^{c}	7.76	0.017
DMI, Kg/d	0.62^{a}	0.37^{c}	0.33^{e}	0.38^{bc}	0.34^{de}	0.39^{b}	0.35^{d}	0.008	<0.001
WI, kg	92.47^{a}	62.40^{b}	45.61^{c}	62.40^{b}	45.61^{c}	62.40^{b}	45.61^{c}	4.12	<0.0001
WI:DMI	2.31^{a}	2.25^{a}	1.87^{b}	2.20^{a}	1.83^{b}	2.21^{a}	1.84^{b}	0.09	0.012

ADG= average daily gain; BW^0.75= metabolic weight; DMI= total dry matter intake; SEM=standard error of mean; WI = water intake; ^{a,b,c}means with different superscripts across the row are significantly different (P<0.05); $^{+}$ 3gVC daily; $^{++}$ 3gVC daily+extra 5gVC every 8th days; W=water restriction

Water restriction levels did not significantly affect ($P>0.05$) the skin temperature, internal gradient, external gradient, and total thermal gradient. The effect of water restriction levels with or without VC supplementation was not significant on the RcT ($P>0.05$). All the water restricted groups had significantly lower RR ($P<0.05$) compared to the control (W0) group. The slight increase in the values recorded for RR in the VC supplemented groups ($W70^{+}$, $W50^{+}$, $W70^{++}$ and $W50^{++}$) did not follow a consistent pattern compared to the untreated groups (W70 and W50).

3.3.3 FAMACHA© and body condition scores (BCs)

The FAMACHA and BCs outputs are shown in Table 3.5. Levels of water restriction, with or without vitamin C, did not significantly ($P>0.05$) affect the FAMACHA scores. However, the BCs were significantly affected ($P<0.05$) by levels of water restriction. VC treated groups ($W70^{+}$, $W50^{+}$, $W70^{++}$ and $W50^{++}$) were not statistically different ($P>0.05$) from the untreated groups (W70 and W50) in their BCs. The BCs of goats under W50 were the most affected ($P<0.05$) compared to other water restricted groups. All water restricted groups had lower BCs compared to the water *ad libitum* groups.

3.3.4 Blood biochemical profile

The plasma osmolality and blood chemistry are shown in Tables 3.6 and 3.7. The effect of water restriction levels was significant in plasma osmolality. Plasma osmolality tends to increase ($P<0.05$) with water restriction levels. However, the high plasma osmolality (Na, K, Mg, Cl, and Ca) due to water restriction levels were lowered following VC administration. The effect of extra VC dosing ($W70^{++}$ and $W50^{++}$) did not ($P>0.05$) additively influence the plasma osmolality compared to single dosing ($W70^{+}$ and $W50^{+}$). Supplementation of VC at both single and multiple doses had no significant effect ($P>0.05$) on bilirubin, cholesterol, high and low-density lipoprotein (HDL and LDL).

Table 3. 2 Body thermal gradients of water-restricted Xhosa goats supplemented with VC

Variable	Day	Treatment								Probability		
		W0	W70	W50	$W70^{+}$	$W50^{+}$	$W70^{++}$	$W50^{++}$	SEM	T	D	TxD
Skin Temperature (°C)									0.322	Ns	***	ns
	15	32.07	32.27	32.27	32.01	32.27	32.28	31.68				
	30	30.88	31.04	31.04	30.98	31.23	31.39	31.01				
	45	25.08	23.87	23.87	25.11	25.15	24.11	23.97				
	60	29.30	28.78	28.78	28.67	28.98	28.73	28.88				
	75	26.36	26.53	26.53	26.48	26.17	26.56	26.33				
Internal gradient									0.418	Ns	***	ns
	15	6.33	6.17	6.57	6.99	6.20	6.45	6.79				
	30	7.76	7.11	7.30	7.19	7.20	7.44	7.56				
	45	12.43	14.16	13.52	12.08	12.85	13.66	14.17				
	60	8.20	9.65	9.30	9.73	9.52	8.34	9.39				
	75	11.81	11.53	11.43	12.09	11.25	11.44	11.87				
External gradient									0.272	Ns	***	ns
	15	16.61	16.64	17.04	17.21	16.67	16.94	16.67				
	30	16.13	15.43	16.06	15.67	15.93	16.33	16.07				
	45	15.86	16.39	15.99	15.56	16.36	15.93	16.49				
	60	16.44	17.37	17.71	17.34	17.44	17.31	17.20				
	75	19.48	19.38	19.41	19.88	19.74	19.31	19.51				
Total gradient									0.322	*	***	ns
	15	10.28	10.47	10.47	10.22	10.47	10.49	9.89				
	30	8.38	8.54	8.76	8.48	8.73	8.89	8.51				
	45	3.42	2.22	2.47	3.47	3.50	2.46	2.33				
	60	8.24	7.72	8.41	7.61	7.92	8.97	7.82				
	75	7.67	7.84	7.98	7.79	8.49	7.86	7.64				

ns, $P>0.05$; * $P<0.05$; **$P<0.001$; ***$P<0.0001$; $^{+}$ 3gVC daily; $^{++}$ 3gVC daily+extra 5gVC every 8th days; W=water restriction; SEM= standard error of mean; T=treatment effect; D=Day effect; TxD=interaction between treatment and day.

Table 3. 3 RcT and RR of water restricted Xhosa goats supplemented with VC

Parameter	Day	Treatments								Probability		
		W0	W70	W50	W70^{+}	W50^{+}	W70^{++}	W50^{++}	SEM	T	D	TxD
Rectal Temperature (RcT) (^{o}C)									0.27	ns	**	ns
	15	38.40	38.43	38.83	38.48	38.67	38.73	38.47				
	30	38.63	38.93	38.57	38.47	38.43	38.83	38.57				
	45	39.50	38.03	37.93	38.20	38.00	37.97	38.13				
	60	38.50	38.43	38.77	38.40	38.50	38.37	38.27				
	75	38.17	38.07	38.10	38.47	38.43	38.00	38.20				
Respiratory rate (RR) (breathe/min)									1.34	**	**	*
	15	38.67^{a}	31.33^{b}	30.53^{b}	32.67^{b}	31.17^{b}	33.05^{b}	31.05^{b}				
	30	39.00^{a}	32.33^{bcd}	30.67^{bc}	33.67^{bc}	30.89^{cd}	34.67^{b}	30.79^{d}				
	45	44.00^{a}	35.33^{b}	31.33^{c}	35.43^{b}	32.00^{c}	36.67^{b}	31.59^{c}				
	60	42.67^{a}	35.39^{b}	33.33^{b}	35.67^{b}	33.67^{b}	35.00^{b}	33.52^{b}				
	75	41.33^{a}	34.51^{b}	31.34^{c}	36.67^{b}	31.67^{c}	35.67^{b}	32.03^{c}				

^{abc} means with different superscript across the row are significantly different ($P<0.05$); ns, $P>0.05$; * $P<0.05$; **$P<0.001$; ***$P<0.0001$; $^{+}$ 3gVC daily; $^{++}$ 3gVC daily+extra 5gVC every 8th days; W=water restriction; SEM = Standard error of mean; T=treatment effect; D=Day effect; TxD=interaction between treatment and day.

Table 3. 4 FAMACHA and BCs of water restricted Xhosa goats supplemented with VC

	Day	Treatments								Probability		
Parameter		W0	W70	W50	$W70^{+}$	$W50^{+}$	$W70^{++}$	$W50^{++}$	SEM	T	D	TxD
FAMACHA									0.293	*	**	ns
	15	2.67^{ab}	2.33^{b}	2.33^{b}	2.67^{ab}	2.67^{ab}	3.00^{a}	2.33^{b}				
	30	2.33^{b}	2.33^{b}	2.00^{b}	2.33^{b}	2.33^{b}	2.33^{b}	3.00^{a}				
	45	3.00^{ab}	2.67^{b}	3.00^{ab}	2.67^{b}	3.00^{ab}	3.00^{ab}	3.33^{a}				
	60	3.00	3.00	3.33	3.33	3.00	3.33	3.33				
	75	2.67	2.67	3.00	3.00	3.00	3.00	2.67				
BCs									0.252	***	*	ns
	15	3.00	2.33	2.33	2.67	2.67	2.33	2.67				
	30	3.33	2.33	2.00	2.33	2.00	2.67	2.33				
	45	2.67^{a}	2.33^{ab}	1.33^{c}	2.33^{ab}	2.33^{ab}	2.33^{ab}	2.00^{bc}				
	60	3.00^{a}	2.00^{b}	2.00^{b}	2.00^{b}	2.00^{b}	2.67^{a}	2.00^{b}				
	75	3.00^{a}	2.67^{ab}	2.00^{c}	2.00^{c}	2.00^{c}	2.33^{bc}	2.00^{c}				

^{abc} means with different superscript across the row are significantly different (P<0.05); ns, P>0.05; * P<0.05; **P<0.001; ***P<0.0001; $^{+}$ 3 gVC daily; $^{++}$ 3 gVC daily+extra 5 gVC every 8th days; W=water restriction; SEM= standard error of mean; T=treatment effect; D=Day effect; TxD=interaction between treatment and day

Table 3. 5 Plasma osmolality of water-restricted Xhosa goats supplemented with VC

Parameter	Day	Treatment								Probability		
		W0	W70	W50	$W70^{+}$	$W50^{+}$	$W70^{++}$	$W50^{++}$	SEM	T	D	TxD
Na (mmol/L)									1.21	**	***	ns
	30	135.33^{b}	138.00^{a}	139.00^{a}	135.00^{b}	137.00^{b}	133.00^{c}	134.67^{c}				
	60	136.67^{bc}	136.00^{c}	138.67^{ab}	136.67^{bc}	139.67^{a}	137.67^{abc}	137.00^{bc}				
	75	136.65^{d}	138.00^{cd}	142.00^{a}	140.33^{bc}	143.67^{a}	137.67^{d}	140.33^{bc}				
K (mmol/L)									0.16	**	***	***
	30	5.40^{c}	5.70^{b}	6.10^{a}	5.43^{c}	4.80^{e}	5.13^{d}	5.23^{cd}				
	60	4.70^{c}	5.00^{b}	4.60^{c}	5.00^{b}	5.00^{b}	5.13^{ab}	5.33^{a}				
	75	4.52^{c}	5.06^{b}	5.13^{b}	5.06^{b}	4.70^{c}	4.47^{c}	5.60^{a}				
Mg(mmol/L)									0.05	**	***	**
	30	0.95^{a}	0.87^{a}	0.65^{c}	0.86^{a}	0.74^{b}	0.87^{a}	0.66^{c}				
	60	1.00^{a}	0.78^{b}	0.65^{c}	0.90^{a}	0.71^{b}	0.93^{a}	0.79^{b}				
	75	1.19^{a}	1.13^{ab}	1.03^{bc}	1.01^{cd}	1.05^{bc}	0.96^{cd}	0.94^{d}				
Cl (mmol/L)									1.42	*	***	*
	30	100.67^{bc}	103.33^{ab}	105.00^{a}	96.67^{de}	95.33^{e}	97.67^{de}	100.33^{ab}				
	60	100.00^{b}	101.67^{b}	104.67^{a}	100.67^{b}	96.00^{c}	100.00^{b}	95.00^{c}				
	75	101.67^{b}	105.00^{b}	105.67^{a}	101.27^{b}	102.00^{ab}	102.67^{ab}	101.52^{b}				
Ca (mmol/L)									0.05	**	***	***

30	2.05^{a}	1.95^{ab}	1.62^{d}	1.86^{b}	1.84^{c}	1.94^{abc}	1.86^{b}
60	2.10^{a}	1.85^{b}	1.73^{c}	1.86^{b}	2.08^{a}	2.08^{a}	2.02^{a}
75	2.12	2.10	2.02	2.07	2.11	2.12	2.05

^{abc} means with different superscript across the row are significantly different ($P<0.05$); ns, $P>0.05$; * $P<0.05$; **$P<0.001$; ***$P<0.0001$; $^{+}$ 3gVC daily; $^{++}$ 3gVC daily+extra 5gVC every 8th days; W=water restriction; SEM= standard error of mean; T=treatment effect; D=Day effect;TxD=interaction between treatment and day

Table 3. 6 Blood chemistry of water restricted Xhosa goats supplemented with VC

Parameter	Day	Treatment								Probability		
		W0	W70	W50	W70^{+}	W50^{+}	W70^{++}	W50^{++}	SEM	T	D	Tx D
Urea (mmol/L)									0.48	**	**	**
	30	5.10^{d}	7.07^{bc}	8.63^{a}	6.85^{c}	7.80^{ab}	7.10^{b}	8.25^{a}				
	60	8.60^{c}	8.73^{c}	10.33^{ab}	7.50^{d}	9.73^{b}	8.55^{c}	11.00^{a}				
	75	8.57^{c}	11.27^{a}	11.77^{a}	9.30^{bc}	11.43^{a}	9.80^{b}	11.73^{a}				
Creatinine (mmol/L)									3.26	**	**	*
	30	53.67	54.00	55.67	56.00	55.33	54.00	56.67				
	60	40.00^{c}	43.33^{bc}	49.33^{ab}	48.33^{ab}	50.00^{a}	40.00^{c}	45.00^{abc}				
	75	43.00	45.00	48.67	44.00	48.00	43.00	46.67				
Glucose (mmol/L)									0.12	**	**	**
	30	3.23^{a}	2.80^{b}	2.37^{c}	2.97^{b}	2.33^{c}	2.40^{c}	2.30^{c}				
	60	2.47^{a}	2.37^{ab}	2.20^{bc}	2.40^{ab}	1.97^{d}	2.42^{a}	2.00^{cd}				
	75	3.00^{a}	2.90^{ab}	2.70^{bcd}	2.97^{b}	2.53^{de}	2.37^{ab}	2.45^{e}				
Total Protein (g/L)									2.20	**	**	**
	30	51.00^{f}	60.67^{cd}	70.00^{a}	56.67^{de}	65.67^{ab}	53.67^{ef}	63.33^{bc}				
	60	52.00^{c}	54.33^{c}	73.67^{a}	54.00^{c}	63.33^{b}	53.00^{c}	65.00^{b}				
	75	50.67^{c}	68.00^{ab}	68.00^{ab}	65.67^{b}	69.67^{ab}	66.33^{b}	71.67^{a}				
Albumin (g/L)									0.71	*	**	ns

	30	13.00^{c}	14.00^{abc}	15.33^{a}	13.67^{bc}	14.00^{abc}	11.67^{d}	14,67^{ab}				
	60	12.00^{b}	12.67	13.00^{b}	12.00^{b}	14.67^{a}	12.33^{b}	14.76^{a}				
	75	14.67^{d}	14.67	15.33^{c}	16.00^{ab}	15.00^{cd}	15.67^{bc}	16.67^{a}				
Globulin (g/L)									2.45	**	**	ns
	30	38.00^{d}	46.67	48.66^{bc}	45.00^{c}	51.67^{ab}	40.00^{d}	56.00^{a}				
	60	40.00^{c}	41.66^{c}	50.33^{b}	42.00^{c}	48.66^{b}	40.67^{c}	61.01^{a}				
	75	36.00^{d}	53.33^{bc}	55.00^{b}	49.65^{c}	54.67^{b}	50.66^{bc}	63.33^{a}				
Bilirubin (µmol/L)									0.68	ns	ns	*
	30	6.67	7.00	7.67	7.00	7.33	7.00	7.33				
	60	5.33	6.67	7.00	6.00	6.33	7.00	7.00				
	75	5.33	7.33	7.33	6.33	7.67	6.33	6.00				
ALT (U/L)									1.27	**	**	ns
	30	17.00	18.00	18.67	19.00	18.00	17.00	19.00				
	60	16.00^{b}	16.33^{b}	21.33^{a}	16.33^{b}	20.33^{a}	19.00^{a}	20.67^{a}				
	75	18.33^{b}	22.00^{a}	22.26^{a}	20.00^{ab}	21.50^{a}	21.00^{ab}	22.07^{a}				
ALP (U/L)									1.54	**	**	**
	30	30.67^{bc}	33.67^{ab}	23.00^{d}	32.00^{bc}	29.00^{c}	36.00^{a}	25.00^{d}				
	60	38.00^{a}	38.33^{a}	28.33^{c}	39.33^{a}	32.00^{b}	40.00^{a}	30.00^{bc}				
	75	44.33^{b}	46.00^{ab}	37.67^{c}	47.67^{a}	40.67^{c}	45.00^{ab}	44.67^{b}				
Cholesterol (mmol/L)									0.15	ns	**	ns
	30	1.30	1.56	1.55	1.36	1.35	1.34	1.47				
	60	1.38	1.39	1.55	1.39	1.47	1.34	1.30				

	75	0.95^{b}	1.11^{ab}	1.34^{a}	1.18^{ab}	1.16^{ab}	1.20^{ab}	1.09^{ab}				
High Density Lipoprotein (mmol/L)									0.08	*	*	ns
	30	0.80^{b}	1.08^{a}	0.95^{ab}	1.03^{ab}	0.95^{ab}	0.83^{b}	1.07^{ab}				
	60	0.83	0.99	1.08	1.05	1.01	0.88	0.98				
	75	0.75	0.93	0.80	0.83	0.97	0.87	0.87				
Low Density Lipoprotein (mmol/L)									0.06	**	**	**
	30	0.30^{b}	0.42^{ab}	0.47^{a}	0.32^{b}	0.37^{ab}	0.30^{b}	0.38^{ab}				
	60	0.23^{b}	0.37^{a}	0.35^{a}	0.32^{ab}	0.35^{a}	0.34^{ab}	0.36^{a}				
	75	0.12	0.13	0.20	0.16	0.25	0.14	0.23				
Triglyceride (mmol/L)									0.04	**	*	ns
	30	0.18^{bcd}	0.24^{ab}	0.16^{cd}	0.25^{ab}	0.12^{d}	0.26^{a}	0.13^{d}				
	60	0.17^{cd}	0.25^{ab}	0.17^{cd}	0.28^{a}	0.18^{bcd}	0.26^{ab}	0.12^{d}				
	75	0.16^{b}	0.29^{a}	0.16^{b}	0.27^{a}	0.22^{ab}	0.23^{ab}	0.15^{b}				

^{abcd} means with different superscript across the row are significantly different ($P<0.05$); ns, $P>0.05$; * $P<0.05$; **$P<0.001$; ***$P<0.0001$

$^{+}$ 3gVC daily; $^{++}$ 3gVC daily+extra 5gVC every 8th days; W=water restriction; SEM= standard error of mean; T=treatment effect; D=Day effect TxD=interaction between treatment and day

However, increased ($P<0.05$) concentrations of urea, total protein, alanine aminotransferase (ALT), alanine phosphate (ALP) following water restriction levels were slightly attenuated in the VC supplemented groups. Both single and extra VC doses significantly influenced ($P<0.05$) creatinine and glucose concentrations. Plasma triglyceride was significantly affected ($P<0.05$) by water restriction levels and VC dosing. The values obtained reduced considerably with increasing levels of water restriction.

3.4 Discussion

The THI experienced by the Xhosa ear-lobe goats was above the threshold values, resulting in severe stress, especially in the afternoon. This connotes that more heat was gained by the animal from the environment. When body thermal gradients (internal and external) are under the thermo-neutral zone, heat is dissipated to the external environment. However, animals' exposure to extreme heat stress results in heat flow from the external environment to the animal’s body (Ames, 1980). The similarity in response observed in the animals' skin's temperature following water restriction suggests that the Xhosa ear-lobe goats can maintain their thermal balance load even at a water restriction level of 50% *ad libitum* intake and during severe heat stress. Despite the combined stress of suboptimal water intake and high thermal load, the total body thermal gradients across the experimental groups were similar. Recently, studies have suggested that ruminants exposed to extreme heat stress and under high thermal load adapt by constricting their thermal gradients between the environment and bodies. This is made possible by the increased blood flow to the skin surface resulting from the elevated vasolidation of the skin capillaries induced by increased skin temperature (Katiyatiya *et al.*, 2017). Limited water intake resulted in body weight loss in this study. Similar results of weight loss resulting from water restriction levels have also been reported in other studies (Ghanem *et al.*, 2008). Also, Alamer and Al-hozab (2004) reported 18% weight loss in Awassi sheep following three days of water deprivation during summer. Reduction in total body water and body solid loss during water deprivation is attributed to the usually observed body weight loss and can be exacerbated when the ambient temperature is very high. Also, the animal falls back to its body reserves due to reduced feed intake induced by suboptimal water intake. As observed in this study, a drop in the dry matter intake agrees with other studies (Casamassima *et al.*, 2016). This adaptive nature to reduce feed intake following water restriction discourages the digestive system's packing of feed. This may be due to the need to optimize food digestion with minimal water that is below the required amount. All vitamin C treated groups tended to lessen weight loss compared to the untreated water-restricted groups. A similar result of improved growth rate in weaned pigs, following ascorbic acid supplementation, has been reported (de Rodas *et al.*, 1998). The WI: DMI ratio decreased as the level of water restriction increases. However, de Santos *et al.* (2019) reported an increase in WI:DMI ratio in animals that underwent water restriction for 24 h, 48 h and 72 h. The observed differences could be because the animals had *ad libitum* access to water every 24 h at the end of each water restrictions' duration. The animals under the present study had their water restricted for the entire duration of the trial. According to NRC (2007), 2.87 l of water will be consumed for each kilogram (kg) of dry matter ingested in animals. This might explain the progressive decrease in the WI:DMI ratio as the water restriction increases.

In this study, the RcT range lies within the 1.1°C reported (Degen, 1977). This tendency of RcT to remain within a particular range has been reported for different environmental conditions (Lucena *et al.*, 2013). Generally, an increase in RcT is often attributed to heat and water stress. However, the water restriction effect was not significant on the RcT in this study, and this agrees with other reports (Hamadeh *et al.*, 2006). This implies that the Xhosa ear-lobe goat breed can adapt to the stressful conditions of limited water intake. The mechanism by which VC reduces rectal temperature is yet to be elucidated. This study's RR values decreased as the water restriction levels increase, especially in the water restricted untreated groups. The RR of Lacauna ewes dropped when water restricted at both W80% and W60% of *ad libitum* water from day 0 to day 14 (Casamassima *et al.*, 2016). During the period of water scarcity and suboptimal water intake, small ruminants adapt by reducing their respiratory activities in an attempt to curb the excessive loss of water and dehydration through pulmonary evaporation (Casamassima *et al.*, 2016). Contrary to this study, Nejad *et al.* (2017) indicated an increase in the RR of Corriedale ewes, water-deprived for 2 h and 3 h compared to the control.

All the water-deprived experimental groups had small body condition scores compared to the water *ad libitum* group. Suboptimal water intake affects feed intake, leading to a loss in body condition (Sejian *et al.*, 2010). The similarity in the BCs among the water restricted groups indicates the goats' ability to maintain their body fat reserves. Supplementation of VC did not improve the BCs as compared to the water restricted untreated groups. The FAMACHA$^{©}$ system helps identify anaemic goats and sheep by checking the ocular mucosa and comparing it with a standard chat. By this assessment, anaemic animals requiring urgent anti-helminthic treatment can easily be identified (Van Wyk and Bath, 2002). Accordingly, scores of 3, 4 and 5 in goats indicate anaemia and require selective treatment (Van Wyk and Bath, 2002). The FAMACHA© scores recorded in this study were ≤ 3.33, representing a very minimal gastrointestinal nematode load. Despite the reduced feed intake and weight loss in the water restricted groups, the low FAMACHA© scores obtained, coupled with good housing care and management, indicate the goats' ability to maintain a good nutritional level still. Supplementation of VC at single or multiple doses did not significantly reduce the FAMACAHA © scores.

