

Soil-Plant-Animal Continuum in Relation to Micro and Micro Mineral Status of Tswana Sheep at Goodhope and Morale Ranches in Bots wana

Bakani Victor Maule

MSc in the Animal Nutrition Stream

September 2014

SOIL-PLANT-ANIMAL CONTINUUM IN RELATION TO MACRO AND MICRO MINERAL STATUS OF TSWANA SHEEP AT GOODHOPE AND MORALE RANCHES IN BOTSWANA

By

BAKANI VICTOR MAULE

A dissertation submitted in partial fulfillment of the requirements for the Masters degree in Animal Science in the Animal Nutrition stream

Department of Animal Science and Production

Botswana College of Agriculture

University of Botswana

Supervisor: Dr M Letso

Co supervisors: Prof F.P. Meulenberg

: Dr W. Mahabile

September 2014



DECLARATION OF ORIGINALITY

I BAKANI VICTOR MAULE do hereby declare that this dissertation represents my own work and has not been previously submitted for the award of a degree or any other qualification at this or another University.

Bakani Victor Maule

BOTSWANA COLLEGE OF AGRICULTURE SPECIAL COLLECTION		
CLASS:	TH 636, 30877	1144
SUPPLIER	BCA	
INVOICE NO	140.00	
DATE:	October 2014	

APPROVAL

This thesis by BAKANI VICTOR MAULE has been approved as fulfilling the requirements for

the award of Masters Degree in Animal Science in the Animal Nutrition stream by the University of Botswana (Botswana College of Agriculture). Signature Supervisor Date 19/09/2014 LETSD, M. Signature Co-supervisors Date W. Mahabite Flora hie Meusebog 19/09/2014 Head of department Signature Date S.J.T. SOSO 19-09-2014 Dean of faculty Date Signature

ABSTRACT

This study reports the concentration of macro and micro minerals in soil, forage, water and blood of Tswana sheep at Morale and Goodhope ranches. The main aim of the study was to evaluate mineral status of soil, forage, water and their absorption by sheep under the influence of season and site in relation to their critical requirement values. Soil, forage, water and blood plasma samples were collected from these two study sites once every two months of wet (November to April) and dry (May to October) seasons. There was a significant (P<0.05) difference between the soil sampled from Goodhope and Morale ranches during the dry season on the concentration of Ca, Na, K, P, Zn, Cu and Fe, while during the wet season the difference between the two sites was on Ca, Mg, Na, K, P, Cu and Fc. Soils Ca, Mg, K, Mn, Zn, Cu, Fe concentration for both locations were found to be within the required range (250 mg/kg, 30mg/kg, 17mg/kg, 5mg/kg, 0.5mg/kg and 0.3mg/kg respectively) for growth of forage while Na and P concentrations were below (62mg/kg and 17mg/kg) the forage growth requirement. There was a significant (P<0.05) difference between the forage sampled from Goodhope and Morale ranches during the dry season on the concentration of Mg, Na, K, P, Mn, Zn, Cu and Fe, while during the wet season the difference between the two sites was on Na, K, P, Mn, Zn and Fe. In water only Na was influenced (P<0.05) by interaction of site and season. Forage K, Mn, Zn and Fe were within critical levels (0.60%, 20ppm, 30ppm, and 50ppm) respectively while Ca, Mg, Na, P, and Cu were below the sheep necessary requirement (0.35%, 0.20%, 0.08%, 0.25%, and 8ppm respectively. There was no noticeable difference (P>0.05) in plasma Ca, Mg, K, Na, Mn, Zn, Fe and Cu concentration between yearling sheep of Morale and of Goodhope ranches during wet and dry season. Plasma K and Fe for adult sheep in Goodhope ranch were influenced (P<0.05) by age during wet season, while plasma Ca, Mg, Na, Mn, Zn and Cu were not influenced (P>0.05). Age had a noteworthy effect (P<0.05) on plasma Na and Cu during the dry season of Goodhope ranch while plasma Ca, Mg, K, Mn, Zn and Fe were not influenced (P>0.05). There was a significant difference (P<0.05) on the concentration of Ca, Na, K, Mn, Zn and Cu for yearling sheep at Goodhope ranch between wet and dry season. At Morale ranch only K, Mn, Zn and Cu showed a noteworthy difference (P<0.05) on yearling sheep between wet and dry season. In Goodhope ranch Ca, Na, K, Mn, Zn and Cu minerals concentration for adult sheep were significantly different (P<0.05) between wet and dry season, while in Morale ranch the difference (P<0.05) was observed on Mn, Cu and Fe. Blood Ca and Mg were not within the adult

and yearling sheep requirement (80mg/l and 20 mg/l respectively) hence supplementation necessary.

DEDICATION

This paper is dedicated to my girlfriend (Pemla Sehudi) and my daughter (Warona Sehudi) who inspired me to live, struggle and achieve in time.

ACKNOWLEDGMENTS

All praise to almighty God who guided the success of these study

My special thanks goes to Prof A.A Aganga who had seen to it that this study is funded. It is with great honor and pleasure for me to express my gratitude to my worthy supervisor Dr M Letso major supervisor, Department of Animal Science and Production, under whose inspiring and scholastic guidance this work was planned, executed and completed. My gratitude to Prof F.P Meulenberg from Department of Crop Science and Production, Dr W Mahabile Department of Agricultural Research members of the supervisory committee for their help and guidance in completion of this research work.

I wish to express my sincere appreciation to Director of Agricultural Research for allowing me to access the laboratory facility in analyzing my soil and forage samples. My gratitude mostly goes to the research plant and soil laboratory technicians and researchers who assisted me in carrying out the laboratory procedures for processing research samples.

My sincere thanks goes to the Director of National Veterinary Laboratory who has allowed me to utilize their laboratory facility. My gratitude mostly goes to the residue laboratory chemists who assisted in analyzing blood plasma minerals.

I will like to thank the biometrician from the Department of Agricultural Research Mr. J Makore for guiding me through the statistical analysis of this study data.

Special appreciation is made to my family for their moral support, patience and understanding and sacrifices they made in per suite of the success of this study.

List of abbreviations

Ca : Calcium:
Cu : Copper

DTPA: Diethylenetriaminepenta-acetic acid pentasodium salt

DMl: Dry matter intake

Fe: Iron

g : Grams

K : PotassiumKg : kilogram

L : Litre

LSM: least square means

Mg : Magnesium mg : Milligrams

mg/kg: milligrams per kilogram

mg/l : migrams per litre

Mn : Manganese

Na : Sodium

P : Phosphorus

P : Probability ppm : Parts per million

SE: Standard error

Zn : Zinc:

% : Percentage

< : Less than

>.... : More than

/ : Per

TABLE OF CONTENTS

CONTENT	PAGE
Abstract	i-ii
Dedication	iii
Acknowledgments	iii
List of abbreviations	iv
Table of contents	
List of figures	
List of tables	viii-ix
CHAPTER 1.0	1
1.0 General introduction	
1.1 The effect of soil fertility on forage quality and animal nutrition	
1.2 Sheep production systems in Botswana	1-3
1.3 Problem statement	3-4
1.4 Justification	4-5
1.5 Objective of the study	5
CHAPTER 2.0	6
LITERATURE REVIEW	6
2.1 Sources of minerals in animal nutrtion	6
2.1.1 Soil	6-7
2.1.2 Forage	8
2.1.3 Water	8
2.2 Mineral nutriton in sheep	8-9
2.3 Mineral functions	9
2.3.1 Structural function	9
2.3.2 Physiological function	9
2.3.3 Enzymes Co factors and activators	9
2.3.4 Constituents of protein	10
2.3.5 Constituents of energy	10
2.4 Dietary recommendation for mineral nutrition	10-12
2.5 Mineral deficiency for sheep	12-14
2.6 Effect of mineral supplementation on animal performance	14-15
CHAPTER 3	16
MINERALS STATUS OF SOIL, FORAGE AND WATER AT MORALE AND C	OODHOPE
RANCHES (BOTSWANA)	16
ABSTRACT	16
3.1 INTRODUCTION	
3.2 Overall objectives	18
3.2.1 Specific objectives	
3.3 Hypotheses	18

3.4 MATERIALS AND METHODS	19
3.4.1 Site description	19
3.4.2 Sample collection	19
3.4.2.1 Soil	19-21
3.4.2.2 Forage	21
3.4.2.3 Water	
3.4.3 Soil analysis	22
3.4.3.1 Extraction of exchangeable basic cations (Ca, Mg, Na and K)	
3.4.3.2 Extraction of micro minerals (Mn, Cu, Zn and Fe)	
3.4.3.3 Extraction of Phosphorus	
3.4.4 Determination of minerals in forage	
3.4.5 Determination of minerals in water	
3.4.6 Analytical procedure for minerals determination	23
3.4.7 Statistical analysis	24
3.4.7.1 Experimental model	
3.5 RESULTS	25
3.5.1 Soil mineral concentration	25-29
3.5.2 Forage mineral concentration	30-33
3.5.3 Water mineral concentration	34
3.6 DISCUSSION	35
3.6.1 Calcium content of soil, forage and water samples from the study sites	35-36
3.6.2 Magnesium content of soil, forage and water samples from the study sites	36-37
3.6.3 Sodium content of soil, forage and water samples from the study sites	
3.6.4 Potassium content of soil, forage and water samples from the study sites	
3.6.5 Phosphorus content of soil and forage samples from the study sites	
3.6.6 Manganese content of soil, forage and water samples from the study sites	
3.6.7 Zinc content of soil and forage samples from the study sites	
3.6.8 Copper content of soil, forage and water samples from the study sites	
3.6.9 Iron content of soil and forage samples from the study sites	
3.7 CONCLUSION	
3.8 RECOMMENDATIONS	
CHAPTER 4.0	
EFFECT OF SEASONAL VARIATION ON THE MINERALS STATUS OF TSWAM	
SHEEP GRAZING NATURAL PASTURES AT MORALE AND GOODHOPE RAN	
BOTSWANA	
ABSTRACT	
4.1 INTRODUCTION	
4.2 OBJECTIVES	
4.2.1Specific objectives	
4.3 HYPOTHESIS	50

4.4 MATERIALS AND METHODS51
4.4.1 Site description
4.4.2 Blood sample collection
4.4.3 Blood sample preparation51
4.4.4 Analytical procedure for determination of minerals
4.4.5 Statistical analysis
4.4.5.1 Experimental model
4.5 RESULTS
4.5.1 Sheep minerals concentration
4.5.1.1 Yearlings sheep minerals concentration54-56
4.5.1.2 Adult sheep minerals concentration57-58
4.6 DISCUSSION
4.6.1 Concentration of Calcium in the blood Plasma of adult and yearling sheep 59
4.6.2 Concentration of Magnesium in the blood Plasma of adult and yearling sheep 60
4.6.3 Concentration of Sodium in the blood Plasma of adult and yearling sheep 60-61
4.6.4 Concentration of Potassium in blood Plasma of adult and yearling sheep
4.6.5 Concentration of Manganese in the blood Plasma of adult and yearling sheep 61
4.6.6 Concentration of Zinc in the blood Plasma of adult and yearling sheep
4.6.7 Concentration of Copper in the blood Plasma of adult and yearling sheep
4.6.8 Concentration of Iron in the blood Plasma of adult and yearling sheep
4.7 CONCLUSION
4.8 RECOMMENDATIONS
CHAPTER 5.065
REFERENCES65-76

LIST OF FIGURES

FIG	TITLE TITLE	PAGE
2.1 5	Summary of the many and varied factors in addition to plants which can	
i	influence the flow of an element from the soil to the grazing and or not meet the animal's	
r	requirements	7
2.2 5	Schematic sequences of pathophysiological changes in livestock given Inadequate	
5	supply of minerals storage and function are linked by bone strength	13
2.3	Schematic dose response relationships between mineral supply and animal	
	production showing marginal bands between adequate and inadequate or toxic dietary	
	concentrations	14
3.1 0	Concentration of macro-minerals of soil at Goodhope and Morale ranches	27
3.2 (Concentration of micro-minerals of soil at Goodhope and Morale	28
3.3 (Cconcentration of Ca, Mg and K in samples collected from Goodhope and Morale ranches during	
1	the wet season (sampled from December to April), and the dry (sampled from June to October)	29
3.4 (Concentration of macro-minerals in forages at Goodhope and Morale ranches	32
3.5 C	Concentration of micro-minerals in the forages at Goodhope and Morale ranches	33
LIS	ST OF TABLES	
TA	ABLE TITLE	PAGE
1.1	Trends of sheep population (Goats and Sheep) 1983-2004	4
2.1	Macro minerals requirements percentage diet dry matter	11
2.2	2 Micro mineral requirements of sheep and maximum tolerance	
	levels (ppm, mg/kg of diet dry matter)	11
3.1	Sampling points coordinate at Goodhope Agricultural ranch	20
3.2	Sampling points coordinate at Morale Agricultural research ranch	21
3.3	Soil macro-mineral concentrations (mg\kg) at Goodhope and Morale ranch	
	during the wet and dry season	26
3.4	Soil micro-mineral concentrations (ppm) at Goodhope and Morale	
	during the wet and dry season	26

3.5 Forage macro-mineral concentrations for pasture samples from Goodhope and
Morale during the wet and dry season,31
3.6 Forage micro-mineral concentrations for pasture samples from Goodhope and
Morale during the wet and dry season31
3.7 Mineral concentrations for water samples from Goodhope and Morale
during the wet and dry season34
4.1 Concentration of Ca, Na, and Fe in sheep blood sampled at Goodhope and Morale
ranches55
4.2 Concentration of Na, Fe, Zn, and Cu in sheep blood sampled at Goodhope and Morale
ranches during the wet season55
4.3 Interaction for the concentration (mg/l) of macro minerals in the blood of yearling sheep at
Goodhope and Morale tested during the wet and dry season56
4.4 Interaction for the concentration (mg/l) of micro minerals in the blood of yearling sheep at
Goodhope and Morale during the wet and dry season56
4.5 Interaction for the concentration (mg/l) of macro minerals in the blood of adult sheep at
Goodhope and Morale during the wet and dry season58
4.6 Interaction for the concentration (mg/l) of micro minerals in the blood of adult sheep at
Goodhope and Morale during the wet and dry season58

CHAPTER 1.0

SOIL-PLANT-ANIMAL CONTINUUM IN RELATION TO MACRO AND MICRO MINERAL STATUS OF TSWANA SHEEP AT GOODHOPE AND MORALE RANCHES IN BOTSWANA

GENERAL INTRODUCTION

1.1 The Effects of Soil Fertility on Forage Quality and Animal Nutrition

The relationship between the content and availability of mineral elements in the soil and mineral composition in plants and the mineral content that passes to animals is very important. Mineral composition of forage plants is affected by soil-plant factors, including soil pH, drainage, fertilization, forage species and forage maturity (Espinoza *et al.*, 1991). The mineral status of soils and forage influences the mineral status of grazing livestock. However many other animal factors such as breed, age, health, production and mineral interactions may also play an important role.