The increased plasma concentrations of urea, total protein, and alanine transaminase (ALT) in response to water restriction levels observed in this study were lowered following VC treatment. A similar outcome was observed in vitamin C treated, water-deprived Awassi ewes (Ghanem *et al.*, 2008). The welfare of animals can be assessed by serum concentration levels of ALT (together with aspartate transaminase). A higher concentration of this enzyme in stressed goats indicates haemoconcentration and adaptive capability (Banerjee *et al.*, 2015). Values obtained for alkaline phosphatase (ALP) in this study decreased with increasing levels of water restriction, with the W50 untreated group the most affected. The serum enzyme ALP in an animal is often associated with metabolic activities. Chaidanya (2015) attributed the generally low enzyme levels in heat-stressed animals to a metabolic shift. Other studies, however, have reported that stress has no significant effect on the plasma ALP concentration (Pragna *et al.*, 2018). The values obtained for serum concentration of albumin and protein decreases in water restricted group following vitamin C supplementation and further decreases at multiple dosages. Contrarily, serum levels of albumin and protein in heat-stressed Japanese quails was reported to increase following vitamin C supplementation (Sahin *et al.*, 2003). The plasma glucose level in this study decreases as the water restriction levels increase, which could be attributed to the reduced availability of nutrients due to low feed intake. When Sudanese desert sheep were watered, every 72 h, a 13% decline in plasma glucose level was reported (Hadjigeorgiou *et al.*, 2000). However, some authors have reported that plasma glucose concentration remains unchanged in small ruminants following water restriction (Hamadeh *et al.*, 2006). Supplementation of single and/or multiple doses of VC to the animals in this study had no significant influence on the plasma glucose. Contrarily, the serum glucose in male rabbit was reported to decrease following VC supplementations (Yousef, 2004). The concentrations of cholesterol, HDL and LDL in this study increased with water restriction levels, and their levels were not reduced in the vitamin C treated groups. Similar reports have been documented in water restricted Yankasa ewes and Sudanese desert sheep (Hamadeh *et al.*, 2006). Supplementation of vitamin C to animals has been reported to lower cholesterol (Yousef, 2004). However, the supposed hypocholesterolemic effect of vitamin C contravenes the documented report in this study. The need to meet the shortfall in dietary energy supply, an indication of fat mobilization, has been linked to the supposed increase in cholesterol following water restriction.

The increased sodium (Na^+) concentration in this study agrees with the finding of Ghanem *et al.* (2008). An increase in aldosterone and vasopressin levels following water restriction usually results in increased renal retention and elevated Na^+ concentrations (Ashour and Benlemlih, 2001). A similar trend is expected for chloride concentrations (Cl^-), giving its passive distribution along the electrical gradients established by the active Na+ transport (Tasker, 1971). A sustained increase in the concentration of Na+ and Cl^- following limited water intake may predispose an animal to salt poisoning as a result of increased accumulation of salt in the nervous tissue (Tasker, 1971). Both single and/or multiple doses of vitamin C lowered the concentration of Na^+ and Cl^- compared to the water restricted untreated group. The increased blood potassium concentration following water restriction levels reported in this study contradicts Ghanem *et al.* (2008). Studies have also found that dehydration could either decrease blood K^+ levels or not affect it (Hamadeh *et al.*, 2006). A decrease in calcium and magnesium concentration following water restriction levels in this study may be attributed to the reduced dietary intake. Vitamin C supplementation, both at single and multiple doses, did not attenuate this decrease in concentration. The modulatory role of vitamin on electrolyte balance is not clear yet. Other studies on electrolyte balance following vitamin C supplementation on Japanese quail and pigs have also been inconclusive (Avci *et al.*, 2005).

3.5 Conclusion

Limited water intake in Xhosa goats under high ambient temperature revealed some significant effects. Goats had reduced body weight, dry matter intake, respiratory rate, body condition scores and increased blood metabolites. However, the body thermal gradients were not affected. Following VC supplementation, body weight loss, dry matter intake, depression and higher blood concentrations were lessened. Contrary to our expectation, multiple VC dosing did not additively improve on the parameters where single daily dosing was positive. Although body weight loss and dry matter intake depression were lessened, there was a need to investigate the effect of water restriction levels with or without vitamin C supplementations on nutrient digestibility, nitrogen and mineral balance to further account for the weight loss observed during water restriction.

3.6 References

Akinmoladun, O.F., Muchenje, V., Fon, F.N. and Mpendulo. C.T. (2019). Small Ruminants: Farmers' Hope in a World Threatened by Water Scarcity**.** *Animals* **9**(7), 456.

Alamer, M. and Al-hozab. A. (2004). Effect of water deprivation and season on feed intake, body weight and thermoregulation in Awassi and Nadji sheep breeds in Saudi Arabia. *J. Arid Environ.* **59**, 71–84.

Ames, D.R. (1980). Thermal environmental affects livestock performance. *Bioscience* **30**, 457-60.

Ashour, G. and Benlemlih. S. (2001). Adaptation of Mediterranean breeds to heat stress and water deprivation. In: Guessous, F., Rihani, N., Ilham, A. (Eds.), Livestock Production and Climatic Uncertainty in the Mediterranean: Proceedings of the Joint ANPA-EAAPCIHEAM-FAO Symposium. Wageningen Pers, Wageningen, pp. 63–74.

Avci, M., Yerturk, M. and Kaplan, O. (2005). Effects of ascorbic acid on the performance and some blood parameters of Japanese quails reared under hot climate conditions. *Turk. J. Vet. Anim. Sci.* **29**, 829–833.

Banerjee, D., Upadhyay, R. CChaudhary, U.B. Kumar, R. Singh, S. Ashutosh Das, T.K. and De, S. (2015). Seasonal variations in physio-biochemical profiles of Indian goats in the paradigm of hot and cold climate. *Biol. Rhythm Res.* **46**(2), 221–236.

Casamassima, D., Vizzarri, F., Nardola, M. and Palazzo M. (2016). Effect of water restriction on various physiological variables on intensively reared Lacauna ewes. *Vet. Med.* **6**, 623–634.

Chaidanya, K. (2015). Impact of heat and nutritional stress on rumen fermentation characteristics and metabolic activity Bucks. Doctoral dissertation Academy of Climate Change Education and research Vellanikkara.

Degen, A.A. (1977). Responses to dehydration in native fat-tailed Awassi and imported German Mutton Merino sheep. *Physiol. Zool.* **50**, 284–293.

de Rodas, B.Z., Maxwell, C.V. Davis, M.E. Mandal, S. Broekman, E. and Stoecker, B.J. (1998). L-ascorbyl-2-polyphosphate as a vitamin C source for segregated and conventionally weaned pigs. *J. Anim. Sci.* **76**, 1636–1643.

Donnenfeld, Z., Crookes, C. and Hedden, S. (2018). A Delicate Balance: Water Scarcity in South Africa; Institute for Security Studies (ISS): Pretoria, South Africa, pp. 1–24.

Dos Santos, F.M., de Araujo, G.G.L., de Souza, L.L., Yamamoto, S.M., Queiroz, M.A.A., Lanna, D.P.D. and de Moraes, S.A. (2019). Impact of water restriction periods on carcass traits and meat quality of feedlot lambs in the Brazilian semi-arid region. *Meat Sci.* **156,** 196-204.

Ghanem, A.M., Barbour, E.K., Hamadeh, S.K., Jaber, L.S. and Abi Said, M. (2008). Physiological and chemical responses in water-deprived Awassi ewes treated with vitamin C. *J. Arid Environ*. **72,** 141-149.

Habibu, B., Kawu, M.U., Makum, H.J. and Aluwong, T. (2017). Influence of seasonal changes on physiological variables, haematology and serum thyroid hormones profile in male Red Sokoto and Sahel goats. *J. Appl. Anim. Res*. **45**, 508–516.

Haliloglu, S. and Serpek, B. (2000). The effects of plasma vitamin C and Ceruplasmin levels and exogen vitamin C supplementation on reproduction in sheep. *Turk. J. Vet. Anim. Sci.* **24**, 403–412.

Hamadeh, S.K., Rawda, N., Jaber, L.S., Habre, A. Abi Said, M. and Barbour, E.K. (2006). Physiological responses to water restriction in dry and lactating Awassi ewes. *Livest. Sci.* **101,** 101–109.

Hashem, N.M., Abd-Elrazek, D., Abo-Elezz, Z.R., Latiff, M.G.A. (2016). Effect of vitamin A or C on physiological and reproductive response of Rahmani ewes during subtropical summer season. *Small Rumin. Res.* **144**, 313-319.

Hidiroglou, M., Batra, T.R. and Zhao, X. (1997). Comparison of vitamin C bioavailability after multiple or single oral dosing of different formulations in sheep. *Reprod. Nutr. Dev*. **37**, 443-448.

Kaplan, R.M., Burke, J.M., Terrill. T.H. *et al.* (2004). Validation of the FAMACHA© eye color chart for detecting clinical anemia in sheep and goats on farms in the southern United States. *Vet. Parasitol*. **123**, 105-120.

Katiyatiya, C.L.F., Bradley, G. and Muchenje, V. (2017). Thermotolerance, health profile and cellular expression of HSP90AB1 in Nguni and Boran cows raised on natural pastures under tropical conditions. *J. Therm. Biol*. **69**, 85-94.

Kurylyk, B.L. and MacQuarrie, K.T.B. (2013). The uncertainty associated with estimating future groundwater recharge: a summary of recent research and an example from a small unconfined aquifer in a northern humid-continental climate. *J. Hydrol*. **492**, 244–253.

Lucena, L.F.A., Furtado, D.A., Nascimento, J.W.B., Medeiros, A.N. and Souza, B.B. (2013). Physiological responses of native goats maintained in a thermoneutral temperature and thermal stress. *Rev. Bras. Eng. Agrıc. Ambient* **17**, 672–679.

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 Rumin. Res*. **71**, 1–12.

McDowell, L.R. (2000). Vitamins in Animal and Human Nutrition, second ed. State University, Ames IA, Iowa, pp. 597–634.

McGregor, B.A. (2011). The role of objective and subjective evaluation in the production and marketing of goats for meat. In: Mahgoub, O., Kadim, I.T., Webb, E.C. (Eds.), Goat Meat Production and Quality. CAB International, Wallingford, Oxfordshire, UK, pp. 209–230.

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

Mohamed, S.S. (2012). Effect of level of feeding and season on rectal temperature and blood metabolites in desert rams (Ovisaries). *Acad. J. Nutr.* **1**(2), 14–18.

Nejad, J.G. and Sung, K. (2017). Behavioural and physiological changes during heat stress in Corriedale ewes exposed to water deprivation. *J. Anim. Sci. Tech.* **59**, 13.

NRC (2007). National Research Council. Nutrients requirements of Small Ruminants. 362p.Washington, D. C: National Academy Press.

Pragna, P., Sejian, V. Soren, N.M., Bagath, M., Krishnan, G., Beena, V., Devi, P.I. and Bhatta, R. (2018). Summer season induced rhythmic alterations in metabolic activities to adapt to heat stress in three indigenous (Osmanabadi Malabari and Salem Black) goat breeds. *Biol. Rhythm Res.* **49** (4), 551–565.

Ranjan, R., Swarup, D., Naresh, R. and Patra, R.C. (2005). Enhanced erythrocytic lipid peroxides and reduced plasma ascorbic acid, and alteration in blood trace elements level in dairy cows with mastitis. *Vet. Res. Commun.* **29**, 27–34.

Richards, S.A. (1973). Temperature Regulation. Wykeham Publications, London, United Kingdom.

Sejian, V., Maurya, V.P. and Naqvi, S.M.K. (2010). Adaptability and growth of Malpura ewes subjected to thermal and nutritional stress. *Trop. Anim. Health Prod*. **42,** 1763-1770.

Silanikove, N. (2000). The physiological basis of adaptation in goats to harsh environments. *Small Rumin. Res.* **35**, 181-193.

Silanikove, N. (1994). The struggle to maintain hydration and osmoregulation in animals experiencing severe dehydration and rapid rehydration: The story of ruminants. *Exp. Physiol.* **79**, 281–300.

Tasker, J.B. (1971). Fluids, electrolytes, and acid-base balance. In: Kaneko, J.J., Cornelius, C.E. (Eds.), Clinical Biochemistry of Domestic Animals, second ed. Academic Press Inc., London, pp. 65–75.

Tyler, P.J. and Cummins, K.A. (2003). Effect of dietary ascorbyl-2-phosphate on immune function after transport to a feeding facility. *J. Dairy Sci.* **86**, 622–629.

Van Wyk, J.A. and Bath, G.F. (2002). The FAMACHA system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. *Vet. Res*. **33**, 509–529.

Yousef, M.I. (2004). Aluminium-induced changes in hemato-biochemical parameters, lipid peroxidation and enzyme activities of male rabbits: protective role of ascorbic acid. *Toxicology* **199**, 47–57.

Zhang, Z., Zhang, H. and Liu, T. (2019). Study on body temperature detection of pig based on infrared technology: A review. *Artif. Intell. Agric*. **1**, 11-26

Chapter Four

Intake, nutrient digestibility, nitrogen and mineral balance of water restricted Xhosa goats supplemented with vitamin C

(Published in *Open Agriculture [De Gruypter]* Journal)

Abstract: The study objective was to evaluate the effects of single and/or extra doses of vitamin C (VC) on water-restricted (WR) Xhosa goats by evaluating their intake, digestibility, nitrogen and mineral balance during summer. Goats (42) were randomly divided into seven groups; forty-two (42) goats were divided equally into seven treatments (75-day trial); without water restriction (W0, control), water restriction of 70% of *ad libitum* water intake WI (W70), water restriction of 50% *ad libitum* WI (W50), water restriction of 70% of *ad libitum* WI plus 3g VC daily ($W70^{+}$), water restriction 50% of *ad libitum* WI plus 3g VC daily ($W50^{+}$), water restriction of 70% of *ad libitum* WI plus 3g VC and extra 5gVC given every eight-day (70^{++}), water restriction of 50% of *ad libitum* WI plus 3g VC and extra 5gVC given every eight-day ($W50^{++}$). The experiment was a complete randomized design. Similar ($P>0.05$) decrease in dry matter, organic matter, and crude protein intakes were observed regardless of water restriction levels and VC supplementation. The Water-restriction effect was not significant ($P>0.05$) on nutrients intake/metabolic weight. Retained nitrogen increased ($P<0.05$) with water-restriction levels but not influenced by VC. The urinary and faecal output decreased ($P<0.05$) with levels of water restrictions. NH_3-N decreased ($P<0.05$) as the level of water restriction increases while the pH was similar ($P>0.05$) across the water restricted groups. Supplementation of VC failed to additively influence the increased ($P<0.05$) apparent digestibility and digestible nutrients induced by water restriction levels. Regardless of VC supplementations, similar decrease ($P>0.05$) in macro minerals (Ca, P, Mg, Na and K) intake was observed in the water restricted groups. The amount of Ca, Mg and K retained increased ($P<0.05$) with water-restriction levels. Under the burden of limited water intake, nutrients and mineral uptake decreased while nutrient digestibility increased.

Keywords: water-restriction; Xhosa goats; nutrient digestibility; mineral balance, ascorbic acid

4.1 Introduction

The multiplicity role of livestock in supporting livelihood, especially to developing nations, ranges from household income generation, food security, and employment opportunities to many socioeconomic attributes (Moyo *et al.*, 2010). These immense attributes and contributions require that their productions be increasingly sustained to meet the ever-growing human population's need. Unfortunately, a considerable percentage of the livestock population, especially ruminants, widely distributed in water-limiting and dry zones of the world, are faced with the challenge of water scarcity and seasonal draught (Marino *et al.*, 2016). This water stress burden is further heightened by fluctuations in weather and rainfall patterns (global warming), consequently limiting water resources volume in most areas (IPCC, 2007).

Drinking water is essential in livestock nutrition, and suboptimal intake can critically impact physiology and productivity. This is due to its role in maintaining body heat balance, efficient digestion, absorption of food and as the primary solvents for both intra and extracellular fluids (Alamer, 2010). In a review on livestock adaptation to water scarcity, small ruminants seem to be more resilient to limited water intake than other livestock species (Akinmoladun *et al.*, 2019). However, the extent to which species can effectively utilize limited water differs with respect to breed and animal type. Desert adaptable breeds in arid regions have evolved survival mechanisms in terms of efficient water-use and body reserves during the period of water shortfalls (Alemneh and Akeberegn, 2019). Unlike monogastric, where body water loss above 15% can be detrimental, water losses of 18%, 20% and 25% of body weight can be tolerated by cattle, sheep and goat and camel, respectively. Such resilience by ruminants to dehydration effects is due to improved feed intake digestibility, lower energy deficiency induced by dry matter intake reduction and the rumen capacity to conserve water up to 15% body weight for use during scarcity (Al-Ramamneh *et al.*, 2012). Despite this adaptive mechanism, ruminants' exposure to limited water intake portends a stressful condition, negatively impacting body weight and production performances in animals (Akinmoladun *et al.,* 2019).

In ruminant nutrition, the administration of vitamin C is a practice that is not common. This is because VC is usually biosynthesized in ruminants and may require no exogenous supplementation. However, stress and disease conditions can quickly deplete plasma VC in livestock (Chambail *et al.*, 2013). As an antioxidant, VC helps in scavenging free oxidative radicals induced by oxidative stress and plays an active role in immunomodulation (Tan *et al.*, 2018). Studies have shown improvements in body weight loss, depression in feed intake, and other physiological variables in water and/transportation-stressed sheep and goats following VC supplementation (Ghanem *et al.,* 2008; Kassab and Mohammed, 2014; Akinmoladun *et al.,* 2020).

Xhosa goat is an indigenous breed, adaptable to the Eastern Cape Province. The summer months in South Africa (December-March) are characterized by hot and dry temperature extremes and water scarcity. It is always difficult for ruminants to maintain their body weights during this period because most water points are dried up. The goats are forced to trek long distances searching for water while grazing the sparsely distributed grasses and browse plants. Variations among species of ruminants to digestibility, water use abilities and feed utilization have been documented (Jaber *et al.*, 2013). It is hypothesized that adaptable breeds may have evolved a different digestive mechanism to water scarcity and marginal feeding circumstances and a possible improvement when vitamin C is supplemented. This might likely result in differences in the efficiency of nutrient utilization. In addition, the limitation of VC bioavailability due to urinary excretion losses may reduce when boosted with extra VC doses. Therefore, this research was undertaken to evaluate the intake, digestibility, rumen products, nitrogen, and mineral balance in water restricted Xhosa goats supplemented with single or extra doses of VC.

4.2 Materials and Methods

4.2.1 Study site and description

The location is as described in 3.2.1.

4.2.2 Experimental animals and management

The management of animals is as described in 3.2.2

4.2.3 Dietary treatments and design

The dietary treatments and design are as described in 3.2.3

4.2.4 Meteorological parameters

The meteorological data is as described in 3.2.6. Diurnal temperature and humidity pattern in the housing units are presented in Figure 1 as average values.

4.2.5 Nutrient intake, digestibility and urine collection

In the last seven days leading to the end of the trial, four goats from each treatment group were randomly sampled and moved to the metabolic cages for digestibility trial. Each goat was housed individually. Feed offered and feed refused for each goat were collected and weighed every morning to estimate feed intake. Total urine and faecal output from each animal were collected daily and weighed. Out of the total faeces collect, 10% was taken and dried in an oven at 70°C for 48 h. Faecal DM and DM intake were calculated as appropriate. The dried samples of faeces and feed were screened through a 1 mm mesh size, sieved, and stored before analysis. Urine samples were collected in plastic containers that have been previously washed with 10% HCL for 4 h, rinsed with de-ionized water and allowed to dry before use. Total urine output was collected daily into 10 L plastic containers containing 6N H_2SO_4 and de-ionized water to prevent the urine N from volatilization. Ten per cent of daily urine aliquot was transferred into a 3 L plastic container during the 7-d collection period and frozen at -20^{o}C until further analysis.

4.2.6 Rumen content sampling for pH and NH_3-N

Rumen contents were sampled from goats for experimental meat studies following slaughtering at a registered abattoir (Adelaide abattoir, South Africa). Goats were electrically stunned for 5 s at 200V to render them unconscious before exsanguinations and evisceration, after which rumen samples were obtained from the reticulo-rumen and squeezed through two layers of cheesecloth. The ruminal pH was determined from fresh rumen fluid before transferring 5 mL into a bottle containing 1 mL of 1% H_2SO_4 and stored at -20 ^{o}C until NH_3-N analysis.

4.2.7 Chemical analysis

Triplicates of feed, refusals and faecal samples were used to determine DM, OM, EE, Ash and CP according to the procedure of AOAC (2000). Approximately 1.0g of each sample was measured in a glass beaker and dried for 48 h at 70^{o}C. Samples were thereafter cooled in a desiccator for 2 h, and the weights of the dried samples were recorded. The dried samples were ashed in a muffle furnace (630 ^{o}C for 6 h), cooled for 2 h in a desiccator and weights recorded. Organic matter intake (OM=100-ash content) was calculated by multiplying the per cent OM of the feed sample with the amount consumed for each goat. A similar thing was done to calculate for faecal OM. Nitrogen (N) was determined using the Kjeldahl method (AOAC, 2000), and crude protein calculated as N x 6.25. Nitrogen retained (NR) was calculated from the amounts of N (g/d) consumed and excreted in the faeces, and the urine as follows: $NR(g/d) = N_{consumed} - N_{faeces} - N_{urine.}$

A dilution rate of 1:60 and 1:9 in 1% nitric acid for urine and faecal samples respectively were used in the analysis of Na, Ca, K and Mg (Richter *et al.,* 2012) with the aid of the optical emission spectrophotometry (ICP; Optima 7000 DV; PerkinElmer, Waltham, MA). Samples were analyzed in triplicate, and the mean values recorded. The mineral content of individual components of feed offered, refusals, total faeces and urine were calculated by multiplying respective values of minerals determined by their total quantities. The daily mineral content of mineral intake, refusals, faecal output and urine output were determined by dividing the total mineral content of the respective components by the number of days of collection. Apparent mineral absorption was calculated by subtracting faecal mineral from consumed mineral and multiplying by 100. The frozen rumen samples were thawed at 4°C. The NH_3-N concentration in rumen fluid was analyzed by the alkaline phenol-hypochlorite colorimetric method (Broderick and Kang, 1980). The coefficient of digestibility (%), digestible nutrient and total digestible nutrient (TDN) were calculated as follows:

Coefficient of digestibility

$$= \frac{Nutrient\ consumed - Nutrient\ in\ faeces}{Nutrient\ consumed} \text{ x } 100 \qquad \text{(Givens } et\ al., \text{ 2000)}$$

Digestible nutrient

$$= \frac{\%\ Nutrient\ amount\ in\ feed\ x\ \%\ of\ nutrient\ digestibility}{100} \qquad \text{(Banerjee, 1998)}$$

TDN = %DCP + %DCF + %DNFE + (%DEE x 2.25); where DCP, digestible crude protein; DCF, digestible crude fibre; DNFE, digestible nitrogen free extract; DEE, digestible ether extract (NRC, 2001).

4.2.8 Statistical analysis

Data for intake, apparent digestibility of dietary constituents, digestible nutrients, nitrogen balance, mineral absorption and rumen products were subjected to PROC GLM procedure of SAS 8.0 (SAS, 2001). Mean treatment differences were determined by Duncan's Multiple Range Test at a probability level of 0.05. The model used was; $Y_{ij} = \mu + T_i + \mathcal{E}_{ij}$, Where Y_{ij} = values of observation; μ = general mean; T_i = effect of water treatments; $\mathcal{E}_{ij}$=residual error.

4.3 Results

The diurnal temperature (^{o}C) and humidity (RH %) in the housing unit is shown in Figure 2.1. The average temperature-humidity index (THI) in the experimental room was 25.57 ± 0.15 throughout the experiment. DMI (g/d), OMI (g/d), and CP (g/d) intake were similar ($P>0.05$) in the entire water-restricted groups but lower ($P<0.05$) than the control (W0) (Table 4.1). The EE and nutrients intake per metabolic weight for both the water restricted groups and control were similar ($P>0.05$). The TDN was significantly higher ($P<0.05$) in the water-restricted treated groups (W70-W70^{++}) than in W0 (Fig. 4.1). Vitamin C supplementation (single and/or extra) did not influence ($P>0.05$) the TDN values. The faecal and urine output decreased ($P<0.05$) with water restriction levels. As expected, the faecal and urine outputs in W0 were significantly higher ($P<0.05$) than all the water restricted groups. Regardless of VC supplementation, similar water restricted groups (W70, W70^{+}, W70^{++} and W50, W50^{+}, W50^{++}) did not differ ($P>0.05$) in their feacal and urine outputs. The total N intake decreased ($P<0.05$) with water restriction levels and linearly increased in the VC treated groups. Faecal N, faecal N/N%, urinary N and urinary N/N% decreased ($P<0.05$) with water restriction levels. The %N retained increases ($P<0.05$) with levels of water restriction but was not affected ($P>0.05$) by VC supplementation. The pH was similar ($P>0.05$) in the water restricted groups, and values obtained were higher ($P<0.05$) than the control (GI). The NH_3-N decreased

($P<0.05$) as the water restriction levels increase and was not influenced ($P>0.05$) by VC supplementation at various doses.