Among different environmental factors soil plays a major role in sheep production and health because sheep obtain their nutrients from the feed and fodder which in turn obtain nutrients from the soil (Kumaresan et al., 2009). The role of the soil and nutritional quality of plants with respect to the health and production of livestock is very important and varies from place to place. Factors that influence the mineral composition in plants vary greatly depending on the location where they are grown. In general the chemical composition of plants is influenced to a greater extent by the inherent soil properties (Kuria et al., 2004).

1.2 Sheep production systems in Botswana

In semi-arid areas like Botswana, the preferred livestock species kept are sheep and goats because of their hardiness (Adogla-Bessa and Aganga, 2000). Their farming is found under two distinct production systems: commercial and traditional (Nsoso *et.al.*, 2004). The traditional system is characterized by use of limited inputs hence less output while commercial sector is characterized by improved management systems. The traditional system is more economically

important as it accounts for larger proportion of country's livestock industry in terms of both human and livestock population (Seleka, 1999). In this system sheep depend largely upon forages to fulfill their mineral requirements. Based on their traditional knowledge, farmers rear sheep on a system where they obtain their nutritional demand from low nutrient feed material.

Feeding of mineral supplement to sheep is an unknown phenomenon on sheep and goats that are raised on communal grazing land (Devendra, 2001). According to Khan *et al.* (2005b) based on a series of forage sampling and other people's work much of the pasture herbage available throughout the different regions of the world cannot completely satisfy the mineral requirements of grazing animals. Farmers keeping sheep in the tropics use natural grazing and crop by products that are available and try to feed their sheep as best as they can with what they have. This can be termed supply driven feeding as sheep are fed according to supply of feeds available, over which the farmer may have little control. Traditional farmers depend entirely on the government to provide subsidized medicine for animal health issues (Peacock, 1996). The farming system does not have an organized marketing structure in place. This is because sheep products are either consumed at home or traded in villages.

The availability of minerals to sheep depends on production system, feeding practices, and environment (Devendra, 2001). Mineral requirements for sheep depend on several factors which include kind and level of production, age, breed, chemical form of feed and animal adaptation. The detection of minerals deficiencies or excesses involves clinical, pathological and analytical criteria as well as response from specific mineral supplementation (Khan *et al.*, 2006). Minerals nutrients (major and minor) are very important for several metabolic functions and their deficiency impair production and reproduction.

The concept of soil-plant-animal interrelationship on minerals implies that a particular sequence of events may lead to imbalance or balance in nutritional quality of feeds for animals. Most naturally occurring mineral deficiencies in herbivores are associated with specific regions and are directly related to soil characteristics. Micronutrients are depleted more from light textured and calcareous soils particularly when high yielding crops are grown under intensive system (McDowell and Valle, 2000; Khan, 2003). When mineral nutrients in herbage are marginal in

respect of animal requirements, changes in concentrations brought about by climatic, managerial or seasonal influences as well as plant maturity can be significant factors in incidence or severity of deficiency states by livestock wholly or largely dependent on these plants (Khan *et al.*, 2005a).

Analysis of soil, water and forages for mineral composition is important for obtaining mineral status of an area with a view to provide mineral supplements to grazing animals. Adequate information on soil characteristics and mineral composition of forages is therefore necessary for livestock production. The objective of this research work was therefore to evaluate mineral status of soil, forage, along with water so as to relate it with the mineral requirement of pastured Tswana sheep and recommend proper supplementation program for each study area.

1.3 Problem statement

Mineral status of soils and forage influence mineral status of grazing livestock, but many other animal factors and mineral interactions also play an important role. As long as grazing livestock have to depend largely upon forages to fulfill their mineral requirements, they will never be completely satisfied for their mineral needs (Khan *et al.*, 2008a). In fact poor animal growth and reproductive problems are common even when forage supply is adequate, and can be directly related to mineral deficiencies caused by the low mineral concentrations in soils and associated forages as concluded by Kumaresan *et al.* (2009). Since minerals are involved in almost all the metabolic processes taking place in the body, their deficiency or toxicity would affect the sheep production negatively. Livestock census conducted by the Central statistics office 2008 (CSO, 2008) in Table 1.1 shows that there has been a declining trend in sheep numbers in Botswana.

Table 1.1 Trends of sheep Population (1983 – 2004) in Botswana

Census Year	Sheep population	(%) Change	į.
1983	165,000		
1993	250,100	51.6	
2004	225,500	-9.8	·

Source (Central Statistics Office, 2008)

These could have been attributed to several production constraints, which include the outbreak of Contagious Bovine Pleuropneumonia (CBPP) in 1995 which led to eradication of affected cattle in Ngamiland. Eradication of cattle in Ngamiland might have led to pressure in the sheep numbers since sheep were the alternative sources of meat when beef was running short. The other factor could have been the growing need for sheep meat by the Muslim community. The biggest constraint to traditional sheep famers is inadequate and poor feed supply (Lundu *et al.*, 2012). Nutrition plays a major role in animal production, in which minerals are involved in almost every metabolic process to keep the animal healthy and able to reproduce. Mineral inadequacy or excessiveness would have a negative impact on animal production and reproduction. Hence minerals outside the optimum range can cause a decline in sheep numbers.

1.4 Justification

Nutritional constraints to improved sheep production include inadequate feed supply, low feeding value of the available feed resources and reduced efficiency of utilization of the available feed resources. Grazing and browsing on natural pastures and poor quality crop residues are the main sources of feed in most parts of Botswana. Due to seasonal rainfall distribution, there is a marked seasonal variation in the quantity and quality of feed supply. It is common knowledge that crops are continuously mining the macro and micro-nutrients from the soil reserves. Increased removal of nutrients leads to their deficiencies. To diagnose an animal's mineral deficiencies, soil, plant tissue and animal fluids analysis are necessary. Lack of readily available data about the mineral status of the soils, forage and animals, to farmers who depend on livestock as their livelihood makes it important for the study to be conducted. Famers cannot determine

mineral deficiency and toxicity based on symptoms shown by animals. This is due to the fact that there are so many minerals interrelationships, therefore it is difficult to authentically indicate which mineral might be implicated. A major problem in formulating precise mineral nutritional requirements by small scale farmers is lack of knowledge. Farmers in most of the developing countries have the quest for information on how they can improve the productivity of their animals for cash and provision of draft power therefore they need legitimate information for guidance. The availability of sensitive analytical equipment and procedures has made it possible for minerals status to be monitored hence making it possible to advise farmers accordingly.

1.5 Objective of the study

The main aim of the study was to evaluate mineral status of soil, forage, water and their absorption by sheep under the influence of season and site in relation to their critical requirement values.

CHAPTER 2.0

LITERATURE REVIEW

2.1 Sources of minerals in animal nutrition

On traditional management systems, sheep use locally available grasses and salty water as sources of mineral supplements, with minimal use of commercial supplements (Aganga *et al* .,1997). Feed sources of required minerals are sometimes divided into normal or natural feed ingredients and minerals supplements (Adogla-Bessa and Aganga, 2000). Under natural conditions sheep have the capacity to choose their forages efficiently, grazing more on grasses than forage trees. Minerals can also be accessed directly from the soil or non-feed contamination (McDowell, 1992). Soil is the source of all mineral elements found on plants. Of all mineral concentration in the soils only a fraction is taken up by the plants.