The coefficient of digestibility and digestible nutrients percentages (CP, CF, NFE, EE, DM and OM) among the differently watered groups (with or without VC treatment) are shown in Table 4.2. In the water-restriction groups (W70-W70^{++}), the digestibility of DM, OM, CF, CP and NFE increased ($P<0.05$) with water restriction levels and were higher than the W0. The digestibility of EE was similar ($P>0.05$) for treatments W70-W70^{++} but lower ($P<0.05$) than in W0. Unlike the EE, the digestible nutrients (CP, CF, NFE, DM and OM) in the water restricted groups were significantly higher ($P<0.05$) than the W0. Supplementation of VC either at 3g or 3g plus extra 5g to the water restricted groups did not improve ($P>0.05$) the digestible nutrients.

Macro mineral intake, excretion, absorption, and retention are reported in Tables 4.3. The macro-minerals (Ca, P, Mg, Na and P) intake significantly decreased ($P<0.05$) due to water- restriction in W70-W70^{++} compared to W0. A similar amount ($P>0.05$) of the macro minerals was consumed regardless of water restriction and vitamin C dosage levels. Compared to other mineral elements, a greater amount of Ca (g/d) and K (g/d) ($P<0.05$) was excreted via urine than faecal. Supplementation of vitamin C either at 3g or 3g plus extra 5g did not create a pattern of influence on the minerals excreted via faces or urine. The apparent absorption of Ca was highest in W50 ($P<0.05$) while W50^{++} had the highest absorption ($P<0.05$) of Mg and K. The retained Ca (%) was highest ($P<0.05$) in W50 and lowest in W0. The retained K (%) increased ($P<0.05$) with water restriction levels. A similar amount of P (%) and Na (%) were retained in W0, W50, W50^{+} and W50^{++}. The retained Mg (%) increased ($P<0.05$) with levels of water restriction. Ca, Mg, and K's retentions increased ($P<0.05$) with water restriction levels.

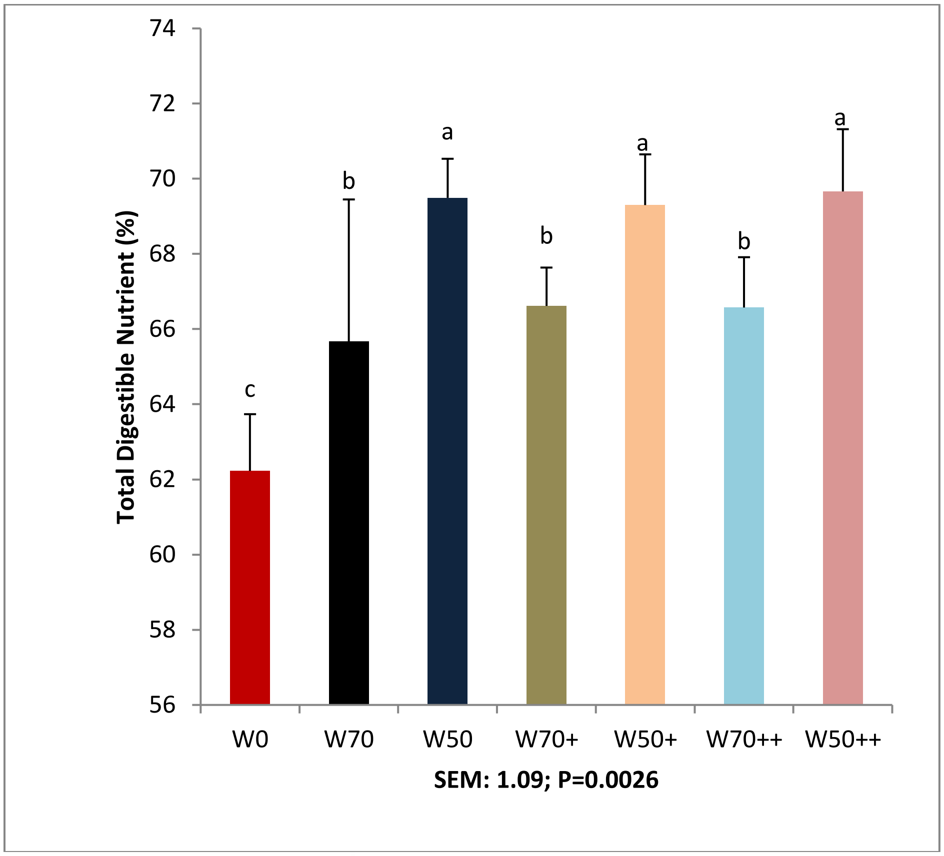


Figure 4. 1 Total digestible nutrients of water restricted Xhosa goats supplemented with single or multiple vitamin C.

Error bars with different alphabet are significantly different ($P<0.05$); $^{+}$ 3gVC daily; $^{++}$ 3gVC daily+extra 5gVC every 8th day; W=water restriction; SEM = Standard error of mean

Table 4. 1 Nutrient intake, output, nitrogen metabolism and rumen product of water restricted Xhosa goats supplemented with vitamin C

Variables	W0	W70	W50	$W70^{+}$	$W50^{+}$	$W70^{++}$	$W50^{++}$	SEM	P-value
Does (n)	4	4	4	4	4	4	4		
Nutrient intake									
DMI, g/d	633.37^{a}	546.94^{b}	536.22^{b}	552.08^{b}	538.68^{b}	551.86^{b}	538.32^{b}	7.18	0.045
DMIg/BW^0.75	74.41	78.88	78.02	73.92	73.42	75.95	73.81	16.72	0.996
OMI, g/d	524.62^{a}	453.03^{b}	444.15^{b}	457.29^{b}	446.19^{b}	457.10^{b}	445.89^{b}	5.95	0.036
OMIg/BW^0.75	61.14	65.34	64.63	61.23	60.81	62.91	61.14	4.37	0.895
CP, g/d	137.25^{a}	118.52^{b}	116.20^{b}	119.64^{b}	116.73^{b}	119.59^{b}	116.65^{b}	1.56	0.035
CP g/ BW^0.75	16.12	17.09	16.91	16.01	15.91	16.46	15.99	0.35	0.766
EE, g/d	11.08	9.57	9.38	9.66	9.42	9.66	9.42	0.13	0.045
EE g/ BW^0.75	1.30	1.38	1.37	1.29	1.28	1.33	1.29	0.13	0.906
Output									
F. output, g/d	199.59^{a}	144.39^{b}	124.23^{c}	143.98^{b}	126.07^{c}	144.10^{b}	123.56^{c}	5.28	<0.001
U. output, ml/d	383.33^{a}	233.95^{b}	156.67^{c}	223.33^{b}	157.38^{c}	226.43^{b}	153.81^{c}	14.94	<0.001
Nitrogen metabolism									
N intake, g/d	21.96^{a}	18.96^{b}	18.59^{c}	19.14^{b}	18.68^{c}	19.13^{b}	18.66^{c}	0.12	0.033
N Faeces, g/d	4.77^{a}	2.25^{b}	2.02^{cd}	2.42^{b}	2.17^{c}	2.19^{c}	1.90^{d}	0.09	0.003
Digest. N, g/d	19.30^{a}	16.95^{b}	16.43^{b}	16.44^{b}	15.91^{b}	16.48^{b}	16.77^{b}	0.54	0.041
N Urine, g/d	1.55^{a}	1.00^{b}	0.31^{d}	0.80^{c}	0.34^{d}	0.92^{b}	0.37^{d}	0.05	0.004
N retentiong/d	15.63^{c}	15.71^{c}	16.26^{ab}	15.92^{c}	16.18^{b}	16.02^{bc}	16.43^{a}	0.15	0.021
Faecal N/N, %	21.70^{a}	11.85^{c}	10.87^{d}	12.62^{b}	11.63^{c}	11.44^{c}	10.17^{e}	0.23	0.003
Urine N/N, %	7.04^{a}	5.28^{b}	1.68^{d}	4.17^{c}	1.83^{d}	4.80^{bc}	1.98^{d}	0.49	0.022
RetainedN/N,%	71.16^{c}	82.86^{b}	87.45^{a}	83.18^{b}	86.60^{a}	83.75^{b}	88.04^{a}	1.05	0.012
Rumen parameters									
pH	6.78^{b}	7.09^{a}	7.02^{a}	7.03^{a}	7.05^{a}	7.10^{a}	7.08^{a}	0.08	0.001
NH_3-N	27.06^{a}	25.02^{b}	22.44^{c}	24.90^{b}	21.42^{d}	27.16^{a}	21.68^{cd}	0.18	<0.001

^{abc}:means with different superscript across the row are significantly different (P<0.05); $^{+}$ 3gVC daily; $^{++}$ 3gVC daily+extra 5gVC every 8th days; W=water restriction; SEM = Standard error of mean; DMI: dry matter intake; OMI: organic matter intake; F: Faecal; U: Urinary.

Table 4. 2 Digestibility coefficients (%) and digestible nutrients (%) of different nutrients in water-restricted Xhosa goats supplemented with vitamin C

Parameters	W0	W70	W50	$W70^{+}$	$W50^{+}$	$W70^{++}$	$W50^{++}$	SEM	P-value
Does (n)	4	4	4	4	4	4	4		
Digestibility coefficients %									
CP	85.21^{d}	86.00^{cd}	88.13^{ab}	85.93^{d}	87.07^{bc}	85.95^{d}	88.99^{a}	0.73	0.023
CF	56.65^{c}	63.31^{b}	68.55^{a}	63.85^{b}	68.14^{a}	64.17^{b}	68.52^{a}	1.56	0.001
EE	76.19^{a}	65.62^{b}	63.62^{b}	66.66^{b}	64.32^{b}	66.11^{b}	63.85^{b}	2.63	0.055
NFE	64.92^{c}	70.05^{b}	75.29^{a}	71.87^{b}	75.52^{a}	71.66^{b}	75.25^{a}	1.32	0.001
DM	68.39^{c}	73.37^{b}	76.85^{a}	73.87^{b}	76.60^{a}	73.89^{b}	77.05^{a}	1.13	0.001
OM	65.82^{c}	70.59^{b}	74.94^{a}	71.67^{b}	74.57^{a}	71.64^{b}	75.46^{a}	1.25	0.001
Digestible nutrients %									
CP	18.47^{d}	18.64^{cd}	19.09^{ab}	18.62^{cd}	18.87^{bc}	18.63^{cd}	19.28^{a}	0.16	0.023
CF	12.19^{c}	13.63^{b}	14.76^{a}	13.75^{b}	14.67^{a}	13.82^{b}	14.75^{a}	0.34	0.001
EE	1.33^{a}	1.15^{b}	1.11^{b}	1.17^{b}	1.13^{b}	1.16^{b}	1.12^{b}	0.05	0.055
NFE	28.57^{c}	30.82^{b}	33.13^{a}	31.62^{b}	33.23^{a}	31.53^{b}	33.11^{a}	0.58	0.001
DM	45.21^{d}	48.49^{c}	50.79^{ab}	48.82^{b}	50.63^{ab}	48.83^{b}	50.93^{a}	0.75	0.001
OM	58.54^{c}	62.79^{b}	66.66^{a}	63.75^{b}	66.33^{a}	63.73^{b}	67.13^{a}	1.15	0.001

^{abc}:means with different superscript across the row are significantly different $^{+}$ 3gVC daily; $^{++}$ 3gVC daily+extra 5gVC every 8th days; W=water restriction; SEM = Standard error of mean

Table 4. 3 Daily macro mineral intake, faecal, urine excretion and apparent absorption and retention in water restricted Xhosa goats supplemented with vitamin C

Variables	W0	W70	W50	W70^{+}	W50^{+}	W70^{++}	W50^{++}	SEM	P-value
Does (n)	4	4	4	4	4	4	4		
Mineral intake (g/d)									
Ca	9.94^{a}	8.58^{b}	8.42^{b}	8.67^{b}	8.46^{b}	8.66^{b}	8.45^{b}	0.32	0.033
P	2.41^{a}	2.08^{b}	2.04^{b}	2.10^{b}	2.06^{b}	2.09^{b}	2.04^{b}	0.08	0.047
Mg	3.80^{a}	3.28^{b}	3.22^{b}	3.31^{b}	3.23^{b}	3.30^{b}	3.22^{b}	0.12	0.048
Na	1.43^{a}	1.24^{b}	1.21^{b}	1.25^{b}	1.22^{b}	1.24^{b}	1.21^{b}	0.05	0.021
K	5.26^{a}	4.54^{b}	4.45^{b}	4.58^{b}	4.47^{b}	4.58^{b}	4.46^{b}	0.17	0.017
Faecal excretion (g/d)									
Ca	2.11^{a}	1.83^{bc}	1.36^{d}	2.47^{a}	1.08^{d}	1.82^{bc}	1.50^{cd}	0.28	0.051
P	1.36^{a}	0.63^{d}	1.00^{c}	1.14^{bc}	1.05^{bc}	1.19^{b}	0.78^{d}	0.08	<0.001
Mg	0.94^{a}	0.72^{b}	0.57^{cd}	0.52^{d}	0.68^{bc}	0.60^{bcd}	0.36^{e}	0.06	<0.001
Na	0.41^{a}	0.37^{ab}	0.30^{bc}	0.35^{ab}	0.33^{ab}	0.16^{d}	0.20^{cd}	0.05	0.048
K	1.33^{ab}	0.90^{cd}	1.11^{bc}	1.48^{a}	1.06^{c}	0.76^{d}	0.69^{d}	0.11	0.002
Urinary excretion (g/d)									
Ca	7.29^{a}	3.77^{bc}	2.77^{c}	4.00^{b}	3.98^{bc}	3.41^{bc}	3.68^{bc}	0.59	0.003
P	0.49^{a}	0.28^{b}	0.22^{bc}	0.50^{a}	0.20^{bc}	0.12^{cd}	0.07^{d}	0.05	<0.001
Mg	0.78^{a}	0.53^{b}	0.24^{d}	0.42^{bc}	0.30^{cd}	0.35^{cd}	0.39^{bcd}	0.07	0.003
Na	0.69^{ab}	0.53^{bc}	0.72^{ab}	0.30^{d}	0.36^{cd}	0.76^{a}	0.81^{a}	0.11	0.044
K	3.71^{a}	2.25^{b}	1.35^{c}	2.85^{b}	2.08^{b}	2.25^{b}	2.17^{b}	0.39	0.023
Apparent absorption (%)									
Ca	78.82^{b}	78.50^{b}	83.88^{ab}	70.75^{c}	87.30^{a}	78.97^{b}	82.26^{ab}	3.70	0.135
P	43.18^{b}	68.67^{a}	50.98^{b}	45.42^{b}	48.49^{b}	43.35^{b}	61.82^{a}	4.23	0.004
Mg	75.32^{e}	78.06^{d}	82.24^{b}	84.39^{b}	78.98^{cd}	81.74^{bc}	89.00^{a}	1.52	0.001
Na	72.41^{b}	70.52^{b}	75.02^{b}	71.84^{b}	72.82^{b}	87.08^{a}	83.84^{a}	3.36	0.019
K	75.03^{b}	80.14^{a}	75.10^{b}	67.33^{c}	76.22^{b}	83.32^{a}	84.66^{a}	2.24	0.001
Mineral retention (%)									
Ca	5.60^{d}	33.87^{bc}	50.65^{a}	24.79^{c}	40.13^{ab}	39.60^{abc}	38.73^{abc}	7.72	0.025
P	23.09^{c}	54.52^{a}	40.13^{b}	21.35^{c}	38.66^{b}	37.56^{b}	58.31^{a}	6.25	0.006

Mg	55.33^{d}	61.87^{c}	74.64^{ab}	71.70^{b}	69.78^{b}	71.20^{b}	77.09^{a}	2.59	0.001
Na	24.25^{bc}	28.61^{abc}	14.77^{c}	47.64^{a}	43.45^{ab}	26.50^{abc}	17.60^{c}	11.28	0.036
K	5.35^{b}	29.29^{a}	44.73^{a}	5.70^{b}	29.61^{b}	34.25^{b}	36.04^{b}	7.68	0.017

^{abc}:means with different superscript across the row are significantly different $^{+}$ 3gVC daily; $^{++}$ 3gVC daily+extra 5gVC every 8th days; W=water restriction; SEM = Standard error of mean

4.4 Discussion

The average THI recorded in the experimental house was above the threshold values (Marai *et al.*, 2007), resulting in severe stress on the goats. This implies a negative heat balance as more heat will be gained by the animal from the environment. Efficient digestion and absorption require a water medium for the physical softening of feed (Doreau *et al.,* 2012). When water supply is adequate, the breakdown of feed is enhanced, thus facilitating fermentative and digestive processes. Giving the correlation between feed intake and water consumption (Thang *et al.,* 2012), the reduced nutrients intake in the water-restricted groups could result from limited water intake. Usually, the amount of feed consumed by ruminants is affected by the osmolality of body fluids. This is because gastric juices and saliva secretion in response to feed intake usually results in hyperosmolality and hypovolemia, urging animals to drink while eating or stop when dehydrated (Langhans *et al.*, 1991). Severe reactions to feed intake under various water restriction regimen has been reported in Turkana goats, fat-tailed sheep and Zebu (Maloiy *et al.*, 2008). Other studies have also reported a drop in DM intake following water restriction (Casamassima *et al.,* 2016).

Regardless of VC supplementation, the reduced urine and faecal output in the water restricted groups could be attributed to suboptimum water intake. During water scarcity, ruminants reduce water loss by reducing faecal water and urine volume, thereby resulting in higher urine osmolality (Naqvi *et al.,* 2015). This drop in urine volume could be attributed to the reduced filtration rate of the glomerulus and renal plasma flow in the water- restricted groups (Schwartz and Furth, 2007). This tendency of generating hyperosmotic urine is attributed to the length of the loops of Henlè, which is longer compared to non-adaptable breeds (McNab, 2002). This greater urine concentration and reduced urine output are the adaptive mechanisms that enhance their chances of survival. The decrease in nitrogen consumption could be attributed to reduced voluntary dry matter intake by the water restricted groups. Similarly, there was a decrease in the amount of nitrogen excreted due to the animal's limited water intake. When expressed as a percentage of N-intake, high nitrogen losses were recorded through faeces compared to urine across the treatment groups. Compared to the water restricted groups, the control group's high faecal nitrogen loss mirrors the high feed consumed and faecal output in the control group. The increased nitrogen retention obtained by the water restricted group in this study was facilitated by low nitrogen excretion. Contrarily, the control group had a negative nitrogen balance, but the water-restricted groups were able to keep hold of a significant amount of the consumed nitrogen. The higher digestible and retained N and CP digestibility in the water restricted groups reflect the low nitrogen excretion in faeces and urine, resulting in positive N retention. This may indicate N recycling via the rumen and saliva wall for microbial synthesis (Nejad *et al.*, 2014). Due to suboptimal water intake, the rumen's degradation process is slowed down, facilitating protein escape. The escaped protein may be digested in the abomasum and small intestine, leading to increased nitrogen uptake from the small intestine. Thus, protein metabolism in the liver is increased, with enhanced nitrogen recycling into the rumen from possible amino acid deamination for non-essential protein synthesis or energy utilization.

The decreased urinary N loss following water-restriction in this study could be attributed to reduced kidney glomeruli's filtration. The decreased urine excretion and urinary N loss in the water restricted groups agrees with Nejad *et al.* (2014), who conducted a study on water restricted Corriedale ewes. The decline in urea output and urinary nitrogen following water restriction in this study was similar to what was reported in a water deprivation study involving sheep and goats (Abdelatiff *et al.*, 2010). Ammonia-N decreased with decreased water intake in this study. According to Mathew *et al.* (2019), when intake is below optimum, rumen ammonia production drops due to a low degradation rate. The increased rumen pH in the water-restricted groups could be due to the combined effect of heat and water stress. Restricted water under high ambient temperature implies lower feed intake, hence fewer solubles being supplied, which means that there will be less volatile fatty acids (propionate). High volatile fatty acids are responsible for a drop in pH (Zenobi *et al.,* 2015). Hence, the higher pH observed in the water restricted animals. Lactating dairy cattle undergoing heat stress had their ruminal pH increased from 5.82 to 6.03 (Hall, 2009) and was slightly different from the 6.78 to 7.10 recorded in this study. In a similar study on growing heat-stressed Korean cattle steers fed rumen-protected fat, the rumen pH was found to slightly increase from 6.88 to 7.02 and 6.74 to 7.10 in July and August, respectively (Kang *et al.,* 2019).

Water restriction of any form would generally enhance the digestibility of nutrients by improving feed utilization by allowing sufficient time for digesta. Increased retention of digesta implies that rumen microbes will have enough time for degradation and synthesis (Casamassima *et al.*, 2016). In this study, the digestibility of nutrients significantly increases as water intake decreases. This is similar to the increased total tract digestibility of DM and OM in water restricted St. Croix sheep (Hussein *et al.*, 2018). However, the findings of Nejad *et al.* (2014) showed no differences in nutrient digestibility between 2 hrs and 3 hrs water restrictions after feeding in Corriedale ewes. This non-significant effect may result from the limited time of exposure to reduced water intake, compared to the 75-days of prolonged exposure in this study where the restricted passage bought more time for nutrients to be properly harvested. In the control group, where the passage rate is higher in this study, only the solubles are extracted, hence the lower digestibility and higher content in faeces. Single and/or extra dosing with vitamin C did not improve nutrient digestibility, as values obtained between treated and untreated water-restricted groups were similar.

The amount of minerals in dietary constituents is minute compared to the animal's protein, carbohydrates, or fat requirement. This does not undermine their importance, as an absence or imbalance may elicit deficiencies and consequently impair performance (Suttle, 2010). In addition, the mineral nutrition of goats is yet to be fully worked out, as recommended mineral consumption values are still based on extrapolations from intermediate values between sheep and cattle (Meschy, 2000). The low mineral intake in the water-restricted groups may be due to reduced feed intake, given the fact that nutrients ingested by livestock are positively correlated with feed intake. As an adaptive mechanism coupled with the role of water for proper digestion, a reduction in feed intake usually accompanies limited water intake in small ruminants (Akinmoladun *et al.*, 2019), consequently impairing the amount of nutrients ingested. However, a marginal improvement in DMI intake following vitamin C supplementation in the water-restricted groups did not significantly translate to higher mineral intake. In this study, the control group ingested higher minerals but excreted more of these minerals, especially Ca, Mg, P and K, in faeces and urine compared to the water restriction groups. The affirmation of Boswald *et al.* (2017) on higher excretion of P in herbivores through faeces agrees with this study's report. However, Ca's excretion in this study is more elevated in urine but decreased with water restriction levels. When dehydrated, animals adapt by reducing faecal water and urine output. This might explain Ca, K, P and Mg's higher retention among the water restricted groups compared to the control. However, a minimum threshold of 45% in apparent Ca absorption is recommended for ruminants (McDowell, 1992). Ca's apparent absorption was higher than the threshold recommended in the current study, regardless of water restriction levels. The increase in Na and K's apparent absorption in the water-restricted groups following multiple vitamin C supplementations could probably facilitate Na flux in the fibroblast cells (Morla *et al.*, 2016) due to the increased bioavailability of this vitamin

(Hidiroglou *et al.*, 1997). The more significant result of Na and K retention than absorption is similar to what was reported by Salles *et al.* (2008). The reason is that urine is the main excretion path for these minerals, and it is usually factored in the calculation for retention. The improvement in the amount of K and Na retained following water stress is very important in the mineral balance. This is due to their role in transporting substrates into and out of the cells and osmotic pressure regulation. A similar improvement in K retention was reported in heat-stressed steers fed diets supplemented with monensin (Salles *et al.*, 2008). Water restriction levels and multiple vitamin C supplementations significantly improve the absorption and retention of Mg. This may be linked to decreased excretion through the urine.

4.5 Conclusions

Exposure of small ruminants to limited water affects nutrients intake and digestibility and can be more pronounced under high thermal load. Supplementation of VC at various doses failed to significantly improve the depression in digestibility and nutrients intake induced by water restriction. The mineral balance was affected by water restriction levels, with higher losses of Ca and K observed in the urine than faeces, compared to other minerals. Limited water intake portends major stressful stimuli, affecting growth performance and nutrients intake. Such stressor may further impair carcass characteristics and meat quality as well cause perturbations on plasma hormonal indices. Hence, the need to further investigate the effect of limited water intake and VC supplementations on hormonal stress indicators, carcass characteristics and meat quality of Xhosa goats.

4.6 References

Akinmoladun, O.F, Fon, F.N., Mpendulo, C.T., Okoh, O. (2020). Performance, heat tolerance and blood metabolites of water restricted Xhosa goat supplemented with vitamin C. *Transl. Anim. Sci*. **4**(2), 1-15.

Akinmoladun, O.F., Muchenje, V., Fon, F.N., Mpendulo, C.T. (2019). Small Ruminants: Farmers' Hope in a World Threatened by Water Scarcity. *Animals* **9**(7), 456.

Alamer, M. (2010). Effect of water restriction on thermoregulation and some biochemical constituents in lactating Aardi goats during got weather conditions. *J. Basic Appl. Scientific Res.* **11**, 189–205.

Alemneh, T. and Akeberegn, D. (2019). Adaptation Strategies of Farm Animals to Water Shortage in Desert Areas. *Am. J. Biomed. Sci. Res*. **2(**6**)**, 245-250.

Al-Ramamneh, D., Riek, A. and Gerken, M. (2012). Effect of water restriction on drinking behaviour and water intake of German black-head mutton sheep and Boer goats. *Animal* **6**, 173–178.

AOAC, (2000). Official Methods of Analysis of the Official Analytical Chemists (W. Horwitz ed) 17th Ed, Association of Official Analytical Chemists, Washington DC USA.

Banerjee, G.C. (1998). Feeds and principles of animal nutrition. Revised edition of Animal Nutrition. New Delhi: Oxford and IBH Publishing Co. Pvt. Ltd.

Boswald, I.F., Dobenecker, B., Clauss, M., Kienzle, E. (2017). A comparative meta-analysis on the relationship of faecal calcium and phosphorus excretion in mammals. *J. Anim. Physiol. An. N*. **102**, 370-379.

Broderick, G.A. and Kang, J.H. (1980). Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and *in vitro* media. *J. Dairy Sci.* **63**, 64–75.

Casamassima, D., Vizzarri, F., Nardola, M. and Palazzo, M. (2016). Effect of water restriction on various physiological variables on intensively reared Lacauna ewes. *Vet. Med.* **6**, 623–634.

Chambial, S., Dwivedi, S., Shukla, K.K., John, P.J. and Sharma, P. (2013). Vitamin C in disease prevention and cure: an overview. *Indian J. Clin. Biochem*. **28**(4), 314-28.

Doreau, M., Corson, M.S. and Wiedemann, S.G. (2012). Water use by livestock: A global perspective for a regional issue? *Anim. Front*. **2**(2), 9-16.

Ghanem, A.M., Barbour, E.K., Hamadeh, S.K., Jaber, L.S. and Abi Said, M. (2008). Physiological and chemical responses in water-deprived Awassi ewes treated with vitamin C. *J. Arid Environ*. **72**, 141-149.

Givens, D.I., Owen, E., Axford, R.F.E. and Omed, H.M. (2000). Forage evaluation in ruminant nutrition. Wallingford, Oxon (UK): CAB International.

Hall, M.B. (2009). Heat stress alters ruminal fermentation and digesta characteristics, and behavior in lactating dairy cattle. In Proceeding of 11th International Symposium on Ruminant Physiology, Y. Chilliard, F. Glasser, Y. Faulconnier, F. Bocquier, I. Veissier, and M. Doreau, ed. Wageningen Academic Publ., Wageningen, The Netherlands, pp. 204.

Hamadeh, S.K., Rawda, N., Jaber, L.S., Habre, A., Abi Said, M. and Barbour, E.K. (2006). Physiological responses to water restriction in dry and lactating Awassi ewes. *Livest. Sci.* **101**, 101–109.

Hidiroglou, M., Batra, T.R. and Zhao, X. (1997). Comparison of vitamin C bioavailability after multiple or single oral dosing of different formulations in sheep. *Reprod. Nutr. Dev.* **37**, 443-448.

Hussein, A., Puchala, R., Gipson, T., Tadesse, D., Wilson, B. and Goetsch, A. (2018). Effects of water restriction on feed intake and digestion by St. Croix sheep. *J. Anim. Sci*. **96**(3), 456-457.

Jaber, L., Chedid, M. and Hamadeh, S. (2013). Water stress in small ruminants. In: Akinci, S. (Ed.). Responses of Organisms to Water Stress. InTech. Jarieza Trdine 9, 51000 Rijeka, Croatia, pp.115-149.

Jaber, L., Hanna, N., Barbour, E.K., Abi Said, M., Rawda, N., Chedid, M. and Hamadeh, S.K. (2011). Fat mobilization in water restricted Awassi ewes supplemented with vitamin C. *J. Arid Environ*. **75**, 625-628.

Kang, H.J., Piao, M.Y., Park, S.J., Na, S.W., Kim, H.J. and Baik, M. (2019). Effects of heat stress and rumen-protected fat supplementation on growth performance, rumen characteristics, and blood parameters in growing Korean cattle steers. *Asian Austral. J. Anim.* **32**(6), 826–833.

Kinambo, A.E., Moiro, J.N., Aboud, A.A.O., Mtenga, L.A., Hveplund, T., Weisbjerg, M., Mgheni, D.M. and Madsen, J. (1999). Potential for nitrogen recycling into the rumen of adult rams. *S. Afr. J. Anim. Sci*. **29**, 248–249.

Langhans, W., Scharre, E. and Meyer, A.H. (1991). Changes in feeding behaviour and plasma vasopressin concentration during water deprivation in goats. *J. Vet. Med*. **38**, 11–20.

Maloiy, G.M.O., Kanui, T.I., Towett, P.K., Wambugu, S.N., Miaron, J.O., Wanyoike, M.M. (2008). Effects of dehydration and heat stress on food intake and dry matter digestibility in East African ruminants. *Comp. Biochem. Physiol.* **151**, 185–190.

Marai, I.F.M., El-Darawany, A.A., Fadiel, A., Abdel-Hafez, M.A.M. (2007). Physiological traits as affected by heat stress in sheep—a review. *Small Rumin Res*. **71**, 1–12.

Marino, R., Atzori, A.S., D'Andrea, M., Iovane, G., Trabalza-Marinucci, M. and Rinaldi, L. (2016). Climate change: Production performance, health issues, greenhouse gas emissions and mitigation strategies in sheep and goat farming. *Small Rumin. Res*. **135**, 50–59.

Mathews, C., Crispie, F., Lewis, E., Reid, M., O'Toole, P.W. and Cotter, P.D. (2019). The rumen microbiome: a crucial consideration when optimizing milk and meat production and nitrogen utilization efficiency. *Gut Microbes* **10(2)**, 115-132.

McDowell, L.R. (1992). Minerals in animal and human nutrition. Academic Press, San Diego

McNab, B.K. (2002). The physiological ecology of vertebrates. Comstock, Cornell University, Ithaca, New York, USA.

Meschy, F. (2000). Recent progress in the assessment of minerals requirements of goats. *Livest. Prod. Sci.* **64,** 9–14.

Morla, L., Edwards, A. and Crambert, G. (2016). New insights into sodium regulation in the distal nephron: Role of G-protein coupled receptors. *World J. Bio. Chem*. **7(1)**, 44-63.

Moyo, S. and Swanepoel, F.J.C. (2010). Multifunctionality of livestock in developing communities. In: The Role of Livestock in Developing Communities: Enhancing Multifunctionality, edited by Frans Swanepoel, Aldo Stroebel and Siboniso Moyo, Co-published by The Technical Centre for Agricultural and Rural Cooperation (CTA) and University of the Free State.

Naqvi, S.M.K., Kumar, D., De, K. and Sejian, V. (2015) Climate Change and Water Availability for Livestock: Impact on Both Quality and Quantity. In: Sejian V., Gaughan J., Baumgard L., Prasad C. (eds) Climate Change Impact on Livestock: Adaptation and Mitigation. Springer, New Delhi.

National Research Council (NRC). (2001). Nutrient Requirements of Dairy Cattle. 7th rev. ed. Na-tional Academy Press, Washington, DC.

National Research Council (NRC). (1981). Nutrient requirements of goats: angora, dairy, and meat goats in temperate and tropical countries. National Academy of Sciences. National Academic Press, Washington, DC.

Nejad, G.J., Lohakare, J.D., West, J.W. and Sung, K.I. (2014). Effects of water restriction after feeding during heat stress on nutrient digestibility, nitrogen balance, blood profile and characteristics in Corriedale ewes. *Anim. Feed Sci. Tech*. **193**, 1-8.

Richter, E.L., Drewnoski, M.E. and Hansen, S.L. (2012). The effect of dietary sulfur on performance, mineral status, rumen hydrogen sulfide, and rumen microbial populations in yearling beef steers. *J. Anim. Sci*. **90**, 3945–3953.

Salles, M.S.V., Zanetti, M.N. and Salles, F.A. (2008). Effect of monensin on mineral balance in growing ruminants reared under different environmental temperatures. *Anim. Feed Sci. Tech.* **141,** 233-245.

SAS, Institute, (2001). SAS/STAT User's Guide. Statistics, Version 8. SAS Institute, Cary, NC.

Schulze, R.E., Lynch, S.D. and Maharaj, M. (2006). Annual precipitation. In: Schulze RE, editor. South African atlas of climatology and agrohydrology. Water Research Commission report 1489/1/06, Section 6.2. Pretoria: Water Research Commission.

Schwartz, G.J. and Furth, S.L. (2007). Glomerular filtration rate measurement and estimation in chronic kidney disease. *Pediatr. Nephrol.* **22**, 1839–1848.

Skotnicha, E., Muszczynski, Z., Dudzinska, W. and Suska, M. (2007). A review of the renal system and diurnal variation of renal activity in livestock. *Irish Vet. J.* **60**(3**)**, 161-168.

Suttle, N.F. (2010). Mineral nutrition of livestock. Cabi. 4th ed. London, UK.

Tan, B.L., Norhaizan, M.E., Liew, W.P. and Sulaiman, R.H. (2018). Antioxidant and Oxidative Stress: A Mutual Interplay in Age-Related Diseases. *Front. Pharmacol.* **9**, 1162.

Thang, T.V. Sunagawa, K., Nagamine, I., Kishi, T. and, Ogura, G.A. (2012). Physiological stimulating factor of water intake during and after forage feeding in large-type goats. *Asian Austral. J. Anim.* **25**, 502–514.

Zenobi, M.G., Lardner, H.A., Jefferson, P.G. and McKinnon, J.J. (2015). Effect of feeding strategically blended feed pellets on rumen fermentation and nutrient digestion. *Can. J. Anim. Sci.* **95**, 243–254.

Chapter Five

Stress indicators, carcass characteristics and meat quality of Xhosa goat meat subjected to different watering regimen and vitamin C supplementation

(Published in *Livestock Science* Journal)

Abstract: Limited water intake in small ruminants induced by water scarcity may result in pre-slaughter stress. A possible management practice could be the supplementation of exogenous antioxidant during the period of water stress. The effect of water restriction (W) levels with or without single and/or multiple vitamin C (VC) supplementations were evaluated on plasma hormones (cortisol, β-estradiol, thyroxine (T_4), tri-iodothyronine (T_3), prolactin, FSH, LH, insulin) carcass and non-carcass components and physicochemical characteristics of the *longissimus thoracis et lumborum* (LTL) muscle of Xhosa ear lobe goats. Forty-two goats were divided equally and randomly assigned into seven treatments; without water restriction (W0, control); water restriction of 70% of *ad libitum* water intake (W70), water restriction of 50% *ad libitum* water intake (W50), water restriction of 70% of *ad libitum* water intake plus 3g VC daily ($W70^{+}$), water restriction 50% of *ad libitum* water intake plus 3g VC daily ($W50^{+}$), water restriction of 70% of *ad libitum* water intake plus 3g VC and extra 5gVC given every eight-day (70^{++}), water restriction of 50% of *ad libitum* water intake plus 3g VC and extra 5gVC given every eight-day ($W50^{++}$). At the end of the 75-day experimental trial, the goats were humanely slaughtered and dressed. Increased plasma cortisol concentration due to water stress was lessened ($P<0.05$) by VC. FSH, LH and insulin were not affected ($P>0.05$) by levels of water restriction. Compared to the control, the W70+ and W50+ increased but the W70 and W50 decrease. The head and spleen weights were not affected by water restriction levels while that of the liver, skin, and kidney decreased significantly ($P<0.05$) with increased water restriction levels. VC treated goats had higher redness (a*) than the untreated (W70 and W50) and control groups (W0). The shear force and cooking loss increased with water restriction levels while thaw loss, pH_{45min} and pH_{24h} were not affected by water restriction levels.

Keywords: water stress, vitamin C, shear force, carcass compactness, meat colour

5.1 Introduction

Goat meat is highly preferred in regions such as Asia and Africa (FAOSTAT, 2016) and is gradually gaining wider acceptance as well as enjoying increasing popularity among consumers in other geographical regions. This excellent market potential and preponderance, especially in the dry and water-scarce zones of the world, compared to other species of livestock, are linked to their browsing habits, adaptation to harsh environmental conditions and general acceptance of the meat as a good protein source across religion diversities (Webb, 2014; Akinmoladun *et al.*, 2019).

Water is an essential nutrient to livestock and regarded as second to oxygen in importance to sustain life (Wakchaure *et al.*, 2015). This is attributed to its multiplicity role in body heat balance, efficient digestion of food, solvents for both intra and extracellular fluids, and its requirements by other nutrients for proper functioning (Alamer, 2010). However, its availability in a sufficient amount is now being threatened by increasing water use due to rising population and climate change (IPCC, 2007). Livestock is the most affected, especially in the arid and dry zones of the globe (Akinmoladun *et al.*, 2019). Water scarcity is a perennial problem in South Africa (Donnenfeld *et al.*, 2018)

Goat production and management in the Eastern Cape Province are mainly under the communal householders. Under this setting, animals search for feed and water by walking long distances through the sparsely distributed pastures and browse plants throughout the year. Animals in the field are therefore faced with dehydration while grazing far from widely spread watering points to meet their nutrient requirements. As reviewed by Akinmoladun *et al.* (2019), small ruminants (as water conservation and adaptive mechanisms) respond to insufficient water intake by reducing feed intake for a lowered metabolic rate.

Despite the efficient water usage abilities of small ruminants (Barbour *et al.*, 2005), inadequate supply of this nutrient in the right amount and quantity induces stress and may impair body weight, normal internal milieu, hormonal secretions, blood metabolites, reproductive physiology and meat quality parameters. As observed, the dehydration effect accompanying water restriction and heat stress may darken the colour of meat due to myofibrils shrinkages (Jacob *et al.,* 2006). Under extreme heatwaves or high thermal load, the severity of limited water intake in animals could be heightened due to the increased adrenaline production. Being a stress hormone, adrenalin stimulates peripheral vasolidation and muscle glycogenolysis (Watt *et al.,* 2001; Wang *et al.*, 2015). When exposure to preslaughter stress is sustained for long, meat from such animals may have higher pH and darker colourations (Lowe *et al.*, 2002).

Vitamin C (VC) or ascorbic acid plays a significant role in cellular metabolism, especially in redox reactions, collagen synthesis and enzyme cofactor (Mandi *et al.*, 2009). However, vitamin requirements for small ruminants have only been specified for A, D and E (NRC, 2007). This is because ruminants can naturally bio-synthesize ascorbate from glucose. However, studies have documented that these antioxidants can be quickly depleted during stressful periods and disease conditions (Ranjan *et al.*, 2005), hence a possibility of an improvement if supplemented. A trial conducted on water-stressed Awassi ewes with ascorbic acid supplementation resulted in improved feed intake and body weight (Ghanem *et al.*, 2008). This improvement may be due to ascorbic acid's ability to effectively scavenge free radicals and reactive oxygen species generated following increased lipid mobilization and metabolic stress induced by negative energy balance in water-limited animals. Data on daily VC requirements for small ruminants as a management procedure, especially during water shortages and high thermal load, are still very scanty. Also, the combined effect of water restriction and vitamin C supplementation on meat quality at pre-mortem is unknown. It is hypothesized that supplementation of vitamin C will improve meat quality parameters as affected by water restriction. This study, therefore, is focused on the effect of water restriction levels with single and/or multiple vitamin C supplementations on stress indicators, carcass and non-carcass components, physical and chemical characteristics of the *longissimus thoracis et lumborum* (LTL) muscle of the Xhosa ear lobe goats.

5.2 Materials and methods

5.2.1 Study area and ethical clearance

The study area and ethical clearance is the same as described in 3.2.1.

5.2.2 Experimental design

The experimental design is the same as described in 3.2.3.

5.2.3 Animal management and diet

The animal management and diet are the same as described in 3.2.3.

5.2.4 Feeding and watering activities

The feeding and watering activities are the same as described in 3.2.4. The efficiency of water use was determined by finding the ratio of water intake to dry matter intake (WI: DMI). Water intake per kg of carcass produced (WI: kg carcass) was estimated as the total water intake ratio (WI) to hot carcass weight.

5.2.5 Stress indices and hormonal assay

On the last day of the experiment (75-d) and at 7:00 h early in the morning, blood was collected from the jugular vein using an 18-gauge needle in anti-coagulant free tubes. The blood was allowed to coagulate after it has settled for 30 minutes at room temperature before centrifuging at 1000 x g for 10 min. The serum samples obtained were stored in propylene tubes and kept at -20^{o}C pending analysis. The concentration of estradiol (E_2) and progesterone (P_4) were determined in serum samples. All hormonal assessments were carried out using the ELISA technique (DiaMetra, Italy). Serum Triiodothyronine (T_3) and Thyroxin (T_4), cortisol and insulin were quantified using a radioimmunoassay kit (Diagnostic Product Cooperation, Los Angeles, CA, US). Sensitivities of the methods were 0.38pg/ml, 0.05ng/ml and below 0.07, 0.25 and 1.1 ng/dL for E_2, P_4, T_3, T_4 and cortisol respectively. The corresponding intra and inter-assay coefficients of variations were 6.3-9.2%, 4.0-9.3%, 2.4-7.8%, 1.5-5.2% and 4.55-6.36% for E_2, P_4, T_3, T_4 and cortisol respectively.

5.2.6 Slaughter and carcass traits

Three goats were randomly selected per treatment for carcass traits. The final body weight (BW) was taken at the end of the trial by weighing the goats and then subjected to solid fasting overnight, a day before slaughter and reweighed to obtain the shrunk body weight (SBW). After that, the goats were transported to Adelaide, a small through-put commercial abattoir under Nxuba Local Municipality, 63.1 km from the university. The goats were stunned electrically (200 V applied for 4 s), slaughtered, and dressed following standard procedures. The dressed carcass were comprised of the body after removing the skin, head at the occipital-atlantal joint, the fore-feet at the carpal-metacarpal joint, the hind feet at the tarsal-metatarsal joint and the viscera. The weights of the digestive tracts (full and empty), liver, skin, heart, head, respiratory apparatus (lung + trachea), spleen and feet (fore and hind) were also recorded. After dressing, the carcasses' weights were immediately taken to obtain hot carcass weights (HCW) and hot carcass yield (HCY) determined. Thereafter, the cold carcass weight (CCW) was obtained and cold carcass yield (CCY) determined by cooling the carcasses at 4 ^{o}C for 24 h and reweighed. Also, the shrinkage loss of the carcass

(SLC) and biological yield (BY) were determined as described by Cartaxo *et al.* (2009). The empty body weight (EBW) was calculated as SBW- digestive content. After cooling, the carcass compactness indices (CCI (kg/cm)) = CCW/internal carcass length was determined (Souza *et al.*, 2013). The ratio of water intake to carcass weight (Figure 5.1) was calculated by dividing the total water intake by the cold carcass weight. The pH (Crison pH 25, Crison Instruments, S.A., Allela, Spain) was measured in the *longissimus thoracis et lumborum* (LTL) muscle between the 12th and 13th ribs 45 min and 24 h post-slaughter. Before use, the pH meter was calibrated with pH_4 and pH_7 standard solutions at room temperature.

5.2.7 Meat quality and physicochemical assessment

Four pieces (2 cm thick) were carefully cut from the LTL muscle of each dressed goat (three per group) at the caudal end of the carcass and randomly assigned for colour, thaw loss, cooking loss and Warner-Bratzler Shear Force (WBSF) determinations. All determinations were done in triplicate, and the mean value was reported.

5.2.7.1 Colour measurement

After blooming for 30 min, colour measurements were carried out on the meat surface at ambient temperature (22.5 ± 1.2 °C). The direction of muscle fibres was rectus (parallel) to the meat surface. Meat instrumental colour (lightness, *L*;* redness, *a**; yellowness, *b**) was measured after 24 hours of slaughter from the *longissimus* muscle using a Minolta colour-guide 45/0 BYK-Gardener GmbH machine, with a 20 mm diameter measurement area and illuminant D65-daylight, 10° observation angle. To obtain a representative average value of the colour, three readings were taken by rotating and replacing the Colour Guide by 90° between each measurement. Colour saturation (Chroma (C*)), $(a^{*2} + b^{*2})^{0.5}$ and the proportion of redness to yellowness (hue angle (H*)), $[\tan^{-1}(b^*/a^*)]$ were also calculated.

5.2.7.2 Thaw and cooking loss determination

Samples for thaw loss were weighed using a portable weighing scale (LBK 12) and subsequently frozen at -20 ^{o}C for 24 h, after which the frozen samples were reweighed and thawed at 4 ^{o}C for 12 h. The representative samples for cooking loss were thawed and weighed (8 g ± 0.05), placed in airtight plastic bags and immersed in a water bath at 80 ^{o}C for 60 min to reach an internal temperature of 80 ^{o}C. The internal temperature was monitored using a handheld digital temperature monitor (Hanna Instruments, Bellville, South Africa). The bags were cooled to ± 4 ^{o}C, and LTL slices removed and blotted dry with paper towels, without any added pressure and re-weighed. Thaw and cooking loss were calculated as follows;

Thaw loss = [(weight before thaw – weight after thaw) ÷ weight before thaw] × 100.

Cooking loss = [(weight of raw LTL slices after thawing- weight of cooked LTL slices) ÷ weight of raw LTL slices after thawing] × 100

5.2.7.3 Warner Bratzler shear force determination

The representative cooked samples for shear force (about 50 g) were chilled at 4 ^{o}C for 6 h for the determination of Warner-Bratzler shear force (WBS) values. Three sub-samples measuring 10 mm core diameter were cored parallel to the grain of the meat. The samples were sheared perpendicular to the fibre direction using a Warner Bratzler (WB) shear device (speed; 400 mm/min) mounted on an Instron (model, 3344; Universal) and equipped with a 500 N load cell to determine the shear force in Newtons.

5.2.8 Statistical analysis

Data obtained were analyzed using the mixed model of the SAS (2013) procedure. For all traits, the model included the fixed effect of treatments and the random effect of animals.

The model used was:

$Y_{\mathrm{ijk}} = \mu + A_{\mathrm{i}} + C_j + \mathcal{E}_{ijk}$

Where Y_{ijk} = slaughter weight, carcass traits or meat quality; μ = overall mean common to all observations; A_{i} = effect of water restriction levels with or without vitamin C; C_j = the random effect of animals; $\mathcal{E}_{ijk}$ = residual error

5.3 Results

5.3.1 Stress and hormonal indices

Table 5.1 shows the plasma hormonal indices of Xhosa goats subjected to different watering treatments. The follicle-stimulating hormone (FSH) and insulin were not affected (P>0.05) by water restriction levels. Amylase and lipase decreased (P<0.05) with levels of water restriction. Tri-iodothyronine (T_3) and thyroxine (T_4) decreased as the water restriction levels increased. Plasma cortisol concentrations increased (P<0.05) in W70 and W50 and decreased in the VC treated groups.

5.3.2 Carcass traits and yield

The carcass traits and yield of Xhosa goats subjected to different watering treatments are shown in Table 5.2. The shrunk body weight (SBW) and empty body weight (EBW) progressively decreased (P<0.05) with the levels of water restriction. However, the SBW and EBW of animals under group $W50^{++}$ were significantly higher compared to W50. There was a significant decrease (P<0.05) in the hot carcass (HCY) and cold carcass yield (CCY) due to levels of water restriction.