2.1.1 Soil

Soil is a heterogeneous material comprising solid, liquid and gaseous phase. The solid phase is the main reservoir of nutrients including Sodium (Na), Iron (Fe), Manganese (Mn), Zinc (Zn) and others (Devendra, 2001; Ahmad *et al.*, 2008). However, optimal concentration of trace elements is required for various physiological and metabolic processes taking place in the body of organisms (McDowell, 2003). Plant uptake of these micro-nutrients is essential for plant growth and development (Koike *et al.*, 2004). Soils have different mineral concentrations depending on the site examined. The effect of season has been shown in the concentration of minerals in the soil. According to Khan *et al.* (2004b) seasonal differences were observed on the concentration of Copper (Cu) in the soils of Soone valley Pakistan. Soils with less than 0.6mg/kg extractable Cu were considered deficient for pasture and other crops use, therefore soil with such low Cu concentration should have crop responding to Cu supplementation.

Figure 2.1 (Underwood and Suttle, 2004) shows how minerals flow from a chain of events. McDowell (1992) reported that soils were usually considered to influence animal nutrition only through quality and quantity of herbage they produce. The fact that soil is ingested by animals

indicate that not only the usual sequence of soil-plant-animal relationship occur but also direct relationship between soil and animal should be considered as shown Figure 2.1.

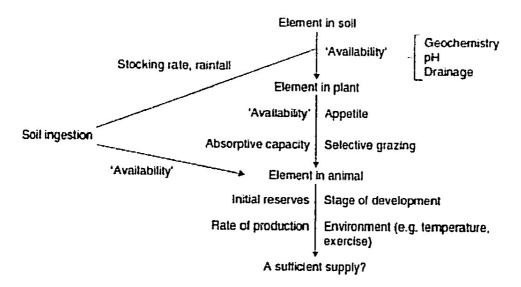


Figure 2.1: Summary of the many and varied factors in addition to plants which can influence the flow of an element from the soil to the grazing animal and whether the supply will meet the animal's requirements

Source: Underwood and Suttle (2004)

The assessment of minerals in the soil, plant and the animal can give conflicting results in adequacy of supply. Devendra (2001), concluded that the utilization of minerals by the grazing animals in particular were likely to vary widely from season to season and from year to year. Changes in mineral concentration brought about by atmospheric, climatic or seasonal influence and by forage maturity and seed shedding, can be significant where mineral nutrients are marginal to animal requirements. Furthermore there is or can be a substantial recycling of minerals supplies via excreta, and there are also withdrawals of minerals from the ecosystem each year in harvested crop and livestock products which are time to time replaced (Aregheore et al., 2007). Changes in management practices can shift mineral balances substantially.

2.1.2 Forages

Factors that influence mineral composition in forage vary greatly depending on the location where they are grown. Mineral concentration may range from toxic to inadequate for livestock production. Generally the concentrations in broadleaf plants including leguminous plants are more than those in grasses and other forages (Belesky et al., 2001; Khan et al., 2006a). Mineral ion concentration decreases with increase in age both in the case of legumes and grasses (Gonzalez et al., 2006). In general the chemical composition of the plants is influenced to a greater extend by inherent soil properties. Season of the year affects mineral intake, which is often greatest during the in dry season. When forages stop growing they lose the green color and become high in fiber and lignin and low in digestibility and mineral availability. As plants mature, mineral content declines (Talat et al., 2009)

2.1.3 Water

McDowell (1992) reported that extensive analyses of surface water and groundwater by geological, agricultural and health agencies have demonstrated the presence of varying concentrations of all essential minerals for humans and animals. On the same matter Underwood and Suttle (2004) reported that drinking water was not normally a major source of minerals to livestock though there could be some exceptions. The concentration of Sulphur from deep aquifers can reach 600mg/L adding 3g\kg of S dry matter to the diet as sulphates. This is far more than any nutritional requirements and may even create problems by inducing copper deficiency. Some hard water may provide some significant amount of Calcium, Magnesium and Sulphur and occasionally other minerals (Blincoe *et al.*, 1973).

2.2 Minerals nutrition of sheep

Mineral requirements are generally expressed in two ways; in amount required per day or in unit of product such as milk, or in proportion of dry matter of the diet consumed (Kafeel et al., 2008). Homeostatic regulation of higher animals that tends to buffer the marginally deficient or marginally excessive intake by changing the efficiency of absorption and excretion should be taken into account when assessing mineral deficiency or toxicity. The actual amount of the mineral may also influence the utilization of the mineral. For instance if there is abundance of Ca in the body than the body needs homeostatic mechanism are brought into play and the efficiency

of absorption is reduced. The minerals status of an animal may be influence by its absorption. A Fe deficient animal is more efficient in the absorption of Iron than an animal with adequate Iron stores (McDowell 1992).

2.3 Mineral Functions

There are four types of functions for minerals; utilization-structural, physiological, catalytic and regulatory.

2.3.1 Structural function

Minerals can form a structural component of the body organs and tissues; as exemplified by minerals such as Ca, P, Mg, Fl and Si in bones and teeth, P and S in muscle proteins. Minerals like Zn and P can also contribute structural stability to the molecule and membrane of which they are part of (McDowell, 1992).

2.3.2 Physiological function

Regarding physiological functions these minerals occur in body fluids and tissues concerned with the balance of osmotic pressure, acid base balance, membrane permeability and tissue irritability. Na, K, Cl, Ca and Mg in the blood, cerebrospinal fluid and gastric juice are examples of minerals which provide physiological functions (Underwood and Suttle, 2004).

2.3.3 Enzymes, co-factors and activators

Minerals can act as an catalyst in enzyme and hormone system as integral and specific component in the structure of metalloenzymes or as less specific activators within those systems. Minerals regulate cell replication and differentiation. Calcium for example influences the transduction process while zinc influences transcription adding to the regulatory roles, such as that of the element iodine as a constituent of thyroxin (Underwood and Suttle, 2004). During the absorption of glucose the first family, Sodium-glucose co-transporters actively transport glucose across the mucosal cells from the lumen and across the kidney tubule.

2.3.4 Constituents of protein

The building blocks of protein are amino acids, essential and non essential. These amino acids includes cystine, mithionine and cysteine. These amino acids comprises of suiphur, cystine and cysteine being essential amino acids. The body contains 0.004% iron of which 70% is present in the hemoglobin, the iron containing pigment of the red blood cells that transport oxygen. Mn is involved in the metabolism of protein(Underwood and Suttle, 2004). Se is part of the enzyme glutathione peroxidase.

2.3.5 Constituents of energy

For metabolism to permit the orderly transfer of energy from feeds to processes requiring energy there must be a common carrier of energy. Something cells can use to exchange goods for service. The primary mechanism by which energy is captured and stored is a compound called adenosine triphosphate (ATP). ATP is a compound formed from the purine adinine, the 5 carborn sugar ribose and 3 phosphate molecules (McDowell, 1992)...

2.4 Dietary Recommendation for Mineral Nutrition of Sheep

Mineral requirement for sheep can be met by utilizing different sources of feeds. For nutritional accuracy feedstuffs should be examined for digestibility and anti-nutritional factors, since total minerals do not guarantee bio availability (Khan *et al.*, 2003). Mineral needs of ruminant animals depend greatly on their physiological makeup, growth rate, age, health and nutritional status (McDowell and Velle, 2000). Functions such as meat and milk production or developing a fetus have higher mineral requirements than dry ewes. Different breeds and strains of animals vary in their mineral requirements and those of new strains developed for improved production may be higher (McDowell, 2003) because of their metabolic rates.

Table 2.1 Macro mineral requirements for sheep (percentage of diet dry matter)

Nutrient	Requirement (%)	Toxic levels
Salt		
(Na) and (Cl)	0.09-0.18	4% DMI
Calcium (Ca)	0.20-0.82	N/A
Phosphorus (P)	0.16-0.38	N/A
Magnesium (Mg)	0.12-0.18	0.8%
Potassium (K)	0.50-0.80	N/A
Sulfur (S)	0.14-0.2	0.4%DMI

Source: NRC (1996)

Table 2.2 Micro mineral requirements of sheep and maximum tolerable Levels (ppm of diet dry

matter) for sheep

Nutrient	Requirements	Maximum Tolerable
		Level (ppm)
Iodine (I)	0.10-0.80	50
Iron (Fe)	30-50	500
Copper (Cu)	7-11 ^a	25 ^b
Molybdenum (Mo)	0.5	10
Cobalt (Co)	0.1-0.2	10
Manganese (Mn)	20-40	1,000
Zinc (Zn)	20-33	750
Selenium (Se)	0.1-0.2	2
Fluorine (Fl)	_	60-150

NRC (1996)

Requirement when dietary Mo concentrations are cl mg/kg DM.

^{*}Lower levels may be toxic under some circumstances.

Although sheep possess a lot of minerals in their bodies only 15 has shown to be of importance. These are Na, Cl, I, Mn, Fe, Cu, Co and Zn contained in trace mineralized salt, plus Ca, P, K, Mg, S, Mo and Se (McDowell, 2003), as shown in Table 2.1 and 2.2. Khan *et al.* (2007b) showed that season affected mineral concentration in the soil and herbage. In the same study it was observed that blood calcium was higher in summer than in winter.

2.5 Mineral Deficiency and Toxicity in Sheep

Major and minor minerals are essential for physiological body activities such as growth, production, reproduction. However, there is greater degree of uncertainty in the mineral requirements of animals depending upon age, breed, and level of production, dietary antagonist, animal adaptation and interrelationship with other nutrients (Singh *et al.*, 2005). Mineral deficiency impairs production and reproduction. The availability of minerals to sheep depends on feeding practices and environment.

According to Pope (2000) soil and plants are the best determinants of minerals in the animal, but this information needs to be incorporated with clinical pathological symptoms. Likewise mineral deficiency can only serve as guidelines since no single deficiency can be authentically linked to a certain mineral deficiency alone. This is so because minerals have got similar symptoms for their deficiency. It would seem that one of the most valuable aids in the detection of mineral deficiencies or excesses is blood analysis. T analyses according to Ahmad *et al.* (2008) could include minerals and enzymes as in the case of Se deficiency or Cu toxicity. Availability of some minerals is influenced by the presence or absence of other minerals and their interaction. For instance when pasture forage has low Ca content it is likely to be low in P and protein and have poor digestibility. These nutritional defects would overshadow any inadequacy in calcium intake (Khan, 2003).

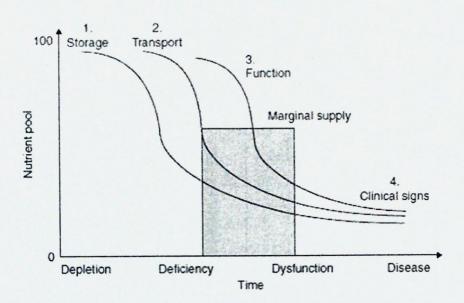


Figure 2.2: Schematic sequence of path physiological changes in livestock given inadequate supply of minerals. Source: Underwood and Suttle (2004)

Underwood and Suttle (2004) demonstrated how the animal's body and its metabolism function from the time of mineral adequacy until there is a serious deficiency (Figure 2.2). It provides a rational basis of the differential diagnosis of disorders due to mineral imbalance. The model divides events into four phases firstly depletion, during which stored minerals in the animal's body are mobilized because of lack of minerals availability in the feeds and water consumed by the livestock. This leads to transport pools of minerals reduced in the second stage called deficiency. Thirdly a dysfunctional stage into which mineral dependent function become rate limiting to particular pathways. Fourthly; diseases stage during which clinical abnormalities become apparent to the naked eye. There may be variable degrees of overlap between phases for example deficiency may occur before the reserves are completely finished (Chelliah et al., 2008)

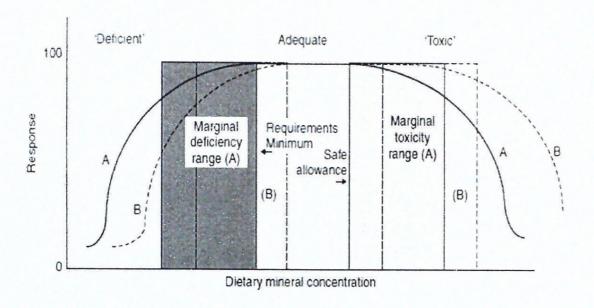


Figure 2.3: Schematic dose-response relationship between mineral supply and animal production showing marginal bands between adequate and inadequate or toxic dietary concentrations. Source: Underwood and Suttle (2004)

Figure 2.3 shows mineral levels from left to right as absorbability of the minerals declines. A basic or minimum requirements for any dietary requirements are those that satisfy all the mineral dietary conditions. Since the exacting conditions rarely apply, there can be no single requirement, but a series of requirements depending on the extent of conditioning factors present, as reported by McDowell (1992). Differences in chemical and physical form into which these minerals are presented affect the requirements and tolerance. The chemical form of the minerals acquire a particular nutritional significance, for non-ruminant animals, e.g. phytates phosphorus and zinc bound to phytate are poorly absorbed by pigs but they are well absorbed by sheep (Johnston *et al.*, 2001). Physical form may be very important for mineral supplement such as magnesium oxide which can vary in particle size and hence nutritive value.

2.6 Effect of Mineral Supplementation on Animal Performance

Mineral supplementation is a very important facet of sheep production, yet often overlooked (Khan et al., 2004b). A sheep's diet rarely provides all of the needed macro- and micro-minerals without supplementation especially in production system that animal depend entirely on natural grazing land for their nutritional requirements. A combination of soil type, plant species and climatic conditions result in consistent deficiencies in certain geographical areas (Kafeel et al.,

2008). Supplementation should be done after the deficient minerals have been identified. During supplementation interrelationships of other minerals with the deficient mineral should be considered as the absence of one mineral could explain that it has been bounded to other minerals (Khan, 2003).

CHAPTER 3.0

MINERALS STATUS OF SOIL, FORAGE AND WATER AT MORALE AND GOODHOPE RANCHES (BOTSWANA)

ABSTRACT

The main aim of the study was to evaluate and compare the mineral status of soil, forage and water from the two ranches of different locations (Morale agricultural research ranch and Goodhope ranch) in relation to seasonal variation and to their critical requirement values. Soil, forage and water samples were collected from these two farms once every two months of the wet (November to April) and dry (May to October) seasons. There was a significant (P<0.05) difference between the soil sample from Goodhope and Morale ranches during the dry season on the concentration of Ca, Na, K, P, Zn, Cu and Fe, while during the wet season the difference(P<0.05) between the two sites was on Ca, Mg, Na, K, P, Cu and Fe. Soil Mg, K, P, Mn, Cu, and Fe were respectively 50%, 93%, 170%, 169%, 161% and 41% higher (P<0.05) at Morale ranch compared with Goodhope ranch. Soils Ca, Mg, K, Mn, Zn, Cu, Fe concentration for both locations were found to be within the required range (250 mg/kg, 30mg/kg, 17mg/kg, 5mg/kg, 0.5mg/kg and 0.3mg/kg respectively) for growth of forage while Na and P concentrations were below (62mg/kg and 17mg/kg) the forage growth requirement. There was a significant (P<0.05) difference between the forage samples from Goodhope and Morale ranches during the dry season on the concentration of Mg, Na, K, P, Mn, Zn, Cu and Fe, while during the wet season the difference between the two sites was on Na, K, P, Mn, Zn and Fe. In water only Na was influenced (P<0.05) by interaction of site and season while Ca, Mg and K were not. Forage K, Mn, Zn and Fe were within critical levels (0.60%, 20ppm, 30ppm, and 50ppm) respectively while Ca, Mg, Na, P, and Cu were below the sheep necessary requirement (0.35%, 0.20%, 0.08%, 0.25%, and 8ppm respectively. Forage minerals that were deficient which includes Na, Mg and P can be met by providing mineral block or mineral salt mixture on pasture. Studies should be carried out to determine the needs and economic benefits of mineral supplementation.