Table 5. 1 Hormonal indices and digestive enzymes of Xhosa goats subjected to different watering treatments

Variable	W0	W70	W50	$W70^{+}$	$W50^{+}$	$W70^{++}$	$W50^{++}$	SEM	P-value
$T_{4,}$ pmol/L	7.50^{a}	7.50^{a}	4.90^{b}	7.57^{a}	6.23^{ab}	6.83^{ab}	5.60^{ab}	0.71	0.011
$T_{3,}$ pmol/lL	3.43^{a}	3.09^{ab}	2.32^{c}	3.00^{ab}	2.41^{c}	2.68^{bc}	2.69^{bc}	0.18	0.001
Prolactin, ug/L	0.52^{b}	0.54^{b}	1.54^{a}	0.46^{b}	0.92^{ab}	0.79^{ab}	0.99^{ab}	0.28	0.013
FSH, IU/L	0.23	0.20	0.23	0.23	0.20	0.20	0.23	0.02	0.658
LH, IU/L	0.23	0.20	0.23	0.23	0.30	0.33	0.33	0.08	0.110
17β. pmol/L	67.00^{a}	56.00^{b}	55.33^{b}	55.33^{b}	56.33^{b}	55.33^{b}	55.33^{b}	2.7	0.032
Cortisol,nmol/L	42.66^{e}	85.67^{d}	143.33^{a}	84.33^{d}	132.00^{b}	79.33^{d}	117.33^{c}	3.62	0.042
Insulin, uIu/ml	1.57	1.43	1.27	1.57	1.23	1.57	1.33	0.18	0.073
Lipase,IU/L	46.00^{a}	39.33^{ab}	31.67^{cd}	38.00^{bc}	28.33^{ab}	30.67^{cd}	27.33^{bc}	2.44	<0.001
Amylase, IU/L	15.67^{a}	15.33^{a}	14.00^{ab}	14.00^{ab}	14.00^{ab}	13.33^{ab}	12.33^{b}	1.16	0.023

$^{+}$ 3gVC daily; $^{++}$ 3gVC daily+extra 5gVC every 8th days; ^{abcde}means with different superscript across the row are significantly different ($P<0.05$); SEM=standard error of mean; LH=luteinizing hormone; FSH=follicle stimulating hormone; T_4=thyroxine; T_3=tri-iodothyronine; 17 β W=water restriction; VC=vitamin C; W=water restriction.

Table 5. 2 Carcass traits and yield of Xhosa goats subjected to different watering treatments

Variable	W0	W70	W50	W70^{+}	W50^{+}	W70^{++}	W50^{++}	SEM	P-value
SBW, kg	22.23^{a}	19.09^{c}	15.53^{f}	19.73^{b}	17.50^{d}	19.50^{b}	17.01^{e}	0.14	<0.001
EBW, kg	16.55^{a}	14.94^{c}	11.39^{e}	15.48^{b}	13.33^{d}	14.93^{c}	13.13^{d}	0.11	<0.001
HCW, kg	9.05^{a}	7.50^{c}	5.80^{g}	7.90^{b}	6.90^{e}	7.30^{d}	6.50^{f}	0.03	<0.001
HCY, %	40.71^{a}	39.29^{b}	37.34^{d}	40.04^{ab}	39.43^{ab}	37.44^{cd}	38.22^{c}	0.24	<0.001
CCW, kg	8.70^{a}	7.30^{c}	5.60^{g}	7.50^{b}	6.60^{e}	7.00^{d}	6.20^{f}	0.03	<0.001
CCY, %	39.14^{a}	38.25^{ab}	36.05^{d}	38.01^{b}	37.71^{b}	36.90^{c}	36.46^{cd}	0.26	<0.001
SLC, %	3.86	3.67	3.44	4.06	3.35	4.10	3.61	0.61	0.199
TBY, %	54.70^{a}	50.19^{cd}	48.89^{d}	51.03^{bc}	49.50^{d}	51.76^{b}	50.95^{bc}	0.40	<0.001
CCI,kg/cm	0.17^{a}	0.15^{b}	0.12^{e}	0.15^{b}	0.14^{c}	0.14^{c}	0.13^{d}	0.002	<0.001

$^{+}$3gVC daily; $^{++}$3gVC daily+extra 5gVC every 8th days; ^{abcefg}means with different superscript across the row are significantly different ($P<0.05$); SEM=standard error of mean; VC=vitamin C; W=water restriction; SBW: shrunk body weight; EBW: empty body weight; HCW: hot carcass weight; HCY: hot carcass yield; CCW: cold carcass weight; CCY: cold carcass yield; SLC: shrinkage loss to cooling; TBY: true biological yield; CCI; carcass compactness index.

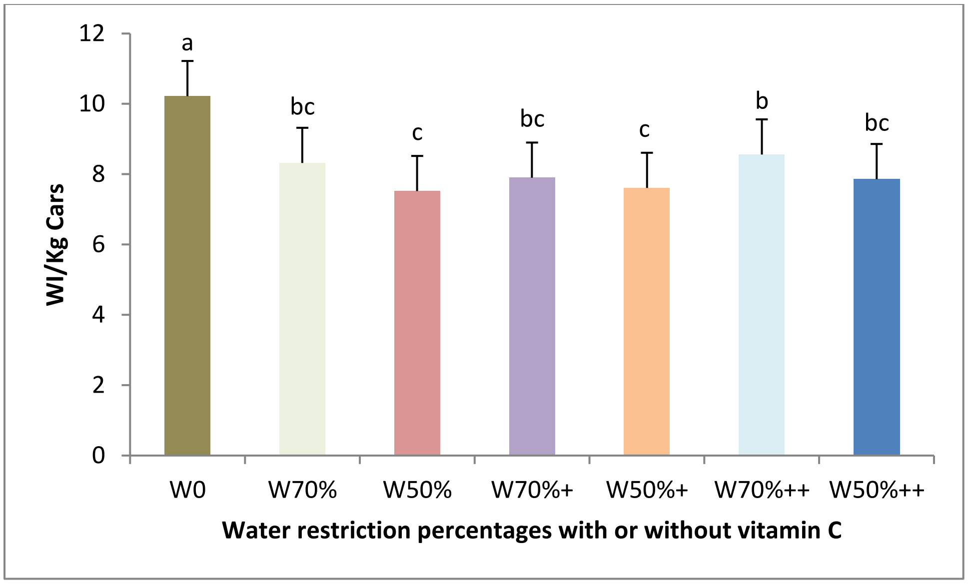
However, the HCY and CCY significantly increased higher in animals under group $W50^{+}$ compared to W50. The true biological yield (TBY) and carcass compactness index (CCI) were influenced considerably ($P<0.05$) by levels of water restriction. The water intake per kg of carcass weight (WI/kgCars) decreased ($P<0.05$) with levels of water restriction for treatments without VC (Figure 5.1). However, animals under groups $W70\%^{++}$ and $W50^{++}$ had higher WI/kgCars compared to $W70\%^{+}$ and $W50^{+}$, and W70 and W50.

5.3.4 Non-carcass components

The non-carcass component is shown in Table 5.3. Regardless of vitamin C supplementation and concentration, the head weight was not affected ($P>0.05$) by water restriction levels. The heart, liver and skin significantly decreased ($P<0.05$) in weight with increased water restriction levels. The weights of the heart in $W70^{+}$ and $W50^{+}$ were higher ($P<0.05$) compared to W70 and W50 groups, respectively. The spleen was not affected ($P>0.05$) by water restriction levels. The kidney's weights were higher ($P<0.001$) in W50, $W50^{+}$ and $W50^{++}$ compared to W0, W70, $W70^{+}$ and $W70^{++}$. All respiratory apparatus were smaller ($P>0.05$) than that of the control. The full and empty digestive tract and gastrointestinal content progressively decreased ($P<0.05$) with increased water restriction levels.

5.3.5 Physicochemical properties

Regardless of VC concentrations or supplementation, the water-restricted groups had decreased ($P<0.05$) L* (lightness), b* (yellowness), and increased ($P<0.05$) a* (redness) values compared to the control (Table 5.4). The colour saturation index, thaw loss, pH_{45min} and pH_{24h} were not affected ($P>0.05$) by water restriction and vitamin C supplementation levels.



SEM=0.46; P-value=<0.001; + 3gVC daily; ++ 3gVC daily plus extra 5gVC every eight day

Figure 5. 1 Ratio of water intake to kg of carcass weight (WI/KgCars) of Xhosa goats subjected to different watering treatments**.**

Table 5. 3 Non-carcass components of Xhosa goats subjected to different watering treatments

Variable	W0	W70	W50	W70^{+}	W50^{+}	W70^{++}	W50^{++}	SEM	P-value
Head, g	1427.33	1438.33	1368.33	1398.33	1348.00	1380.00	1355.00	42.55	0.146
Heart, g	84.00^{a}	71.00^{b}	65.00^{c}	81.00^{a}	71.00^{b}	69.67^{b}	63.30^{c}	1.40	<0.001
Liver, g	310.00^{a}	280.00^{b}	266.67^{c}	307.00^{a}	274.00^{b}	304.00^{a}	277.00^{b}	3.06	<0.001
Spleen, g	45.00	42.00	41.93	44.33	42.00	43.00	43.33	1.54	0.528
L+T, g	201.00^{a}	149.00^{b}	145.67^{b}	142.00^{b}	147.67^{b}	148.32^{b}	139.00^{b}	6.40	<0.001
Skin, g	1476.67^{a}	1376.67^{bc}	1226.67^{de}	1448.33^{ab}	1211.67^{de}	1303.33^{cd}	1195.00^{e}	46.02	<0.001
Kidney, g	65.00^{c}	64.00^{c}	75.00^{a}	63.00^{c}	70.00^{b}	65.00^{c}	70.00^{b}	0.735	0.001
Feet, g	645.00^{a}	615.00^{ab}	545.00^{c}	555.00^{c}	540.00^{c}	555.00^{c}	575.00^{c}	20.88	<0.001
FDT, kg	7.16^{a}	5.61^{d}	5.46^{g}	5.73^{c}	5.59^{e}	5.94^{b}	5.52^{f}	0.01	<0.001
EDT, kg	1.48^{a}	1.46^{ab}	1.31^{d}	1.48^{a}	1.42^{c}	1.47^{a}	1.44^{bc}	0.01	<0.001
GIC, kg	5.68^{a}	4.15^{de}	4.14^{e}	4.24^{c}	4.17^{d}	4.27^{b}	4.18^{d}	0.01	<0.001

$^{+}$3gVC daily; $^{++}$3gVC daily+extra 5gVC every 8th days; ^{abc}means with different superscript across the row are significantly different (P<0.05); SEM=standard error of mean; L+T=lung+trachea; FDT=full digestive tract; EDT=empty digestive tract; GIC= gastro-intestinal content; feet (both fore and hind feet); W=water restriction;

Table 5. 4 Physico-chemical properties of Xhosa goats subjected to different watering treatment

Variable	W0	W70	W50	$W70^{+}$	$W50^{+}$	$W70^{++}$	$W50^{++}$	SEM	P-value
L^{*}	38.94^{a}	37.09^{b}	34.60^{c}	32.29^{d}	31.76^{d}	34.48^{c}	31.86^{d}	0.83	<0.001
$a*$	13.31^{b}	13.98^{ab}	13.51^{b}	14.67^{a}	14.27^{ab}	14.10^{ab}	14.27^{ab}	0.56	0.005
$b*$	7.97^{a}	7.21^{ab}	6.86^{b}	7.45^{ab}	7.35^{ab}	7.50^{ab}	7.19^{ab}	0.42	0.005
$C*$	15.35	15.73	15.69	16.47	15.50	15.97	15.83	0.59	0.450
H^{*}	27.29^{bc}	30.57^{a}	31.10^{a}	27.08^{bc}	26.96^{c}	28.01^{b}	30.99^{a}	0.43	0.013
$pH_{45\ min}$	7.01	6.95	6.97	6.98	7.02	6.99	6.98	0.05	0.337
$pH_{24\ h}$	5.87	5.89	5.89	5.90	5.90	5.89	5.90	0.01	0.088
TL%	9.88	9.58	8.68	9.77	8.79	9.90	8.98	0.43	0.066
CL%	32.53^{c}	34.34^{bc}	37.55^{a}	31.04^{c}	36.72^{ab}	33.42^{bc}	34.29^{bc}	1.93	0.014
WBSF	25.03^{d}	26.40^{cd}	31.43^{ab}	28.87^{bc}	33.31^{a}	27.13^{cd}	33.19^{a}	1.67	0.015

$^{+}$3gVC daily; $^{++}$3gVC daily+extra 5gVC every 8th days; ^{abc}means with different superscript across the row are significantly different (P<0.05); SEM=standard error of mean; CL=cookoing loss; TL= Thaw loss; WBSF= Warner Bratzler shear force. WR=water restriction; VC=vitamin C; W=water restriction.

The effect of water restriction levels was significant ($P<0.05$) on cooking loss and shear force. Changes in cooking loss and shear force under the vitamin C treated groups did not follow a consistent pattern.

5.4 Discussion

This study showed cortisol concentrations increased with water restriction levels and were reduced following single or extra VC dosing. This contradicts what was reported about the non-significant change in cortisol in water-stressed sheep (Parker *et al.,* 2003). A 1% increase in ascorbic acid intake will usually result in a 22% and 27% decrease in adrenal and plasma corticosterone, respectively (Civen *et al.,* 1980). The variability in cortisol response to VC supplementation has been partly linked to handling and blood collection processes and elevated ambient temperature (Wingfield and Romero, 2001). The values recorded for T_4 in this study did not seem to differ under water restriction. However, both T_4 and T_3 had lower values in response to increased levels of water restriction. According to Mullur *et al.* (2014), the basal metabolic rates are primarily determined by T_3 and T_4, and these positively correlate to tissue production and weight gain. The response of T_3 and T_4 to stress may sometimes be prolonged and can take several days to arrive at a steady-state (Silanikove *et al.*, 2000). The decrease in thyroid activity in ruminants undergoing thermal stress is an attempt to reduce heat production and restoring homeothermy (Kahl *et al.*, 2015). A similar trend was reported in vitamin C treated-water-restricted Awassi ewes (Jaber *et al.*, 2011). The non-significant effect of water restriction on prolactin in this study could be an adaptive mechanism employed by the goats during limited water intake. The increase in prolactin following stressful conditions has been described in different species of animals such as donkey, camel, sheep, and cattle (Kataria and Kataria, 2010; Yayou *et al.*, 2010; Kataria and Kataria, 2011). The modulatory role of prolactin during water balance in an animal undergoing heat stress is conflicting. The inconsistencies can be attributed to thermal load's graveness, concealing any damaging effects on water intake induced by prolactin suppression (Alamer, 2011). Lipase and amylase values in this study were reduced with increasing levels of water restriction. Changes in digestive physiology in response to particular physiological needs have been demonstrated in birds and mammals concerning nutrient absorption and organ structure (Karasov and McWilliam, 2005). Decreased feed intake induced either by feed restriction or suboptimal water intake can lower digestive enzymes' activities. Such digestive adaptation to reduced feed intake has been reported in broiler chickens (Pinheiro *et al.*, 2004). All the water restricted groups had a reduced level of oestrogen compared to the control group. The survival instinct of adapting to the demand for an unfavourable environment in animals requires that endocrine hormones suppress growth and reproductive functions. During periods of stress, the ovarian follicular development was reported to diminish due to repression in peripheral gonadotropins levels (Roth *et al.*, 2000). In other reports, there was a depression of plasma estradiol levels in water restricted Malpura ewes (Kumar *et al.*, 2016) and undernourished ewes (Kiyma *et al.*, 2004).

The notable changes in DMI, final weight and body weight gain of goats subjected to different watering treatments were reflected and consistent in their hot and cold carcass weights and yields. With higher DMI, final live weight and body weight gain in animals, it is expected that the deposition of muscle will be greater, and ultimately the weight at slaughter will be higher, including heavier carcasses (Mapiye *et al.*, 2009). With lower DMI and weight gain in animals, the same cannot be said. Because of suboptimal water intake in ruminants, it has been shown that body water loss and feed intake reduction are partly responsible for body weight reduction (Hamadeh, 2006; Alamer, 2009). Besides, energy needs require that fat be mobilized from adipose tissues to compensate for the reduced feed and nutrient intake in animals following water restriction. Regardless of VC supplementation, the non-significant effect of the different watering treatments on the shrinkage loss to cooling (SLC) agrees with dos Santos *et al.* (2019). In lambs, cooling losses usually vary from 3.0 to 4.0 %, depending on sex, chilling temperature, fat cover and weight (Reis *et al.*, 2001). The range of values for SLC in this study is 3.44 to 4.10. The SLC indicates sufficient fat cover and can also act as a shock absorber to the refrigeration chamber's cooling effect, thus preventing cold shortening and excessive water loss from meat (Safari *et al.*, 2001). Though there was a slight depression in the true biological yield (TBY), especially for animals grouped under W50, the range of values for all the goats subjected to different watering treatments was not outside the 40 to 60 % range, based on reported studies (Sanudo and Sierra, 1986). There was a decrease in the carcass compactness indices (CCI) and was not influenced by vitamin C supplementation. The CCI provides a measure of the amount of meat stored in the carcass and leg. This study, however, showed that the storage capacity decreased with increasing water restriction and did not improve following vitamin C supplementation. The intake per kilogram carcass (WI/kgCars) estimates the amount of water ingested per kg of the carcass. With a similar amount of consumed water, animals under W50^{++} produced 7.86 kg of the carcass, compared to 7.52 kg in W50. This improvement in the kilograms of carcass produced could be attributed to an improved feed intake following VC supplementation.

The non-significant effect of water restriction levels on the head weight suggests that the body's different parts have different growth patterns (Rosa *et al.*, 2002). In the allometry growth pattern, the head is usually formed at the early or initial phase of growth, and the effect due to diet may be very minimal. This result agrees with dos Santos *et al.* (2019), who subjected feedlot lamb to different water restriction periods. The skin, heart and liver decreased with an increase in water restriction level. The skin is regarded as one of the essential non-carcass components, and it is estimated to be about 10 % of the total animal value. The skin's growth rate is expected to follow a similar body weight pattern (isogonic growth). This might explain the decrease in skin weight with respect to water restriction levels. Depression in the heart's weight in W70 and W50 was reduced by 14 % and 6.15 % in W70$^+$ and W50$^+$, respectively. The liver, as an organ, is very instrumental in the various metabolic activities of the body. The reduced liver weight with respect to an increase in water restriction levels could result from the reduction in metabolic rate usually induced by limited water intake (Camilo *et al.*, 2012). Also, ruminants' adaptive response to reduce feed intake during water stress might result in gradual liver atrophy when the received feed intake is below the maintenance requirements. As observed, with more water ingested, the weight of the liver increased. The supposed higher liver weights in the treated groups compared to the untreated groups may be attributed to higher feed intake. The reported higher weight values in the kidney of goats subjected to more increased watering restriction levels (W50, W50$^+$ and W50^{++}) might result from the kidney's increased activity to reabsorbed more water from the renal collecting tubules under the stimulation of the anti-diuretic hormone (ADH). This thirst activity centre or mechanism may result in growth stimulation and weight gain (Naves *et al.*, 2003). As Alamer (2009) described, to reduce water loss in ruminants, several physiological mechanisms are activated in response to water shortages, including lowering the respiratory apparatus's weights. The decrease in the weight of respiratory apparatus (lung+trachea) in the water restricted groups compared to the control may be due to an attempt to reduce water loss by respiration and save some nutrients.

The colour of meat is regarded as an essential selection criterion when meat products are purchased because meat colour and freshness seem to be intricately connected (Rani *et al.*, 2013). Based on studies, an induced oxidative environment in stressed animals before slaughter can decrease lipids and colour stability (Rowe *et al.*, 2004; Miranda-de la Lama *et al.*, 2011). Supplementation of antioxidants (e.g. VC) pre-mortem can improve meat colour and lipid stability by prolonging the reducing environment through limiting the activities of reactive oxygen species (Harris *et al.*, 2001). The values obtained for $b*$ and $L*$ significantly decreased while $a*$ increased in the water restricted groups compared to the control. During stress, catecholamines and adrenocortical steroid are released, and their activities may result in dark-cutting meat (Miranda-de la Lama *et al.*, 2011). In addition, the observed low $L*$ values (darker meat) in the water restriction groups may be attributed to an increase in myoglobin content and a sharp decline in muscle glycogen. The lack of difference in a* values across the treated-water- restricted groups may result from a similarity in the muscle's VC content. The hue-angle value linearly increased with respect to water restriction levels, while the chroma values were not significantly affected. The quality of meat is partly affected by the pH of muscles, as darker meat usually results from an increased pH_{24} or ultimate pH (pHu). Other meat quality attributes influenced by pHu include water-holding capacity, tenderness, and collagen (Kadim *et al.*, 2006; Ekiz *et al.*, 2012). Regardless of vitamin C supplementation, the effect of water restriction levels in this study did not affect the pH_{24}. However, Nikbin *et al.* (2016) reported an increase in pH_{24} of Boer goats subjected to high stocking density transportation compared to the un-transported control. According to Muchenje *et al.* (2008**),** increased meat pH values, and consequently, darker meat are usually the outcomes of depleted glycogen levels resulting from pre-slaughter stress. An oxidative environment induced by pre-slaughter stress usually decreases protein degradation and increases WBSF (Green and Price, 1974). In this study, tenderness decreased as the water restriction level increase. Other studies have also affirmed that less stressed animals have more tendered meat than those from highly stressed animals (Ferguson and Warner 2008). Besides, the depression in feed intake induced by suboptimal water intake may reduce glycogen storage in the muscle. This muscle glycogen depletion enhanced rapid protein denaturation, glycolytic activity and rigour mortis process, consequently toughening the meat (Carragher and Mathews, 1996). According to the tenderness scale suggested by Cezar and Sousa (2007), medium-tender meat has a shear force range of 22.4 to 35.6N/cm^2, while hard meat ranges from 35.7 to 53.4 N/cm^2. In this study, the entire water restricted group's meat could still be classified under medium tenderness, as the values obtained ranged from 26.40 to 33.31 N/cm^2. This is not surprising since the ultimate pHu across the entire treatment groups were low (<6.0) and sufficient to produce a more tendered meat with lower shear force and better colourimetric values (Simela *et al.*, 2004).

The increase in cooking loss values to water restriction levels resonates with other studies' report (Kadim *et al.*, 2006; Nikbin *et al.*, 2016). However, some studies have reported lower cooking loss (Apple *et al.*, 1995) or a non-significant effect (Kannan *et al.*, 2003; dos Santos *et al.*, 2019) of pre-slaughter stress on cooking loss. Such differences in the cooking loss from various reports may be due to stress and ambient temperature. Water restriction levels did not influence the thaw loss values. In a similar study on feedlot lambs subjected to 24 h, 48 h and 72 h water restriction periods, dos Santos *et al.* (2019) found out that thaw loss was not affected. On the contrary, Boer goats subjected to pre-slaughter transportation and high stocking density stress had higher values compared to the control (Nikbin *et al.*, 2016). Apart from contributing to juiciness, the shelf life and storage quality of meat depends on its ability to retain a significant amount of water (Henchion *et al.*, 2014). As observed in this study, this ability of goats to still maintain a similar amount of water even at a water intake level of W50% of *ad libitum* intake may probably indicate that the muscle pH and the isoelectric protein point at postmortem were not affected (Munasinghe and Sakai, 2004).

5.5 Conclusion

The increased plasma cortisol concentration induced by water stress was lowered with VC supplementation. The hot and cold carcass weights were reduced at both water restriction levels but improved with a single VC dose. Unlike the heart and liver, the kidney's weight rose with water restriction levels, with the highest value recorded at W50. With a single dose of VC, the depression in the liver and heart weights was reduced but failed to modulate the kidney's weight even at multiple dosages. The pH_{45min}, pH_{24h} and saturation index and thaw loss were not affected by the watering treatments. All VC treated groups had improved redness. Regardless of VC supplementation, the shear force and cooking loss increased with levels of water restriction. Limited water intake induced pre-slaughter stress in animals. However, a single dose of VC promoted the highest significance, where improvements were observed and may be considered a management practice during water stress. Further experimentation on fatty acids, lipids oxidation and sensory attributes of *Longissimus lumborum* muscles of Xhosa goats will add more value to the carcass characteristics that have been observed in this chapter.