15

3.1 INTRODUCTION

Soil is the source of all minerals elements found in plants. The status of these minerals is dependent on several mineral interactions. Most naturally occurring mineral deficiencies in livestock are associated with specific season and are directly related to both soil mineral concentration and soil characteristics (Khan *et al.*, 2003). Of all the mineral concentration in soils, only a fraction is taken up by plants. The bioavailability of minerals in soils depends upon their effective concentration in soil solution (Aregheore *et al.*, 2007). Soil-plant factors which include pH, drainage, fertilization, forage species, forage maturity and interaction among minerals affect mineral composition of forage plants (Fardous *et al.*, 2010).

In Botswana traditional farmers raise sheep on natural vegetation without any supplementation for their minerals requirements (Seleka et al., 1999). Minerals derived from naturally available feedstuffs are sometimes insufficient to meet animal requirements. According to Khan et al. (2009) the quantitative control of mineral elements in animals depend directly on several environmental factors such as soil, water and plants.

Due to climatic variations of climate between seasons in Botswana it is expected that there would be large variation in nutrient uptake. Mineral concentrations in plants differ from species to species and vary from one place to another as influenced by soil-plant factors. The concentration of minerals in soil is varying depending seasonal changes (Khan *et al.*, 2006). Phosphorus concentrations of crop and forage plants decline markedly with advancing maturity, although the decline is less in legumes than in grasses (McDowell *et al.*, 1993).

The concentrations of many other elements (e.g. cobalt, copper, iron, potassium, magnesium, manganese, molybdenum and zinc) also decline, but rarely to the same extent as that of phosphorus. Such changes often reflect increases in the proportion of stem to leaf and old to new leaves, with stems and old leaves having lower mineral concentrations than young leaves (Minson, 1990), although this does not apply to manganese. Minerals are lost with the shedding of seed and the remaining stem or straw is low in most minerals (Suttle, 1991). Furthermore, standing straw is subject to leaching of phosphorus and potassium. Seasonal variation is always

reflected by the mineral level of ruminants (Khan et al., 2008a). It is therefore important to know the mineral concentrations of grazing forage because forage is the primary source of nutrients for livestock in rangelands (McDowell, 1992) and because minerals present in the soil are transported to livestock through the forages on which they feed.

3.2 Overall objectives

To evaluate the mineral status of soil, forage and water from two ranches at different locations (Morale agricultural research ranch and Goodhope agricultural research ranch) in relation to seasonal variation.

3.2.1 Specific objectives

To determine the amount of Ca, P, Mg, Na, K, Zn, Cu, Fe and Mn in soil, forages and water sampled at Morale ranch and Goodhope ranch.

To compare the concentrations of the macro and micro minerals on the two farms of study with optimal requirement levels for sheep.

3.3 Hypotheses:

Ho: The concentration of macro and micro minerals in the soil, forage and water is not influenced by site, season and sampling time.

H_A: The concentration of macro and micro minerals in the soil, forage and water is influenced by site, season and sampling time.

3.4 MATERIALS AND METHODS

3.4.1 Site description

This study was conducted from 2010 to 2011 on land grazed by Tswana breed of sheep at two sites namely Morale ranch and Goodhope ranch. Morale ranch is located in the Central District of Botswana (23° 06′ 32, 30° S; 26° 49, 33 26° E (Google Earth, 2010). Its vegetation type is tree savanna with abundance of *Acacia* species. The soil type is ferric or chromic-Luvisols/Cambisols with rainfall of up to 655mm. The soils are loamy, well-drained and moderately deep (60-90cm) (Anon, 2001). The Goodhope ranch is located in the Southern District of Botswana coordinates 25° 06′ 32, 30 S; 26° 49′33, 26 E. The area has tree savanna vegetation which consists mostly of acacia species with sandy to loamy soils. Annual rainfall is up to 630mm (Anon, 2001).

3.4.2 Sample collection

Soil, forage and water samples were collected from the two study sites once every two months for both the wet and the dry season. In the wet season samples were collected in the months of December, February and April, while in the dry season sampling was done in June, August and October. Sheep grazing was controlled, where sheep were designated to graze on a particular paddock at a time. Samples were collected in paddocks where the animals were grazing at a particular time. Since the mineral status of the soil differs from place to place, soil samples were collected along with forage sample. The samples were collected in intervals as shown by the coordinates to form a Z shape at the designated grazing paddocks (Aganga and Letso, 1999).

3.4.2.1 Soil

Soil samples were collected from Goodhope ranch and Morale ranch at a depth of 15 to 20cm using a stainless steel sampling auger. Sampling paper bags were used to store these samples for transportation from the field to the laboratory. Samples were air dried, and then ground using a Wiley mill with a 2mm sieve and stored in labeled plastic bottles while awaiting analysis. The samples were collected at geographical coordinates shown in Table 3.1 and 3.2 at every sampling period.

Table 3.1 Coordinates of sampling points at Goodhope ranch.

Sampling coordinates	Season	Month
S 25 468 20, E 25 464 71	Wet season sampling	December, February, April
S25 485 28, E 25 463 77	Wet season sampling	December, February, April
S25 485 59, E 25 462 72	Wet season sampling	December, February, April
S25 485 33, E 25 461 80	Wet season sampling	December, February, April
S25 485 06, E 25 460 76	Wet season sampling	December, February, April
S25 485 73, E 25 459 58	Wet season sampling	December, February, April
S25 487 43, E25 459 90	Wet season sampling	December, February, April
S25 488 34, E 25 460 27	Wet season sampling	December, February, April
S25 489 73, E26 460 60	Wet season sampling	December, February, April
S25 491 03, E25 461 15	Wet season sampling	December, February, April
S25 492 33, E25 461 48	Wet season sampling	December, February, April
S25 492 66, E25 460 33	Wet season sampling	December, February, April
S25 492 43, E25 459 41	Wet season sampling	December, February, April
S25 492 14, E25 458 35	Wet season sampling	December, February, April
S25 491 93, E25 457 44	Dry season sampling	June, August, October
S25 489 10, E25 469 94	Dry season sampling	June, August, October
S25 494 03, E25 467 34	Dry season sampling	June, August, October
S 25 500 81, E25 469 94	Dry season sampling	June, August, October
S 25 500 09, E25 467 43	Dry season sampling	June, August, October
S25 498 29, E25 469 53	Dry season sampling	June, August, October
S25 495 12, E25 470 86	Dry season sampling	June, August, October
S25 493 62, E25 473 74	Dry season sampling	June, August, October
S25 494 87, E25 475 34	Dry season sampling	June, August, October
S25 499 13, E25 479 15	Dry season sampling	June, August, October
S25 502 52, E25 472 76	Dry season sampling	June, August, October

Table 3.2 Coordinates of sampling points at Morale ranch.

Sampling coordinates	Season	Months
S23 193 26, E26 824 20	Wet and dry season sampling	December, February, April June, August, October
S23 192 37, E26 824 65	Wet and dry season sampling	December, February, April June, August, October
S23 191 61, E26 825 17	Wet and dry season sampling	December, February, April ,June, August, October
S23 193 25, E26 825 84	Wet and dry season sampling	December, February, April ,June, August, October
S23 193 46, E26 826 30	Wet and dry season sampling	December, February, April ,June, August, October
S23 195 51, E26 826 83	Wet and dry season sampling	December, February, April ,June, August, October
S23 194 89, E26 827 40	Wet and dry season sampling	December, February, April June, August, October
S23 194 28, E26 827 92	Wet and dry season sampling	December, February, April ,June, August, October
S23 193 74, E26 828 48	Wet and dry season sampling	December, February, April ,June, August, October

3.4.2.2 Forage

Using a 1m² quadrant and a grass scissor, all grass from the quadrant was cut at grazing level on coordinates corresponding to that of soils for the two respective ranches. The cut grass from each location was put in labeled paper bags and transported to the laboratory. The samples were then dried in an oven at 70°C for 24 hours, ground to powder to pass through a 2mm sieve and stored in clean dried plastic bottles pending chemical analysis.

3.4.2.3 Water

The water that was supplied to the animals was borehole water, and was provided to the animals on water troughs. Water samples were collected from the site where it was supplied to the animals. Samples were collected before animals were allowed to drink. Water was collected using the sample bottles from the point of drinking. These water (250mL) samples were stored in labeled plastic bottles on shelves awaiting chemical analysis.

3.4.3 Soil analysis

3.4.3.1 Extraction of exchangeable basic cations (Ca, Mg, Na and K)

Exchangeable basic cations (Ca, Mg, Na, and K) were determined using 1N ammonium acetate solution buffered at pH 7.0 as described by (Thomas, 1982). A sample of 2.5 g of air dried soil was put in to the bottom of the extraction syringe and 20 ml of 1M ammonium acetate was added to the syringe and 45 mL to the top syringe. It was then left to extract for at least 2 hours using ammonium acetate. The leachate was collected into a 100mL volumetric flask and filled to the mark with 1 molar ammonium acetate.

3.4.3.2 Extraction of micro minerals (Mn, Cu, Zn and Fe)

Micro minerals in the soil samples were extracted by diethylenetriaminepentacetic acid (DTPA) chelation method as described by Lindsay and Norvell (1978). A 10g air dried soil sample was put into 100mL plastic bottles and then 20mL of DTPA was added and shaken using a shaker for 2 hours. A clear supernatant was filtered into vials through a Whatman no 42 filter paper. The supernatant was collected into a 100mL volumetric flask and filled to the mark using DTPA.

3.4.3.3 Extraction of Phosphorus

Phosphorus (P) in the soil sample was determined by the Bray-1 method. This method involves extracting soil P with 0.03N NH₄F + 0.1N (HCL) with the Bray-1 solution. Soil samples weighing 3g were put into a 50mL extraction cups, then 30mL ammonium fluoride extracting solution (0.03N NH₄F + 0.1NH₄) was then added and stirred for 40 seconds. The mixture was filtered into plastic vials using the Whatman filter paper number 42.Using the semi calumniated dispenser 5mL of extractant plus 20mL Bray-1 solution was pipetted into a 50mL vials. The colour developing solution was added. The samples were left for 40 minutes for the colour to develop before analyzing with UV spectrometry (Bray and Kurtz, 1945).

3.4.4 Determination of minerals in forage

Forage samples were digested through Kjeldahl wet digestion procedure. The digestion reagents were prepared by weighing 10g of selenium metal powder into a 5L volumetric flask, into which 2.5mL of sulphuric acid H₂SO₄ (98%) was added then heated for 4 hours until it was well dissolved. A 1.25g forage sample was put into a digestion tube into which 20 mL of the digestion solution was added, then heated at 330°C for 2 hours, after which the samples were allowed to cool at room temperature. After cooling 4mL of hydrogen peroxide solution was added, and the samples heated at 330°C for another 2 hours under a fuming hood until the material was clear. When the digestion was complete, the sample was cooled drown, and collected in to the 100mL volumetric flask. Distilled water was used to fill the volumetric flask to the mark (AOAC, 1996).

3.4.5 Determination of minerals in water

The 250mL water samples collected from troughs from at the site where it was being supplied to animals was filtered using a $0.45~\mu m$ filter paper then stored in sealed plastic bottles at room temperature awaiting chemical analysis.

3.4.6 Analytical procedure for minerals determination

Combinations of standards were prepared (Na and K), (Mg and Ca), (Mn, Fe, Zn and Cu) to calibrate their respective curves on an inductively coupled plasma atomic emission spectrometry (ICP-AES). Prepared samples were analyzed for their respective mineral elements using ICP-AES. To ensure quality a check sample was run at the beginning of the analyses. Phosphorus was analyzed using the UV spectrometry (Allen *et al.*, 1974). Samples were analyzed after a phosphorus calibration curve was made at wavelength 670nm.

3.4.7 Statistical analysis

A split-split plot design was used to analyse the data collected in the study. The main treatment was the location (Morale and Goodhope). The sub treatment was the seasons (dry season and wet season), and the sub-sub treatment were the sampling time (beginning of the season, mid-season and end of the season). Multiple comparison were made on least square means of sampling times for each study site using PDIFF option of the LS MEANS statement in SAS at (P-value of $\alpha = 0.05$.

3.4.7.1 Experimental model

 $Y_{ijk} = \mu + S.t_i + S_j + (S.t \times S)_{ij} + T_k + (S.t \times T)_{ik} + (S \times T)_{jk} + (S.t \times S \times T)_{ijk} + \mathcal{E}_{ijk}$ Where:

 Y_{ijk} = observation from site i, season j, and sampling time k

μ = overall mean

 $S.t_i = effect of i^{th} type of site.$

 $S_i = effect of j^{th}$ type of season.

 $(S.t \times S)_{ij}$ = interaction between site and season

 $T_k = effect of k^{th} sampling time$

 $(S.t \times T)_{ik}$ = interaction between site and sampling time

 $(S \times T)_{jk}$ = interaction between season and sampling time

 $(S.t \times S \times T)_{ijk}$ = interaction between site, season and sampling time

 E_{iik} = random error

3.5 RESULTS

3.5.1 Soil Mineral Concentration

Soil fertility was different at the two sites. Soil mineral concentrations at the two ranches are presented in Figures 3.1 and 3.2. Soil Mg, K, P, Mn, Cu, and Fe were respectively 50%, 93%, 170%, 169%, 161% and 41% higher (P<0.05) at Morale ranch compared with Goodhope ranch. Only soil concentrations of Ca and Na were similar at the two sites and Zn was about 6% higher at Goodhope compared with Morale. However, there was a seasonal effect (P = 0.01) on soil Ca concentration. Additionally, there were numerical increases in soil Mg (P = 0.08) and soil K (P = 0.07) during the dry season (Figure 3.3).