5.6 References

Akinmoladun, O.F., Muchenje, V., Fon, F.N. and Mpendulo, C.T. (2019). Small ruminants: Farmers' hope in a world threatened by water scarcity. *Animals* **9**(7), 456.

Alamer, M. (2009). Effect of water restriction on lactation performance of Aardi goats under heat stress conditions. *Small Rumin. Res.* **84**, 76–81.

Alamer, M.E. (2010). Effect of water restriction on thermoregulation and some biochemical constituents in lactating Aardi goats during hot weather conditions. *J. Basic Appl. Sci. Res.* **11**, 189–205.

Alamer, M.E. (2011). The role of prolactin in thermoregulation and water balance during heat stress in domestic ruminants. *Asian J. Anim. Vet. Adv.* **6**(2), 1153-1169.

AOAC. (2000). Official Methods of Analysis of the Official Analytical Chemists (W. Horwitz ed) 17th Ed, Association of Official Analytical Chemists, Washington DC USA

Apple, J.K., Dikeman, M.E., Minton, J.E., McMurphy, R.M., Fedde, M.R., Leith, D.E. and Unruh J.A. (1995). Effects of restraint and isolation stress and epidural blockade on endocrine and blood metabolite status, muscle glycogen metabolism and incidence of dark-cutting longissimus muscle of sheep. *J. Anim. Sci.* **73**, 2295–2307.

Barbour, E., Rawda, N., Banat, G., Jaber, L., Sleiman, F.T. and Hamadeh, S. (2005). Comparison of immunosuppression in dry and lactating Awassi ewes due to water deprivation stress. *Vet. Res. Com.* **29**, 47–60.

Camilo, D.A., Pereira, E.S., Pimentel, P.G., Costa, M.R.G.F., Mizubuti, I.Y. Ribeiro, E.L.A. and Moreno, G.M.B. (2012). Weight and yield of non-carcass components of Morada Nova lambs fed with different levels of metabolizable energy. *Agric. Sci.* **33**, 2429–2440.

Cartaxo, F.Q., Cezar, M.F., Sousa, W.H., Gonzaga Neto, S.G., Pereira Filho, J.M.P. and Cunha, M.G.G. (2009). Quantitative traits of carcass from lambs finished in feedlot system and slaughtered at different body conditions. *Rev. Bras. Zootecn.* **38**, 697–704.

Carragher, J.F. and Matthews, L.R. (1996). Animal behavior and stress: Impacts on meat quality. Proceedings of the New Zealand Society of Animal Production **56**, 162–216.

Cezar, M.F. and Sousa, W.H. (2007). Carcaças Ovinas e Caprinas: Obtenção-avaliaçãoclassificação. Uberaba: *Agropecuaria Tropical*. *pp. 232*

Civen, M., Leeb, J.E., Wishnow, R.M. and Morin, R. (1980). Effect of dietary ascorbic acid and vitamin E deficiency on rat adrenal cholesterol ester metabolism and corticosteroidogenesis. *Int. J. Vitam. Nutr. Res.* **50**, 70–78.

Donnenfeld, Z., Crookes, C. and Hedden, S. (2018). A Delicate Balance: Water Scarcity in South Africa; Institute for Security Studies (ISS): Pretoria, South Africa, pp. 1–24.

dos Santos, F.M., de Araujo, G.G.L., de Souza L.L., Yamamoto, S.M., Queiroz, M.A.A., Lanna, D.P.D. and de Moraes, S.A. (2019). Impact of water restriction periods on carcass traits and meat quality of feedlot lambs in the Brazilian semi-arid region. *Meat Sci.* **156**, 196-204.

Ekiz, B., Ergul Ekiz, E., Kocak, O., Yalcintan, H. and Yilmaz, A. (2012). Effect of pre-slaughter management regarding transportation and time in lairage on certain stress parameters, carcass and meat quality characteristics in Kivircik lambs. *Meat Sci.* **90**, 967–976.

FAOSTAT, Food and Agriculture Organization of the United Nations, (2016). FAOSTAT, Food and Agriculture Organization of the United Nations. Retrieved from: http://www.fao.org/faostat/en/#data/QL2016.

Ferguson, D.M. and Warner, R.D. (2008). Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants? *Meat Sci.* **80**, 12–19.

Ghanem, A.M., Barbour, E.K., Hamadeh, S.K., Jaber, L.S. and Abi Said. M. (2008). Physiological and chemical responses in water-deprived Awassi ewes treated with vitamin C. *J. Arid Environ.* **72**, 141-149.

Greene, B. E. and Price, L.G. (1975). Oxidation induced color and flavor changes in meat. *J. Agric. Food Chem.* **23**, 164-167.

Hamadeh, S.K., Rawda, N., Jaber, L.S., Habre, A., Abi Said, M. and Barbour, E.K. (2006). Physiological responses to water restriction in dry and lactating Awassi ewes. *Livest. Sci.* **101**, 101–109.

Harris, S.E., Huff-Lonergan, E., Lonergan, S.M., Jones, W.R. and Rankins, D. (2001). Antioxidant status affects color stability and tenderness of calcium chloride-injected beef. *J. Anim. Sci.* **79**, 666-677.

Henchion, M., McCarthy, M., Resconi, V. C. and Troy, D. (2014). Meat consumption: Trends and quality matters. *Meat Sci.* **98**, 561–568.

Hidiroglou, M., Batra, T.R. and Zhao, X. (1997). Comparison of vitamin C bioavailability after multiple or single oral dosing of different formulations in sheep. *Reprod. Nutr. Dev.* **37**, 443-448.

IPCC (Intergovernmental Panel on Climate Change), (2007). Synthesis Report. In Contribution of Working Groups I, II and II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change; Pachauri, R.K., Reisinger, A., Eds.; IPCC: Geneva, Switzerland, p. 104.

Jaber, L.S., Hanna, N., Barbour, E.K., Abi Said, M., Rawda, N., Chedid, M. and Hamadeh, S.K. (2011). Fat mobilization in water restricted Awassi ewes supplemented with vitamin C. *J. Arid Environ.* **75**, 625-628.

Jacob, R.H., Pethick, D.W., Clark, P., D'Souza, D.N., Hopkins, D.L. and White, J. (2006). Quantifying the hydration status of lambs in relation to carcass characteristics. *Anim. Prod. Sci.* **46**(4), 429-437.

Kadim, I.T., Mahgoub, O., Al-Kindi, A., Al-Marzooqi, W. and Al-Saqri, N.M. (2006). Effect of transportation at high ambient temperatures on physiological responses, carcass and meat quality characteristics of three breeds of Omani goats. *Meat Sci.* **73,** 626–634.

Kahl, S., Elsasser, T.H, Rhoads, R.P., Collier, R.J. and Baumgards, L.H. (2015). Environmental heat stress modulates thyroid status and its response to repeated endotoxin challenge in steers. *Domest. Anim. Endocrinol*. **52**, 43-50.

Kannan, G., Kouakou, B., Terrill, T.H. and Gelaye, S. (2003). Endocrine, blood metabolite, and meat quality changes in goats as influenced by short-term, preslaughter stress. *J. Anim. Sci.* **81**, 1499–1507.

Karasov, W.H. and McWilliam, S.R. (2005). Physiological and ecological adaptation to feeding in vertebrates. Enfield, New Hampshire. Eds: Starck, J.M. and Wang T.

Kataria, N. and Kataria, A.K. (2011). Use of prolactin as an indicator of stress in Marwari sheep from arid tracts in India. Comp. *Clin. Pathol.* **20**, 333-336.

Kataria, N. and Kataria, A.K. (2010). Assessment of stress due to hot ambience in donkeys from arid tracts in India. *J. Stress Physiol. Biochem*. **6**, 12-17.

Khliji, S., Van de Ven, R., Lamb, T., Lanza, M. and Hopkins, D. (2010). Relationship between consumer ranking of lamb colour and objective measures of colour. *Meat Sci.* **85**, 224–229.

Kiyma, Z., Alexander, B.M., Van Kirk, E.A., Murdoch, W.J., Hallford, D.M. and Moss, G.E. (2004). Effect of feed restriction on reproductive and metabolic hormones in ewes. *J. Anim. Sci.* **82**, 2548–2557.

Koohmaraie, M., Kent, M.P., Shackelford, S.D., Veiseth, E. and Wheeler, T.L. (2002). Meat tenderness and muscle growth: is there any relationship? *Meat Sci.* **62**, 345–352.

Kumar, D., De K., Singh, A.K., Kumar, K., Sahoo, A. and Naqvi, S.M.K. (2016). Effect of water restriction on physiological responses and certain reproductive traits of Malpura ewes in a semiarid tropical environment. *J. Vet. Behav*. **12**, 54–59.

Lowe, T.E., Gregory, N.G., Fisher, A.D. and Payne, S.R. (2002). The effect of temperature elevation and water deprivation on lamb physiology, welfare and meat quality. *Aust. J. Agric. Res.* **53**, 707-714.

Mandl, J., Szarka, A. and Bánhegyi, G. (2009). Vitamin C: update on physiology and pharmacology. *Br. J. Pharmacol.* **157**(7), 1097–1110.

Mapiye, C., Chimonyo, M., Dzama, K., Strydom, P.E. Muchenje, V. and Marufu, M.C. (2009). Nutritional status, growth performance and carcass characteristics of Nguni steers supplemented with Acacia Karroo leaf-meal. *Livest. Sci.* **126**, 206-214.

Miranda-de la Lama, G.C., Monge, P., Villarroel, M., Olleta, J.L., Garcıa-Belenguer, S. and Marıa, G.A. (2011). Effects of road type during transport on lamb welfare and meat quality in dry hot climates. *Trop. Anim. Health Prod.* **43**, 915–922.

Muchenje, V., Dzama, K., Chimonyo, M., Raats, J.G. and Strydom, P.E. (2008). Meat quality of Nguni, Bonsmara and Aberdeen Angus steers raised on natural pasture in the Eastern Cape, South Africa. *Meat Sci.*, **79**, 20-28.

Mullur, R., Liu, Y. and Brent, G.A. (2014). Thyroid hormone regulation of metabolism. *Physio. Rev*. **94**(2), 355-382.

Munasinghe, D. M. S. and Sakai, T. (2004). Sodium chloride as a preferred protein extractant for pork lean meat. *Meat Sci.* **67**, 697–703.

Naves, L. A., Vilar, L., Costa, A. C. F., Domingues, L. and Casulari, L.A. (2003). Disorders in the secretion and action of antidiuretic hormone. *Arch. Endocrin. Meta.* **47**, 467–481.

Nikbin, S., Panandam, J.M. and Awis Q.S. (2016). Influence of preslaughter and storage density on carcass and meat quality characteristics of Boer goats. *Ital. J. Anim. Sci.* **15**(3), 504-511.

National Research Council (NRC) (1981). Nutrient requirements of goats: angora, dairy, and meat goats in temperate and tropical countries. National Academy of Sciences. National Academic Press, Washington, DC.

National Research Council (NRC) (2007). Nutrients requirements of Small Ruminants. 362p.Washington, D. C: National Academy Press.

Rani, Z.T., Hugo, A. and Muchenje V. (2013). Perceptions of rural consumers on the quality of mutton in the Eastern Cape Province, South Africa. *Sci. Res. Essays* **8**, 921-931.

Parker, A.J., Hamlin, G.P., Colemn, C.J. and Fitzpatrick, L.A. (2003). Quantitative analysis of acid-base balance in *Bos indicus* steers subjected to transportation of long duration. *J. Anim. Sci.* **81**, 1234-1239.

Pinheiro, D.F., Cruz, V.C., Sartori, J.R. and Vicentini Paulino, M.L.M. (2004). Effect of early feed restriction and enzyme supplementation on digestive enzyme activities in broilers. *Poult. Sci.* **83**, 1544-1550.

Ranjan, R., Swarup, D., Naresh, R. and Patra, R.C. (2005). Enhanced erythrocytic lipid peroxides and reduced plasma ascorbic acid, and alteration in blood trace elements level in dairy cows with mastitis. *Vet. Res. Commun.* **29**, 27–34.

Reis, W., Jobim, C.C., Macedo, F.A.F., Martins, E.N., Cecato, U. and Silveira, A. (2001). Performance of feedlot lambs fed high-moisture grain corn silage or reconstituted grain corn silage in replacement of dry corn grain in the diet. *Rev. Bras. Zootecn.* **30**, 525–532.

Rosa, G.T., Pires, C.C., Silva, J.H.S. and Motta, O.S. (2002). Proportions and coefficients of growth components, non-carcass of the live weight of male and female lambs under different feeding methods. *Rev. Bras. Zootecn.* **31**, 2290–2298.

Roth, Z., Meidan, R., Braw-Tal, R. and Wolfenson, D. (2000). Immediate and delayed effects of heat stress on follicular development and its association with plasma FSH and inhibin concentration in cows. *J. Reprod. Fertil.* **120**, 83–90.

Rowe, L.J., Maddock, K.R., Lonergan, S.M. and Hoff-Lonergan, E. (2004). Influence of early postmortem protein oxidation on beef quality. *J. Anim. Sci.* **82**, 785–793.

Safari, E., Fogarty, N. M., Ferrier, G. R., Hopkins, L.D. and Gilmour, A. (2001). Diverse lamb genotypes: Eating quality and the relationship between its objective measurement and sensory assessment. *Meat Sci.* **57**, 153–159.

Sanudo, C. and Sierra, I. (1986). Calidad de la canal en la especie ovina. *Ovino* **1**, 127–153.

SAS Institute Inc. (2013). SAS System for Windows.Release 9.4.

Silanikove, N., Shamy, A., Shinder, D. and Moran, A. (2000). Stress down regulates milk yield in cows by plasmin induced beta-casein product that blocks K+ channels on the apical membranes. *Life Sci.* **67**, 2201–221.

Simela, L., Webb, E.C. and Frylinck, L. (2004). Effect of sex, age and pre-slaughter conditioning on pH, temperature, tenderness and colour of indigenous South African goats. *S. Afr. J. Anim. Sci.*, **24**(1), 208–211.

Souza, D.A., Selaive-Villarroel, A.B., Pereira, E.S., Osorio, J.C.S. and Texeira, A. (2013). Growth performance, feed efficiency and carcass characteristics of lambs produced from Dorper sheep crossed with Santa Ines or Brazilian Somali sheep. *Small Rumin. Res.* **114**, 51–55.

Tibin, M.A.M., Bushara, I., Elemam, M.B., Tibin, I.M. and Jadalla, J.B. (2012). Carcass characteristics of desert sheep under range conditions in North Kordofan State, Sudan. *Online J. Anim. Feed Res.* **2**, 439–444.

Tyler, P.J. and Cummins, K.A. (2003). Effect of dietary ascorbyl-2-phosphate on immune function after transport to a feeding facility. *J. Dairy Sci.* **86**, 622–629.

Vernon, R.G. (1992). Effects of diet on lipolysis and its regulation. *Proc. Nutr. Soc.* **51**, 397-408.

Vernon, R.G., Clegg, R.A. and Flint, D.J. (1981). Metabolism of sheep adipose tissue during pregnancy and lactation. Adaptation and regulation. *Biochem. J.* **200**, 307-314.

Wakchaure, R., Ganguly, S. and Praveen, P.K. (2015). Role of water in livestock. *Rec. Adv. Acad. J.* **1,** 53-56.

Wang, L.I., Liu, F., Luo, Y., Zhu, L. and Li, G. (2015). Effect of acute heat stress on adrenocorticotropic hormone, cortisol, interleukin-2, interleukin-12 and apoptosis gene expression in rats. *Biomed. Rep.* **3(**3), 425-429.

Watt, M.J., Howlett, K.F., Febbraio, M.A., Spriet, L.L. and Hargreaves, M. (2001). Adrenaline increases skeletal muscle glycogenolysis, pyruvate dehdrogenase activation and carbohydrate oxidation during moderate exercise in humans. *J. Physiol.* **534**(Pt 1), 269-278.

Webb, E.C. (2014). Goat meat production, composition and quality. *Anim. Front.* **4(**4), 33-37.

Wingfield, J.C. and Romero, L.M. (2001). Adrenocortical responses to stress and their modulation in free-living vertebrates. In: McEwen, B.S., Goodman, H.M. (Eds.), Handbook of Physiology; Section 7: The Endocrine System; Coping with the Environment: Neural and Endocrine Mechanisms, Vol. IV. Oxford University Press, pp. 211–234.

Yayou, K.L., Ito, S., Yamamoto, N., Kitagawa, S. and Okamura, H. (2010). Relationship of stress responses with plasma oxytocin and prolactin in heifer calves. *Physiol. Behav.* **99**, 362-369.

Chapter Six

Fatty acid profile, oxidative stability of lipids and sensory attributes of water restricted Xhosa goat meat supplemented with vitamin C

(Published in *Animal Production Science* Journal)

Abstract: Water scarcity, often accompanied by limited water intake in livestock, may result in pre-slaughter stress, thereby affecting meat quality parameters. This study was conducted to determine the effect of vitamin C (VC) supplementations of Xhosa goats subjected to different watering regimes on fatty acid, lipids oxidation and sensory attributes of *Longissimus lumborum* muscles. A total of forty-two goats were randomly assigned into seven treatments; without water restriction (W0, control), water restriction of 70% of *ad libitum* water intake WI (W70), water restriction of 50% *ad libitum* WI (W50), water restriction of 70% of *ad libitum* WI plus 3g VC daily ($W70^{+}$), water restriction 50% of *ad libitum* WI intake plus 3g VC daily ($W50^{+}$), water restriction of 70% of *ad libitum* WI plus 3g VC and extra 5gVC given every eight-day ($W70^{++}$); water restriction of 50% of *ad libitum* WI plus 3g VC and extra 5gVC given every eight-day ($W50^{++}$). The goats were fed for 75 days and slaughtered following standard procedures, and the LD samples obtained were subjected to a 24 h chill storage (4±1°C). Thiobarbituric acid reactive substance (TBARS), moisture and fat content, sensory attributes and fatty profile of the meat were evaluated. The treatment effect was not significant ($P>0.05$) on TBARS values. The moisture and fat content in the water restricted groups were lower ($P<0.05$) than W0. The meat sensory appearance was affected ($P<0.05$) by water restrictions. Regardless of VC concentration or supplementation, vaccenic and docosahexaenoic acid increased ($P<0.05$) while linolenic acid decreased as the water restriction levels increased. Nonetheless, treatment did not affect ($P>0.05$) the saturated and unsaturated fatty acids. Compared with W0, there were no difference in the meat's lipid oxidation and fatty acid profile under restricted water of 70% and 50% of *ad libitum* WI and with or without VC.

Keywords: Lipid peroxidation, oxidative stress, water scarcity, pre-slaughter stress, antioxidants

6.1 Introduction

Climate change and increasing water use due to rising populations have limited the amount of fresh-water available to the populace, with a far-reaching effect in water-scarce and dry zones of the world (IPCC, 2007; Akinmoladun *et al.*, 2019). Livestock is the most affected as animal agriculture is known to be water-intensive (Gerbens-Leenes *et al.*, 2013), and the availability of drinking water in sufficient amount is now a concern to farmers. Drinking water is limiting. Therefore, it is not surprising for livestock, especially ruminants in arid and water-limited or drought-hit regions, to trek long distances in search of water and pasture that are potentially richer in moisture content and nutritional value.

Small ruminants adaptively respond to limited water intake by reducing their feed intake to allow for low metabolic rate and less heat during the digestive process. This enables them to minimise dissipation due to evapotranspiration when the ambient temperature is high (Akinmoladun *et al.*, 2019). Compared to other livestock species, they tend to be more resilient to suboptimal water intake as losses >20% of body water can be tolerated due to rumen capacity to store water for later use (Jaber *et al.*, 2004). Despite this water use efficiency, suboptimal water intake in small ruminant affects animal performance and homeostasis, especially average daily intake and body weight, while promoting oxidative stress (Akinmoladun *et al.*, 2019). The excessive free radicals precipitated by oxidative stress not only affect productivity and overall performance of livestock but also impair cellular biomacromolecules (e.g. DNA, lipids and proteins) and mitochondrial integrity, thus accelerating lipid and protein peroxidation during ageing of meat and in processed meat products (Bekhit *et al.*, 2013; Estevez, 2015). Besides, there is a tendency for the quality of meat to be impaired by changing meat colour from red to dark red due to myofibres shrinkage, thus enhancing shrinkage loss and meat dryness (Jacob *et al.*, 2006).

Acceptance of meat products by consumers depends primarily on meat flavour and tenderness (O’Quinn *et al.*, 2018), and these characteristics may easily be affected by pre-slaughter stress. Antioxidants additives, such as vitamin C (VC), could be instrumental, when supplemented to live animals or added during meat processing, in combating the development of an oxidative environment, thus extending colour, lipid stability and prolonging shelf life (Rowe *et al.*, 2004).

Though the NRC (1981, 2007) did not describe clearly daily VC requirements for small ruminants because they have the potential to biosynthesize VC from glucose in the liver, plasma ascorbate concentrations were found to decrease following stressful and disease conditions (Ranjan *et al.*, 2005). Positive outcomes have been reported in improved feed efficiency and less bodyweight reduction following vitamin C supplementations in heat-stressed poultry and swine (de Rodas *et al.*, 1998; Sahin *et al.*, 2003) and water-stressed Awassi ewes (Ghanem *et al.*, 2008), respectively. It was hypothesized that supplemental VC would enhance a reducing environment by limiting lipids' oxidation within the postmortem muscle and improving the meat's sensory attributes. Therefore, this study evaluated the effect of vitamin C supplementation to water restricted Xhosa goats by evaluating its meat fatty acid profile, lipid oxidation and sensory attributes.

6.2 Materials and methods

6.2.1 Study site and ethical approval

This is the same as described in 3.2.1

6.2.2 Animal management and treatment designs

This is the same as described in 3.2.2.

6.2.3 Feeding and watering activities

This is the same as described in 3.2.4.

6.2.4 Slaughter procedures

At the end of the trial, three goats per treatment group were randomly selected for slaughtering. Slaughtering and dressing of the animals were carried out at a small through-put abattoir in Adelaide (63.1 km from the University of Fort Hare), under Nxuba Local Municipality, following standard procedures. Carcasses were split, weighed, and then chilled (0-4 ^{o}C) before being processed the day after slaughter. Thereafter, samples (100 mm thick piece) of *m. longissimus thoracis et lumborum* (LTL) of the right side were collected from 10th rib in the right direction of the rump and the right LTL's posterior side. Subsequently, the connective tissue and subcutaneous fat were removed and vacuum-packaged at 0-4 ^{o}C for chemical composition, lipid oxidation and fatty acid analysis.

6.2.5 Determination of Lipid Oxidation (TBARS)

The acid-precipitation technique of TBARS (with slight modification) was used to determine lipids' oxidation in the meat samples. Briefly, 2 g of sample was weighed (in triplicate) into 50 mL tubes and homogenised (Ultraturax) for 20 sec after the addition of 6.25 ml each of trichloroacetic acid (TCA, 0.001M) and distilled water (dH_2O). The homogenate was filtered using a Whatman no1 filter paper. A standard curve was constructed (in triplicate) using 0, 5, 10 and 20 µL TMP (1,1,3,3-Tetramethoxypropane (TMP), 0.001 M) in 1 mL of dH_2O. There were three tubes allocated for each sample, and 1 ml of the filtrate was added to each tube. To each prepared standard and two tubes of filtrate samples, 1 mL of Thiobarbituric acid (TBA) was added, while 1 mL of dH_2O was added to the third filtrate sample tube to act as a turbidity blank at 70 ^{o}C for 1 h.

Thereafter, samples were allowed to cool, and the absorbance was read at 530 nm (Spectrostar Nano, BMG Labtech, Ortenberg, Germany). TBARS, expressed as mg of malondialdehyde (MDA)/kg meat, was calculated as;

TBARS (mg MDA/Kg meat) =

$$\frac{absorbance * molar\ mass\ of\ MDA * volume\ of\ extract * dilution\ factor}{sample\ mass * slope\ of\ standard\ curve}$$

6.2.6 Sensory characteristics

To assess the meat sensory attributes, some pieces (approximately 3 cm^3) were cut (per treatment group) from the LTL muscle, wrapped with aluminium foil and cooked in an oven (pre-heated to 200^oC) using a baking pan. Cooking was deemed complete when the geometric centre of the samples reached 75 ^oC. Thereafter, samples were labelled and the sequence of serving randomized. Sensory attributes like colour, texture, taste-odour and acceptability were considered when evaluating the samples. Each sample was evaluated by twelve panellists using a 9-point hedonic scale. The nine-point descriptive scales used in the evaluation were designed as follows: colour (1 = extremely light red to 9 =dark brown), taste-aroma (1 = extremely bland to 9 = extremely intense); appearance (1 = dislike extremely to 9 = like extremely), texture (1 = extremely soft to 9 = extremely hard) and acceptability (1 = dislike extremely to 9 = like extremely) (Pena *et al.*, 2009). The panellist used had over five years of experience in sensory evaluation.

6.2.7 Determination of fatty acid profile

Quantitative extraction of total muscle lipid from LTL samples (Folch *et al.*, 1957) was done using a mixture of methanol and chloroform (ratio of 1:2), followed by the addition of butylated hydroxytoluene (concentration of 0.001 %). The fat extract was dried overnight with the aid of a rotary evaporator and phosphorous pentoxide (moisture adsorbent) under a vacuum oven (50 ^{o}C). The extracted fat was used to quantify the total extractable intramuscular (expressed as per cent fat (w/w) per 100 g tissue). To determine fat-free dry matter (FFDM) content, the residue was weighed on a pre-weighed filter paper after drying and expressed as % FFDM (w/w) per 100 g tissue after calculating weight differences. To determine the moisture content (expressed as % moisture (w/w) per 100 g tissue), per cent FFDM and lipid were both subtracted from 100. The extracted fat was kept in a polytop (glass vial, with push-in top) beneath nitrogen blanket and frozen (–20°C) pending fatty acid analyses. To avoid CLA isomerisation, muscle lipid aliquot (±30 mg) was converted to methyl esters by base-catalysed transesterification for 2 h at 30 °C with sodium methoxide (0.5 M solution in anhydrous methanol) (Park *et al.*, 2001; Kramer *et al.*, 2002; Alfaia *et al.*, 2007). Thereafter, the fatty acid methyl esters (FAMEs) from the muscle were quantified using a Varian 430 flame ionization GC, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 µm film thicknesses). The analysis was performed using an initial isothermic period (40 °C for 2 minutes) while the temperature increased to 230 ^{o}C at a rate of 4 °C/minute and was maintained at this temperature for 10 minutes. FAMEs in n-hexane (1µl) were injected into the column using a Varian CP 8400 Autosampler. The injection port and detector were maintained at 250 °C with hydrogen (45 psi) and nitrogen functioning as the carrier and makeup gas. Galaxy Chromatography Data System Software recorded chromatograms reading. Retention times of FAME peaks from samples were compared to standards obtained from Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma-Aldrich Aston Manor, Pretoria, South Africa) to identify fatty acid methyl ester samples. Conjugated linoleic acid (CLA) standards were obtained from Matreya Inc. (Pleasant Gap, Unites States). These standards included cis-9, trans-11and trans-10, cis-12-18:2 isomers. Fatty acids were expressed as the proportion of each fatty acid to all the sample's fatty acids. Fatty acid data were used to calculate the following ratios of FAs: total SFAs; total MUFAs; total PUFAs; PUFA/SFA; Δ^9 desaturase index (C18:1*c*9/C18:0); total omega-6 (*n*-6); total omega-3 (*n*-3); the ratio of omega-6 to omega-3 (*n*-6)/(*n*-3) FAs. Atherogenicity index (AI) was calculated as: AI = (C12:0 + 4 x C14:0 + C16:0) / (MUFA + PUFA) (Chilliard *et al.* 2003).

6.2.8 Statistical analysis

Data obtained was analyzed using the GLM procedure of SAS (2013). The sensory scores were transformed using the arcsine transformation to achieve normality and reported as back-transformed means.The linear model was:

$Y_{ij} = \mu + B_i + \varepsilon_{ij}$

Where *Yij* = dependent variables; μ = overall mean; B_i = effect of diet; ε_{ij} = residual error. Where differences exist, the means were separated using Fishers' least significance difference (LSD) method of SAS, with a significance level of $P<0.05$.

6.3 Results

6.3.1 Oxidative stability of lipids and proximate analysis

The lipid oxidation values (as determined by TBARS), free-fat dry matter, fat and moisture content are shown in Table 6.1. The effect of water restriction levels and vitamin C supplementation were not significant ($P>0.05$) on TBARS (mg/kg). The moisture and fat content of the entire water restricted groups were lower ($P<0.05$) than the control.

6.3.2 Sensory attributes

The effect of different watering treatments on the sensory attributes of the *Longissimus lumborum* of Xhosa goat is shown in Table 6.2.

Table 6. 1 Effect of different watering treatment on malonaldehyde content, ultimate pH, thaw loss and proximate of the longissimus lumborum of Xhosa goats

Variable	W0	W70	W50	W70^{+}	W50^{+}	W70^{++}	W50^{++}	SEM	P-value
TBARS, mg/kg	0.66	0.79	0.77	0.69	0.71	0.70	0.77	0.08	0.101
Moisture, %	79.12^{a}	76.37^{b}	75.43^{b}	77.87^{ab}	75.93^{b}	78.10^{ab}	77.66^{ab}	1.23	0.023
Fat, %	2.14^{a}	1.36^{b}	1.34^{b}	1.41^{b}	1.42^{b}	1.50^{b}	1.51^{b}	0.16	<0.001
FFDM, %	18.73^{c}	22.26^{a}	23.24^{a}	20.71^{b}	22.64^{a}	20.41^{b}	20.83^{b}	0.58	0.042

SEM= standard error of mean; FFDM= fat-free dry matter; W= water restriction; DMI=dry matter intake; WI=water intake; $^{+}$ 3gVC daily; $^{++}$ 3gVC daily+extra 5gVC every 8th days

Table 6. 2 Average points for the effect of different watering treatments on the sensory characteristics of the longissimus lumborum of Xhosa goats

Variable	W0	W70	W50	W70^{+}	W50^{+}	W70^{++}	W50^{++}	SEM	P-value
Colour	6.40	6.60	6.80	6.60	6.80	7.00	6.40	0.49	0.157
Taste+aroma	6.60	6.80	6.80	6.80	6.40	6.40	6.60	0.34	0.105
Appearance	6.60^{a}	6.00^{abc}	5.60^{c}	6.40^{ab}	5.80^{bc}	6.00^{abc}	6.00^{abc}	0.38	0.009
Texture	5.40	5.50	5.60	5.40	5.60	5.60	5.60	0.50	0.116
Acceptability	6.40	6.00	5.60	5.80	5.60	5.80	5.60	0.46	0.075

SEM=standard error of mean; ^{abc}means with different superscript across the row are significantly different ($P<0.05$); $^{+}$ 3gVC daily; $^{++}$ 3gVC daily+extra 5gVC every 8th days; W=water restriction

Table 6. 3 Fatty acid composition in Longissimus lumborum muscle (% of total fatty acid) of Xhosa goats subjected to different watering treatments

Variable	W0	W70	W50	W70^{+}	W50^{+}	W70^{++}	W50^{++}	SEM	P-value
Saturated (SFA)									
Myristic (C14:0)	2.20	1.84	1.99	2.30	1.75	1.81	1.38	0.505	0.163
Pentadecylic (15:0)	0.40	0.38	0.40	0.42	0.35	0.35	0.32	0.050	0.268
Palmitic (C16:0)	26.58	25.54	25.41	27.23	25.57	25.63	25.44	1.187	0.068
Margaric (C17:0)	1.45^{a}	1.39^{b}	1.33^{c}	1.41^{ab}	1.21^{d}	1.46^{a}	1.15^{e}	0.023	<0.001
Stearic (C18:0)	19.83	20.06	19.45	19.39	20.98	20.08	20.89	0.556	0.101
Nonoadecanoic (C19:0)	0.06	0.08	0.07	0.06	0.08	0.08	0.08	0.012	0.184
Arachidic (C20:0)	0.08	0.09	0.08	0.08	0.08	0.09	0.08	0.010	0.070
Monounsaturated (MUFA)									
Palmitoleic (C16:1c9)	0.82^{c}	0.75^{c}	0.79^{c}	1.62^{a}	1.27^{b}	0.86^{c}	0.84^{c}	0.149	0.002
Heptadecenoic (C17:1c1)	0.16	0.18	0.18	0.16	0.17	0.17	0.16	0.016	0.071
Oleic (C18:1c9)	33.32	32.98	32.07	33.84	32.60	35.67	35.74	1.709	0.557
Vaccenic (C18:1t11)	1.55^{c}	1.77^{b}	2.13^{a}	1.55^{c}	1.72^{b}	1.70^{bc}	1.61^{bc}	0.088	0.002
Polyunsaturated (PUFA)									
Linoleic (C18:2n-6)	7.00	7.58	6.99	6.64	6.45	6.64	6.56	0.859	0.078
Linolenic (C18:3n-3)	1.90^{a}	1.65^{b}	1.57^{b}	1.55^{b}	1.49^{b}	2.00^{a}	1.49^{b}	0.085	0.003
CLA^{a}	0.08	0.08	0.07	0.09	0.08	0.08	0.06	0.023	0.018
EDA^{b}	0.06	0.06	0.05	0.05	0.05	0.05	0.05	0.007	0.707
Arachidonic (C20:4n-6)	7.00	8.96	8.55	7.56	7.20	8.01	7.97	1.876	0.103
EPA^{c} (C20:5n-3)	2.70	3.31	3.70	2.13	2.65	2.88	3.12	0.393	0.198
DPA^{d} (C22:4n-6)	2.71	2.72	2.64	2.10	2.29	2.50	2.54	0.339	0.118
DHA^{e} (C22:6n-3)	0.62^{b}	0.74^{b}	0.93^{a}	0.57^{b}	0.58^{b}	0.96^{a}	1.06^{a}	0.087	0.001
Sums and ratios									
ΣSFA	48.15	48.92	50.69	48.61	48.63	49.05	49.42	1.725	0.268
ΣMUFA	26.93^{cd}	26.97^{cd}	25.60^{d}	32.82^{a}	31.09^{ab}	29.08^{bc}	28.84^{bc}	1.690	0.005

ΣPUFA	22.38	22.88	25.48	23.57	24.86	23.32	21.74	3.366	0.092
Σn-6	14.09	14.71	13.75	12.79	11.38	14.81	13.65	2.610	0.091
Σn-3	8.29	8.18	7.73	7.17	7.07	8.51	8.08	0.880	0.127
PUFA/SFA	0.47	0.46	0.50	0.48	0.50	0.47	0.44	0.087	0.094
PUFA/MUFA	0.82	0.85	0.98	0.72	0.81	0.80	0.75	0.199	0.057
Σn-6/Σn-3	1.67	1.80	1.79	1.78	1.60	1.74	1.69	0.179	0.164
AI[f]	0.70	0.63	0.68	0.71	0.62	0.61	0.61	0.089	0.205
DI[g]	1.18	0.94	1.14	1.14	1.17	1.31	1.22	0.190	0.770

SEM=Standard error of mean; ^{abc}means with different superscript acroos the row are significantly different ($P<0.05$); $^{+}$ 3gVC daily; $^{++}$ 3gVC daily+extra 5gVC every 8th days; W=water restriction; [a] Conjugated linolenic acid (C18:2c9,t11(n-6); [b]Eicosadienoic acid (C20:2c11,14(n-6); [c]Eicosapentanoic acid; [d]Docopentanoic acid; [e]Docosahexanoic acid; [f]Atherogenecity index; [g] Desaturase index

Regardless of VC supplementation, the colour, odour and texture were not affected ($P>0.05$) by water restriction levels. However, regardless of vitamin C supplementation, the sensory meat appearance decreased ($P<0.05$) with water restriction levels.

6.3.3 Fatty acids

The fatty acid composition in the Longissimus lumborum muscle of Xhosa goats subjected to different watering treatments is shown in Table 6.3. The water restriction levels and vitamin C supplementation had no effect ($P>0.05$) on myristic (C14:0), pentadecylic (15:0), palmitic (C16:0), heptadecenoic (C17:1c1), stearic (C18:0), oleic (C18:1c9) and linoleic (C18:2td,12(n-6)). Regardless of vitamin C supplementation and/or concentration, proportions of vaccenic (C18:1t11), docosahexaenoic (C22:6c4,7,10,13,16,19(n-3)) increased ($P<0.05$) while palmitoleic (C16:1c9), α-Linolenic (C18:3c9,12,15(n-3), conjugated linoleic acid (C18:2c9,t11(n-6)) and docosahexaenoic (C22:6c4,7,10,13,16,19 (n-3) decreased with increasing levels of water restriction. The total FA, sums and ratios of fatty acids, n-6, n-3, atherogenicity index and desaturase indices were not affected ($P>0.05$) by water restriction and vitamin C. Vitamin C supplementation, regardless of dose, increased ($P<0.05$) the sum of monounsaturated fatty acid in the water restricted treated groups.

6.4 Discussion

Non-microbial quality degradation in meat or its products, specifically under pro-oxidative conditions, is mainly due to lipid oxidation (Behkit *et al.*, 2013). However, the TBARS values have now been widely accepted as an indicator of oxidation of muscle lipids at the biochemical level (Lorenzo and Gomez, 2012). Regardless of vitamin C concentrations or supplementation, the TBARS values were not affected by water restriction levels. These values were consistent with the results for PUFA in the *longissimus lumborum* muscle in this study, thus justifying the similarity in the TBARS values. The oxidative stability of lipid in meat is dependent on the equilibrium between pro-oxidants (including the concentrations of ROS, PUFA and heme pigment) and antioxidants (e.g. some carotenoids and α-tocopherol) (Bekhit *et al.*, 2013). The higher the level of unsaturated lipids in meat compared to saturated, the more prone is the meat to lipid oxidation. In a similar study, the oxidative stability of lipids of grass-fed ground beef treated with vitamin C was not affected (Realini *et al.*, 2004). Also, studies have reported lipid oxidation to be unaffected by stress induced by slaughtering methods in rabbits (Nakyinsige *et al.*, 2014) and lambs (Linares *et al.*, 2007). The water content of meat is essential in food analysis due to its influence on carcass dressing, storage, packaging and processing (Jimenez Colmenero, 1996). The similarity in the meat's moisture content between those watered with W50 and W70 (treated and untreated) evinced goats' adaptive nature during water scarcity, an attribute linked to the rumen and its ability to conserve water during shortfalls (Silanikove, 2000). Nonetheless, the mean (moisture content) value of 76.89% in all the water restricted groups in this study exceed 75% and 73% in sheep (Madruga *et al.*, 2008) and water restricted Brazilian goats (dos Santos *et al.*, 2019), respectively. The low crude fat in the water restricted groups compared to the control could be attributed to fat mobilization from adipose tissues in response to reduced feed intake induced by limited water intake. According to Jaber *et al.* (2011), fat is mobilized during suboptimum water intake to meet the animal's energy requirements in response to decreased feed intake and weight loss.

One of the meat quality parameters that interest consumers and producers, and retailers are the meat sensory attributes. Aroma or flavour (aroma+taste) is usually affected by the age of the animal and the type and nature of feed offered, and those differences may influence consumer acceptability (Font *et al.*, 2009; Watkins *et al.*, 2013). However, most sensorial parameters assessed (colour, odour, acceptability and texture) in this study were not affected by the pre-slaughter stress and dry matter intake differentials induced by limited water intake. This lends credence to the report of Miranda-de la Lama *et al.* (2012) that a much higher stress level than the one that can affect physicochemical and meat quality parameters is required to induce significant sensory changes attributes. The effect of water restriction levels induced significant changes in the appearance of the meat. The panelist observed that the appearance scores for all the water restricted groups oscillated between 'like slightly' (score 6) to 'neither like nor dislike' (score 5) and with a progressive tendency to dislike meat from an animal with higher water limitations. This may probably be attributed to the water intake differences and, consequently, the meat's water content. Moisture retention of fresh meat is arguably an essential quality characteristic of raw products. Though the effect of water restriction levels was not significant on thaw loss, the values obtained linearly decreased as the water restriction level increase. Water is usually held in myofibrils in the space between filaments, bounding small protein proportions by electrostatic attraction (Ponnampalam *et al.*, 2017). Meat with low moisture, probably due to limited water intake, affected the appearance and dislikes consumers.

The fatty acid (FA) profile of goat meat, compared to other types of red meat, reveals the existence of essential fatty acids (low saturated fatty acids), high concentrations of oleic acid, low concentrations of myristic, lauric and palmitic acids (Lopes *et al.*, 2014). This is seen to be highly beneficial to human health if consumed. Regardless of water restriction levels and VC supplementation, the most abundant FA were the oleic (18:1c9), palmitic (C16:0) and stearic (C18:0) in that order, and their concentrations were not affected by the quantity of water ingested. Similar studies have reported Oleic (C18:1c9) concentrations to be the most abundant in the muscle of sheep, goats and cattle (Oliveira *et al.*, 2012; Roy *et al.*, 2013; Ferreira *et al.* 2014).

Regardless of VC supplementation, the observed similar concentrations of oleic acid in the water restricted and control groups is suggestive that the conversion (de novo) of C18:0 to C18:1c9 by Δ 9 desaturase enzyme (Adeyemi *et al.,* 2015) was not compromised by limited water intake. Oleic acid, the most abundant (about 30-43%) of the mono-unsaturated fatty acids (MUFA), is hypocholesterolemic in its *cis* form and can be influenced by the dietary type and the amount of voluntary feed intake (Melton *et al.*, 1982; Adeyemi *et al.,* 2015). In this study, the percentage of oleic acid was the highest of all the MUFAs and with a similar range (32.07-35.74%).

The non-significant effect of the medium-chain fatty acids (myristic and palmitic acids) in response to water restriction levels could be that their synthesis or incorporation from adipose tissue or diet by lipogenic enzymes were not inhibited (Kim *et al.*, 2007). A similar outcome was reported by dos Santos *et al.* (2019) in feedlot lamb subjected to a different watering period of 24, 48 and 72 h. Myristic and palmitic acids are considered hypercholesterolemic, capable of raising the concentration of low-density lipoprotein (LDL), while stearic acid (long-chain fatty acids) are neutral for plasma cholesterol (Scollan *et al.*, 2001).

The concentration of vaccenic acid (C18:1t11) increased with increased water restriction levels, especially at the W50 (untreated). Both vaccenic acid and CLA are intermediate products of ruminal biohydrogenation of unsaturated fatty acids to saturated fatty acids from the diet (Kim *et al.*, 2007). The reduction in dry matter intake induced by suboptimal water intake may affect the bacteria population responsible for the complete conversion to saturated fatty acids, thereby accumulating higher concentrations of vaccenic acid with water restriction levels. Vaccenic acid (C18:1t11) is subsequently absorbed from the small intestine and deposited in muscle tissue (Adeyemi *et al.,* 2015). Based on studies, CLA is produced from Δ 9 desaturations of vaccenic acid (C18:1t11) in adipose tissue (Adeyemi *et al.*, 2015). The progressive increase in vaccenic acid with respect to water restriction levels was expected to increase CLA. However, the CLA values obtained in this study were not affected by water restriction levels or vitamin C supplementation. Contrarily, dos Santos *et al.* (2019) reported a decreasing trend in CLA of *Longissimus lumborum* of feedlot lambs subjected to water restriction. The observed differences may be due to an imbalance in the mRNA synthesis of Δ-9 desaturase enzyme (Baumgard *et al.,* 2001), induced by the reduced intake of water and feed, thereby affecting CLA concentrations in the muscle of animals with less water.

Both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are essential precursors of eicosanoids, modulating several cellular functions and integrity of the cardiovascular system. DHA increased with increased water restriction levels while EPA remains unaffected. EPA is formed by desaturation and stretching of α-linolenic acid (Calder, 2013). Hence, the non-significant effect on water restriction levels may be that the synthesis of Δ6 desaturase and elongases enzymes required for converting α-linolenic acid into its long derivates was not compromised (Pawlosky *et al.*, 2003). While on the other hand, the water and feed intake differentials may have induced linolenic acid conversion into DHA by increasing the required enzymes' synthesis. DHA is vital for the proper functioning of the cell membrane and is also involved in retina and brain development (Ramakrishnan *et al.*, 2010).

The effect of water intake differentials with or without VC supplementation was not significant on ΣSFA, ΣPUFA and also ΣPUFA:ΣSFA ratio. The content and composition of fatty acids in the muscles, especially PUFA, are partly influenced by dietary fatty acids, which can easily be affected by rumen microorganisms. Based on their location in cell membranes and role as a precursor of different eicosanoids, PUFA is an essential metabolic regulator, a messenger of the cell, and is usually implicated in cardiovascular diseases (Parodi, 2016). Recently studies have indicated that higher consumption of omega 3 fatty acids and less SFA can lower the chances of developing cardiovascular diseases (Ramakrishnan *et al.*, 2010). Supplementation of VC and water restriction levels in the present study did not influence the ratio of omega 6-to-omega 3 fatty acids. The ratio of Σn-6:Σn-3 fatty acids is one of the three criteria for assessing the quality of fat, with values less than 4.0 regarded as the recommended range or limit (Parodi, 2016). The ratios (Σn-6:Σn-3) recorded in this study was found to be <4. The other two criteria include; total lipid content, the ratio of unsaturated to saturated fatty acid, which should be greater than 4 (Wood *et al.*, 2003). The ratio of ΣPUFA: ΣSFA in this study were not significantly affected by water restriction or VC supplementation. However, values recorded across the experimental groups were ≥4, indicating that the meat is nutritionally good despite limited water intake. The atherogenicity and desaturase indexes were not affected by levels of water restriction and VC supplementation. AI usually assesses the capacity of the blood vessels to form plaque. Lower AI values imply higher anti-atherogenic FA in the lipids, thereby resulting in a better chance of preventing cardiovascular disease (Woutersen *et al.*, 1999). The lower AI values recorded in this study indicated that meat from water restricted animals would not compromise consumers' health integrity. This implies that Xhosa goats at 50% water restriction can survive and produce meat, good enough for consumption, especially to the rural poor and small farm holders' farmers of the Eastern Cape.

6.5 Conclusion

This study demonstrated that water restriction levels of 70% and 50%, with or without single VC supplementation, did not impact negatively on the meat quality parameters like oxidative instability of lipids. This implies that the Xhosa goats can be recommended for farming by the rural poor of South Africa as a breed that can withstand water restriction periods, especially with the recent droughts that have hit the country, and still produce meat that is healthy for human consumption.

6.6 References

Adeyemi. K.D., Ebrahimi, M., Samsudin, A.A., Alimon, A., Karim, R., Karsani, S.A. *et al.* (2015). Influence of Carotino oil on in vitro rumen fermentation, metabolism and apparent biohydrogenation of fatty acids. *Anim. Sci. J*. **86,** 270–278.

Akinmoladun, O.F., Muchenje, V., Fon, F.N. and Mpendulo, C.T. (2019). Small ruminants: Farmers' hope in a world threatened by water scarcity. *Animals* **9** (7), 456.

Alfaia, C.M.M., Castro, M.L.F., Martins, S.I.V., Portugal, A.P.V., Alves, S.P.A., Fontes, C.M.G.A., Bessa, R.J.B. and Prates, J.A.M. (2007). Effect of slaughter season on fatty acid composition, conjugated linoleic acid isomers and nutritional value of intramuscular fat in Barrosã-PDO veal. *Meat Sci.* **75**, 44–52.

AOAC (2000). Official Methods of Analysis of the Official Analytical Chemists (W. Horwitz ed) 17th Ed, Association of Official Analytical Chemists, Washington DC USA.

Baumgard, L.H., Sangster, J.K. and Bauman, D.E. (2001). Milk fat synthesis in dairy cows is progressively reduced by increasing supplemental amounts of trans-10, cis-12 conjugated linoleic acid. *J. Nutr.* **131**, 1764–1769.

Bekhit, A.E.D., Hopkins, D.L., Fahri, F.T. and Ponnampalam, E.N. (2013). Oxidative processes in muscle systems and fresh meat: Sources, markers and remedies. *Compr. Rev. Food Sci.* **12**, 565–597.

Calder, P.C. (2013). Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? *British J. Clin. Pharm*. **75**(3), 645-662

Chilliard, Y., Ferlay, A., Rouel, J. and Lamberet, G. (2003). A review of nutritional and physiological factors affecting goat milk lipid synthesis and lypolysis. *J. Dairy Sci.* **86**(5), 1751-1770.

de Rodas, B.Z., Maxwell, C.V., Davis, M.E., Mandal, S., Broekman, E. and Stoecker, B.J. (1998). L-ascorbyl-2-polyphosphate as a vitamin C source for segregated and conventionally weaned pigs. *J. Anim. Sci.* **76**, 1636–1643.

dos Santos, F.M., de Araujo, G.G.L., de Souza, L.L., Yamamoto, S.M., Queiroz, M.A.A., Lanna, D.P.D. and de Moraes, S.A. (2019). Impact of water restriction periods on carcass traits and meat quality of feedlot lambs in the Brazilian semi-arid region. *Meat Sci.* **156**, 196-204.

Ekiz, B., Ergul-Ekiz, E., Kocak, O., Yalcintan, H. and Yilmaz, A. (2012). Effect of pre-slaughter management regarding transportation and time in lairage on certain stress parameters, carcass and meat quality characteristics in Kivircik lambs. *Meat Sci.* **90**, 967–976.

Estevez M. (2015). Oxidative damage to poultry: From farm to fork. *Poult. Sci.* **94**(6), 1368–1378.

Ferreira, E., Pires, A., Susin, I., Gentil, R., Parente, M., Nolli, C., *et al.* (2014). Growth, feed intake, carcass characteristics, and meat fatty acid profile of lambs fed soybean oil partially replaced by fish oil blend. *Anim. Feed Sci. Tech.* **187**, 9–18.

Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissue. *J. Biol. Chem.* **226**, 497-509.

Font, I.F.M., Realini, C.E., Guerrero, L., Oliver, M.A., Sañudo, C., Campo, M.M., Nute, G.R., Cañeque, V., Álvarez, I., San Julián, R. *et al.* (2009). Acceptability of lamb fed on pasture, concentrate or combinations of both systems by European consumers. *Meat Sci.* **81**, 196–202.

Gerbens-Leenes, R.W., Mekonnen, M.M. and Hoekstra, A.Y. (2013). The water footprint of poultry, pork and beef: A comparative study in different countries and production system. *Water Resour. Ind.* **1-2**, 25-36.

Ghanem, A.M., Barbour, E.K., Hamadeh, S.K., Jaber, L.S. and Abi Said, M. (2008). Physiological and chemical responses in water-deprived Awassi ewes treated with vitamin C. *J. Arid Environ.* **72**, 141-149.

Hidiroglou, M., Batra, T.R. and Zhao, X. (1997). Comparison of vitamin C bioavailability after multiple or single oral dosing of different formulations in sheep. *Reprod. Nutr. Dev*. **37,** 443-448.

IPCC (Intergovernmental Panel on Climate Change) (2007). Synthesis Report. In Contribution of Working Groups I, II and II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change; Pachauri, R.K., Reisinger, A., Eds.; IPCC: Geneva, Switzerland, p. 104.

Jaber, L.S., Habre, A., Rawda, N., Abi Said, M., Barbour, E.K. and Hamadeh, S.K. (2004). The effect of water restriction on certain physiological parameters in Awassi sheep. *Small Rumin. Res*. **54**, 115–120.

Jaber, L.S., Hanna, N., Barbour, E.K., Abi Said M., Rawda, N., Chedid, M. and Hamadeh, S.K. (2011). Fat mobilization in water restricted Awassi ewes supplemented with vitamin C. *J. Arid Environ.* **75**, 625-628.

Jacob, R.H., Pethick, D.W., Clark, P., D'Souza, D.N., Hopkins, D.L. and White, J. (2006). Quantifying the hydration status of lambs in relation to carcass characteristics. *Anim. Prod. Sci.* **46**(4), 429-437.

Jimenez Colmenero, F. (1996). Technologies for developing low-fat meat products. *Trends Food Sci.Technol.* **7**, 41–48.

Kim, S.C., Adesogan, A.T., Badinga, L. and Staples, C.R. (2007). Effects of dietary n-6: n-3 fatty acid ratio on feed intake, digestibility, and fatty acid profiles of the ruminal contents, liver, and muscle of growing lambs. *J. Anim. Sci.* **85,** 706–16.

Kramer, J.K., Blackadar, C.B. and Zhou, J. (2002). Evaluation of two GC columns (60m Supelcowax 10 and 100 m CP Sil 88) for analysis of milkfat with emphasis on CLA, 18:1, 18:2 and 18:3 isomers, and short and long-chain fatty acids. *Lipids* **37**(8), 823–835.

Linares, M.B., Berruga, M.I., Bórnez, R. and Vergara, H. (2007). Lipid oxidation in lambmeat: Effect of the weight, handling previous slaughter and modified atmospheres. *Meat Sci.* **76**(4), 715–720.

Lopes, L.S., Martins, S.R., Chizzotti, M.L., Busato, K.C., Oliveira, I.M., Machado Neto, O.R., Paulino, P.V.R., Lanna, D.P.D. and Ladeira, M.M. (2014). Meat quality and fatty acid profile of Brazilian goats subjected to different nutritional treatments. *Meat Sci.* **97**, 602-608.

Lorenzo, J.M. and Gómez, M. (2012). Shelf life of fresh foal meat under MAP, overwrap and vacuum packaging conditions. *Meat Sci.* **92**, 610–618.

Madruga, M.S., Vieira, T.R.L., Cunha, M.G.G., Pereira Filho, J.M.P., Queiroga, R.C.R.E. and Sousa, W.H. (2008). Effect of diets with increasing levels of whole cotton seed on chemical composition and fatty acid profile of Santa Inez (Santa Ines) lamb meat. *Rev. Bras. Zootecn.* **37**, 1496–1502.

Melton, S.L., Amiri, M., Davis, G.W. and Backus, W.R. (1982). Flavor and chemical characteristics of ground beef form grass-forage–grain- and grain-finished steers. *J. Anim. Sci.* **55**, 77–87.

Mirand-de la Lama, G.C., Villarroel, M., Campo, M.M., Olleta, J.L., Sanudo, C. and Maria, G.A. (2012). Effect of double transport and season on sensorial aspects of lamb's meat quality in dry climates. *Trop. Anim. Health Prod.* **44**, 21-27.

Mouzo, D., Rodriguez-Vazquez, R., Lorenzo, J.M., Franco, D., Zapata, C. and Maria-Pedrouso, M. (2020). Proteomic application in predicting food quality relating to animal welfare. A review. *Trends Food Sci. Technol.* **99**, 520-530.

Nakyinsige, K. (2014). Physiological stress responses and meat quality of rabbits subjected to different road transportation and slaughter methods. Ph.D. Thesis research, UniversitiPutra Malaysia. Accessed 28 August 2019. Available from URL:http://www.lib.upm.edu.my/.

National Research Council (NRC), (2007). Nutrients requirements of Small Ruminants. 362p.Washington, D. C: National Academy Press.

National Research Council (NRC), (1981). Nutrient requirements of goats: angora, dairy, and meat goats in temperate and tropical countries. National Academy of Sciences. National Academic Press, Washington, DC.

Oliveira, E., Sampaio, A., Henrique, W., Pivaro, T., Rosa, B., Fernandes, A. *et al.* (2012). Quality traits and lipid composition of meat from Nellore young bulls fed with different oils either protected or unprotected from rumen degradation. *Meat Sci.* **90,** 28–35.

O'Quinn, T.G., Legako, J.F., Brooks, J.C. and Miller, M.F. (2018). Evaluation of the contribution of tenderness, juiciness, and flavor to the overall consumer beef eating experience. *Transl. Anim. Sci.* **2**(1), 26–36.

Park, Y., Albright, K.J., Cai, Z.Y. and Pariza, M.W. (2001). Comparison of methylation rocedures for conjugated linoleic acid and artefact formation by commercial (trimethylsilyl) diazomethane. *J. Agric. Food Chem.* **49(**3), 1158–1164.

Parodi, P.W. (2016). Dietary guidelines for saturated fatty acids are not supported by the evidence. *Int. Dairy J.* **52,** 115–123.

Pawlosky, R.J., Hibbeln, J.R., Lin, Y., Goodson, S., Riggs, P., Sebring, N., Salem, N.J. (2003). Effects of beef- and fish-based diets on the kinetics of n-3 fatty acid metabolism in human subjects. *Am. J. Clin. Nutr.* **77**, 565–572.

Pena, F., Bonvillani, A., Freire, B., Juarez, M., Perea, J. and Gomez, G. (2009). Effects of genotype and slaughter weight on the meat quality of criollo cordobes and Anglonubian kids produced under extensive feeding conditions. *Meat Sci.* **83,** 417-422.

Ponnampalam, E.N., Hopkins, D.L., Bruce, H., Li, D., Baldi, G. and Bekhit, A.E. (2017). Causes and contributing factors to 'dark cutting' meat: current trends and future directions: A review. *Compr. Rev. Food Sci.* **16**, 400-430.

Ramakrishnan, U., Stein, A.D., Parra-Cabrera, S., Wang, M., Imhoff-Kunsch, B., Juarez-Marquez, S. and Martorell, R. (2010). Effects of docosahexaenoic acid supplementation during pregnancy on gestational age and size at birth: Randomized, double blind, placebo-controlled trial in Mexico. *Food Nutr. Bull.* **31**, 108–116.

Ranjan, R., Swarup, D., Naresh, R. and Patra, R.C. (2005). Enhanced erythrocytic lipid peroxides and reduced plasma ascorbic acid, and alteration in blood trace elements level in dairy cows with mastitis. *Vet. Res. Commun.* **29,** 27–34.

Realini, C.E., Duckett, S.K. and Windham, W.R. (2004). Effect of vitamin C addition to ground beef from grass-fed or grain-fed sources on colour and lipid stability, and prediction of fatty acid compositions by near-infrared reflectance analysis. *Meat Sci.* **68**, 35-43.

Rowe, L.J., Maddock, K.R., Lonergan, S.M. and Huff-Lonergan, E. (2004). Oxidative environments decrease tenderization of beef steaks through inactivation of μ-calpain. *J. Anim. Sci.* **82**, 3254-3266.

Roy, A., Mandal, G. and Patra, A. (2013). Evaluating the performance, carcass traits and conjugated linoleic acid content in muscle and adipose tissues of black Bengal goats fed soybean oil and sunflower oil. *Anim Feed Sci Tech*. **185,** 43–52.

Sahin, K., Sahin, N. and Kucuk, O. (2003). Effects of chromium and ascorbic acid supplementation on growth, carcass traits, serum metabolites and antioxidant status of broiler chickens reared at a high ambient temperature (32 °C). *Nutr. Res.* **23**(2), 225-238.

SAS Institute Inc. (2013). SAS System for Windows.Release 9.4.

Scollan, N.D., Choi, N.J., Kurt, E., Fisher, A.V., Enser, M. and Wood, J.D. (2001). Manipulating the fatty acid composition of muscle and adipose tissue in beef cattle. *Br. J. Nutr.* **85**, 115–124.

Silanikove, N. (2000). The physiological basis of adaptation in goats to harsh environments. *Small Rumin. Res.* **35**, 181–193.

Tyler, P.J. and Cummins, K.A. (2003). Effect of dietary ascorbyl-2-phosphate on immune function after transport to a feeding facility. *J. Dairy Sci.* **86**, 622–629.

Watkins, P.J., Frank, D., Singh, T.K., Young, O.A. and Warner, R.D. (2013). Sheep meat flavor and the effect of different feeding systems: A review. *J. Agric. Food Chem.* **61**, 3561–3579.

Wood, J.D., Richardson, R.I., Nute, G.R., Fisher, A.V., Campo, M.M., Kasapidou, E.P.R., Sheard, P.R. and Enser, M. (2003). Effects of fatty acids on meat quality: A review. *Meat Sci*. **66**, 21-32.

Woutersen, R.A., Appel, M.J., Garderen-Hoetmer, A.V. and Wijnands, M.V.W. (1999). Dietary fat and carcinogenesis. *Mutat. Res*. **443,** 111–127.

Chapter Seven

General discussion, conclusion and recommendation

Livestock production, among other things, depends on the quantity and quality of water, with the extensive system of animal husbandry requiring more water per animal (Bezabih and Berhane, 2014). Because most ruminants, especially small ruminants, are ‘extensively’ managed under smallholder and resource-limited farms, water scarcity can be a burden to farmers due to the economic losses emanating from stress-induced (heat and water scarcity) mortalities. Some small ruminants are more resilient to limited water intake, and thus their preference to other livestock in drought-prone and water-scarce areas (Akinmoladun *et al.,* 2019). However, this vital nutrient's suboptimum consumption impairs homeostasis, productivity and immunity (Alamer, 2010). Therefore, to reduce the stress induced by suboptimum water intake in small ruminants, exogenous supplementation of vitamin C could be a potential and viable option.

This study showed that induced water scarcity (W70 and W50) reduced weight gain and feed matter intake in Xhosa goats but was lessened with VC supplementation. Depression in body weight has been attributed to body water loss and increased fat mobilization (Jaber *et al.*, 2011; Akinmoladun *et al.*, 2019). These animals’ adaptive nature is reflected in their heat tolerance and rectal temperature, as values obtained were not affected by water stress and vitamin C supplementation. Most of the blood metabolites assayed in this study significantly increased in response to limited water intake. Usually, higher blood metabolites' concentrations indicate haemoconcentration and their adaptive capability (Banerjee *et al.,* 2015). However, VC treated groups had their urea, total protein and ALT lowered, similar to Ghanem *et al.* (2008). Although there was haemoconcentration of blood metabolites (due to water scarcity), there was a need to investigate intake and nutrient digestibility.

The decreased nutrients intake in the goats subjected to water restrictions (W70, W50, W70+, W50^{+}, W70^{++} and W50^{++}) further confirmed the decrease in weight gain and the final body weight observed in chapter 3. Regardless of VC supplementation or concentration, nutrients intake decreased in proportion to the amount of water consumed. Effective digestion and absorption of feed require a water medium, which was disadvantaged in the water scarcity induced groups. A similar reduction in urine and faecal output were observed with water-restriction levels. This reduction in output and the attendant urinary osmolality have been attributed to ruminants' adaptive mechanism during water shortfalls (Naqvi *et al.*, 2015). Similarly, water restriction levels resulted in increased nitrogen retention and nutrient digestibility and were not influenced by vitamin C supplementations. Similar outcomes following water-restriction in sheep and goats have been reported (Abdelatiff *et al.*, 2010; Nejad *et al.*, 2014). Mineral intake in the water restricted groups decreased due to reduced feed intake. However, more minerals (Ca, Mg, P and K) were excreted (from urine and faeces) in control compared to the water restricted groups. The increased retention of Mg, Na and K with water restriction levels was influenced by VC supplementation.

The dehydration effect, coupled with heat stress during the post-mortem, may cause perturbations of plasma hormones, darken the colour of meat and increase meat pH following prolonged exposure. The result from this study showed that the increased cortisol (chief stress hormone) due to water restriction levels were lowered with VC. According to Karanth *et al.* (2000), ascorbic acid plays an inhibitory role in cortisol synthesis and thermoregulation. This is attributed to its activities as an inhibitory vitaminergic neurotransmitter in the hypothalamus. The depression in feed intake and body weight in the water restricted groups reflected in their carcass yields and weights. Mapiye *et al.* (2009) affirmed that final live weight, weight gains and carcass weight after slaughter are co-related. Unlike the head (was not affected) and kidney (increased), the other non-carcass parameters (skin, liver and heart) decreased with water restriction levels and were not influenced by vitamin C. A similar response of non-carcass parameters to water restriction levels was reported in feedlot lambs (dos Santos *et al.*, 2019). The kidney's increased activities to reabsorb more water from the renal tubules in the water restricted groups could explain the increased weight (Naves *et al.*, 2003). The shear force and cooking loss increased with water restriction levels and were not significantly influenced by vitamin C supplementation. Glycogen depletion due to reduced feed intake induced by water restriction may enhance glycolytic activity and rigour mortis process, consequently toughening the meat (Carragher and Mathews, 1996). Meat redness (a*) increased with vitamin C supplementation.

The TBARS (an index of lipid oxidation) values from this study were not affected by water restriction and VC supplementation. Similarly, the oxidative stability of lipids of grass-fed ground beef treated with VC was not affected (Realini *et al.*, 2004). Stress-induced by slaughtering methods on lambs did not affect lipid oxidation (Linares *et al.*, 2007). The meat moisture content and crude fat in the water restricted groups decreased compared to the control. The increased energy needs (induced by feed intake reduction) require that fat be mobilized from adipose tissues (Jaber *et al.*, 2011). This might explain the reduced crude fat content in the water-restricted groups. Regardless of VC, only the sensory appearance of the meat was affected by water intake differentials. Panellist seems to progressively dislike meat with higher water limitation or reduced meat water content. Regardless of VC supplementation, the saturated fatty acids (excluding margaric acid) were not affected by water restriction levels. However, vaccenic acid (mono-unsaturated fatty acids) increased while conjugated linolenic acid remains unaffected with water restriction levels. As an intermediate product of ruminal hydrogenation of unsaturated to saturated fatty acids, the increased concentrations of vaccenic acid could result from changes in the rumen bacteria community (affected by reduced feed intake) which are responsible for the complete conversion (Adeyemi *et al.*, 2015). The ratio of Σn-6: Σn-3 fatty acids obtained in this study were less than 4. This shows that the water restriction effect with or without VC supplementation did not affect the fat quality. According to Parodi (2016), the recommended range for quality animal fat must be less than 4.

The results of the present investigation confirm the capability of Xhosa goats to withstand intermittent watering usually occasioned by water shortages. This tolerance to water restriction allows them to maximize feeds as evidenced by the increased digestibility. The use of supplemental vitamin C at a daily dose of 3g/d to the water restricted goats was resourceful at minimizing the body weight loss and increased cortisol concentrations, induced by water scacrcity.

7.5 Conclusion

Vitamin C supplementation reduced body weight loss in water restricted goats. Regardless of vitamin C supplementation, th nutrient intake decreased, while the apparent and nutrient digestibility increased with water restriction levels. As a consequence of reduced body weight due water restriction (reduced body water and nutrient intake), carcass parameters were reduced in Xhosa goats. Due to reduced water intake in the water restricted group, meat sensory appearance was disliked by panelist. Xhosa goats seem to be a good choice of goat breed for farmers in dry and water limiting zones, giving their water tolerance potentials. However, oral supplementation of vitamin C at 3g per day during water scarcity could offer a safe and cost- effective practice in amanaging body weight depressions.

7.6 Recommendation

Given the differences in small ruminants' adaptability to limited water intake, there is a need to continue exploring indigenous goats' water tolerance capacity. Behavioural responses and direct observation using cameras could further assist in studying small ruminants undergoing water stress. This study was conducted during the summer season. Future studies might want to consider the effect of water stress with or without vitamin C during the winter season. Due to this study's limitation, weekly evaluation of plasma ascorbic acid concentration following supplementation in the goats is now suggested for future research. This is intended to give a clue to the extent of bioavailability. Other routes of VC administration for increased bioavailability and probably improved stress management are recommended for future research.

7.7 References

Adeyemi. K.D., Ebrahimi, M., Samsudin, A.A., Alimon, A., Karim, R., Karsani, S.A. *et al.* (2015). influence of Carotino oil on in vitro rumen fermentation, metabolism and apparent biohydrogenation of fatty acids. *Anim. Sci. J.* **86,** 270–278.

Akinmoladun, O.F., Muchenje, V., Fon, F.N. and Mpendulo, C.T. (2019). Small ruminants: farmers' hope in a world threatened by water scarcity. *Animals* **9(7**), 456.

Alamer, M. (2010). Effect of water restriction on thermoregulation and some biochemical constituents in lactating Aardi goats during got weather conditions. *J. Basic Appl. Scientific Res.* **11**, 189–205.

Bezabih, M.Y. and Berhane, G. (2014). Livestock production systems analysis. *Am. Int. J. Contemp. Sci. Res***.** **1**, 16–51.

Carragher, J.F. and Matthews, L.R. (1996). Animal behavior and stress: Impacts on meat quality. *Proceedings of the New Zealand Society of Animal Production* **56**, 162–216.

dos Santos, F.M., de Araujo, G.G.L., de Souza L.L., Yamamoto, S.M., Queiroz, M.A.A., Lanna, D.P.D. and de Moraes, S.A. (2019). Impact of water restriction periods on carcass traits and meat quality of feedlot lambs in the Brazilian semi-arid region. *Meat Sci.* **156**, 196-204.

Ghanem, A.M., Barbour, E.K., Hamadeh, S.K., Jaber, L.S. and Abi Said, M. (2008). Physiological and chemical responses in water-deprived Awassi ewes treated with vitamin C. *J. Arid Environ.* **72**, 141-149.

Jaber, L.S., Hanna, N., Barbour, E.K., Abi Said M., Rawda, N., Chedid, M. and Hamadeh, S.K. (2011). Fat mobilization in water restricted Awassi ewes supplemented with vitamin C. *J. Arid Environ.* **75**, 625-628.

Karanth, S., Yu, W.H., Walczewska, A., Mastronardi, C., McCann, M. (2000). Ascorbic acid acts as an inhibitory transmitter in the hypothalamus to inhibit stimulated luteining hormone-releasing hormone release by scavenging nitric oxide. *PNAS***, 97**, 1891–1896.

Linares, M.B., Berruga, M.I., Bórnez, R. and Vergara, H. (2007). Lipid oxidation in lambmeat: Effect of the weight, handling previous slaughter and modified atmospheres. *Meat Sci.* **76**(4), 715–720.

Mapiye, C., Chimonyo, M., Dzama, K., Strydom, P.E. Muchenje, V. and Marufu, M.C. (2009). Nutritional status, growth performance and carcass characteristics of Nguni steers supplemented with Acacia Karroo leaf-meal. *Livest. Sci.* **126**, 206-214.

Naqvi, S.M.K., Kumar, D., De, K. and Sejian, V. (2015) Climate Change and Water Availability for Livestock: Impact on Both Quality and Quantity. In: Sejian V., Gaughan J., Baumgard L., Prasad C. (eds) Climate Change Impact on Livestock: Adaptation and Mitigation. Springer, New Delhi.

Naves, L. A., Vilar, L., Costa, A. C. F., Domingues, L. and Casulari, L.A. (2003). Disorders in the secretion and action of antidiuretic hormone. *Arch. Endocrin. Meta.* **47**, 467–481.

Nejad, G.J., Lohakare, J.D., West, J.W. and Sung, K.I. (2014). Effects of water restriction after feeding during heat stress on nutrient digestibility, nitrogen balance, blood profile and characteristics in Corriedale ewes. *Anim. Feed Sci. Tech*. **193**, 1-8.

Parodi, P.W. (2016). Dietary guidelines for saturated fatty acids are not supported by the evidence. *Int. Dairy J*. **52,** 115–123.

University of Fort Hare
Together in Excellence

ETHICAL CLEARANCE CERTIFICATE
AREC-150311-008

Certificate Reference Number: MUC011SAKI01

Project title: **Supplementation of vitamin C to the diet of indigenous goats during the summer season: Effect on performance, oxidative metabolites and meat quality.**

Nature of Project PHD in Animal Science

Principal Researcher: Festus Oluwakamisi Akinmoladun

Supervisor: Prof V Muchenje

Co-supervisor: Dr. C.T Mpendula

On behalf of the University of Fort Hare’s Animal Research Ethics Committee (AREC) I hereby give ethical approval in respect of the undertakings contained in the above-mentioned project and research instrument(s). Should any other instruments be used, these require separate authorization. The Researcher may therefore commence with the research as from the date of this certificate, using the reference number indicated above.

Please note that the AREC must be informed immediately of

- Any material change in the conditions or undertakings mentioned in the document;
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

The Principal Researcher must report to the AREC in the prescribed format, where applicable, annually, and at the end of the project, in respect of ethical compliance.

The AREC retains the right to

- Withdraw or amend this Ethical Clearance Certificate if
  - Any unethical principal or practices are revealed or suspected;
  - Relevant information has been withheld or misrepresented;
  - Regulatory changes of whatsoever nature so require;
  - The conditions contained in the Certificate have not been adhered to.
- Request access to any information or data at any time during the course or after completion of the project.
- In addition to the need to comply with the highest level of ethical conduct principle investigators must report back annually as an evaluation and monitoring mechanism on the progress being made by the research. Such a report must be sent to the Dean of Research's office.

The Animal Research Ethics Committee wishes you well in your research.

Yours sincerely

Dr. Craig Tambling
AREC Chairperson

18 February 2019