The soil macro-mineral concentrations for the two sites during the two seasons are presented in Table 3.3. There were season x site interactions in most of the soil macro-mineral concentration and changes in concentration from the dry to wet season at the two sites were 86% vs 56% for Ca, 5% vs 60% for Mg, -18% vs 13% for Na, 46% vs 51% for K, and 47% vs 77% for P for comparisons between Goodhope ranch and Morale ranch, respectively.

The soil micro-mineral concentrations for the two sites during the two seasons are presented in Table 3.4. There were season x site interactions in some of the soil micro-mineral concentration and changes in concentration from the dry to wet season at the two sites were 55% vs70% for Mn, 12% vs55% for Zn, 52% vs68% for Cu, and 35% vs26% for Fe for comparisons between Goodhope ranch and Morale ranch, respectively.

Table3.3 Soil macro-mineral concentrations (mg/kg) at Goodhope and Morale during the wet and dry season.

		Ca .	Mg		Na		K		Р	
Season	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
	981.3 ±88.65**	137.2±2.38 ^{by}	155.6±13.45	148.5±0.98y	9.0±1.57y	10.6±1.28y	438.1±31.36**	235.6±25.61by	16.3±0.84**	8.6±0.68hv
Morale	604.7±95.37**	268.6±87.29bz	166.8±14.47*	66.19±13.24bz	17.83±1.69 ^z	15.6±1.55²	297.7±33.74**	146.0±30.88bt	18.9±0.90**	4.4±0.83bz

^{*}Numbers in the same row for each mineral concentration with different letters are significantly different at P<0.05.

Table3.4 Soil micro-mineral concentrations (ppm) at Goodhope and Morale during the wet and dry season.

		Mn		Zn		Cu		Fe
Scason	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Goodhope	136.2±6.70*7	61.93±5.47 ^{by}	1.57±0.17*y	1.38±0.14 ^{By}	2.16±0.13*7	1.04±0.10 ^{by}	49.94±3.74**	32.73±3.05 ^{bs}
Morale	153.5±7.20*7	45.92±6.59 ^{by}	2.17±0.19 ^{ss}	0.98±0.17 ^{by}	3.17±0.14 ^{sz}	1.01±0.13 ^{by}	84.95±4.02**	63.14±3.68 ^k

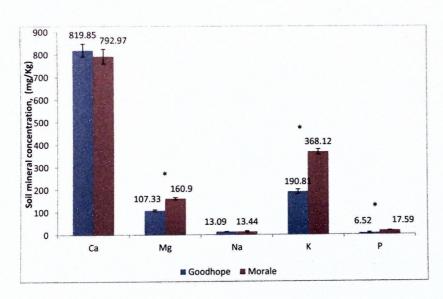
²⁴Numbers in the same column for each mineral concentration with different letters are significantly different at P<0.05

Values for minerals are got from (LSM \pm SE)

Numbers in the same row for each mineral concentration with different letters are significantly different at P<0.05.

Numbers in the same column for each mineral concentration with different letters are significantly different at P<0.05

Values for minerals are got from (LSM ± SE)



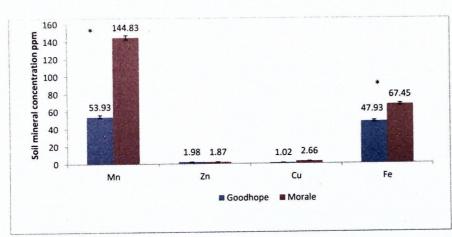


Figure 3.2 Concentration of micro-minerals of soil at Goodhope and Morale ranches (LSM + SE) Mineral bar graphs with an asterix above them are significantly different at P<0.05)

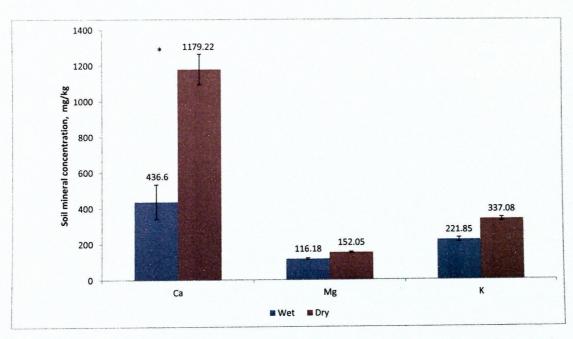


Figure 3.3 Soil concentration of Ca, Mg and K in samples collected from Goodhope and Morale ranches during the wet season (sampled from December to April), and the dry (sampled from June to October).

(Mineral bar graphs with an asterix above them are significantly different at P<0.05) There were numerical increases in soil Mg (P = 0.08) and soil K (P = 0.07) during the dry season.

3.5.2 Forage Mineral Concentration

Forage mineral content was different at the two sites. Forage mineral concentrations at the two ranches are presented in Figures 3.4 and 3.5. Forage Mg, K, P, and Fe concentrations were respectively 167%, 59%, 218%, and 10% higher (P<0.05) at Morale ranch compared with Goodhope ranch. Forage Ca and Na concentrations were similar at the two sites and averaged 0.26%, 0.01%, respectively; and forage Mn, Zn, and Cu were also similar at the two sites and averaged 65.82, 124.22 and 3.31 ppm.

Season did not affect on most forage minerals except for Mn, which was higher in forages harvested during the wet season compared to the dry season LS means + SE were 83.08 \pm 0.2 ppm vs 48.55 \pm 0.18 ppm for the dry and wet seasons, respectively).

The forage macro-mineral concentrations at the two sites during the two seasons are presented in Table 3.5. There were season x site interactions in most of the forage macro-mineral concentration. Wet season forage Ca and Na concentrations were 14% and > 1000% higher (P<0.05) than those of samples collected during the dry season at Goodhope ranch whereas dry season forage Ca and Na concentrations were higher (P<0.05) by 19% and 25%, respectively at Morale ranch. This trend was similar to that of the other forage macro-minerals, which were higher (P<0.05) for dry season forages compared to wet season forage samples; and the respective concentrations for Mg, K, and P were 43% and 63%, 33 and 42%, and 69 and 55%, for at Goodhope and Morale ranches, respectively.

Forage micro-mineral concentrations for the two sites during the two seasons are presented in Table 3.6. There were season x site interactions in some of the soil micro-mineral concentration and changes in concentration from the dry to wet season at the two sites were 55% vs 70% for Mn, 12% vs 55% for Zn, 52% vs 68% for Cu, and 35% vs 26% for Fe for comparisons between Goodhope ranch and Morale ranch, respectively.

Table 3.5 Forage macro-mineral concentrations (%) for pasture samples from Goodhope and Morale during the wet and dry season.

	Ca	, %	Mg, %		N	Na, %		K, %		P, %	
Season	Dry	Wet	Dry	Wet	Dry	Wct	Dry	Wet	Dry	Wet	
Goodhope	0.21±0.03**	0.24 ±0.03 ^{by}	0.07 ±0.01°	0.04 ±0.01°y	0.009 ± 0.0008 ^{ay}	0.1 ± 0.0007*9	0.91 ± 0.05"	0.61 ± 0.04 ^{by}	0.16 ± 0.005**	0.05 ± 0.004**	
Morale	0.21 ±0.05 ^{ay}	0.17 ±0.03**	0.08 ±0.013 st	0.03 ±0.01**	0.012 ± 0.0008**	0.009 ± 0.0008 ^{by}	0.38 ± 0.054	0.22± 0.05bz	0.08 ± 0.05**	0.036 ± 0.005 ^{kg}	

Values for minerals are got from (LSM ± SE)

Table 3.6 Forage micro-mineral concentrations (ppm) for pasture samples from Goodhope and Morale tested during the wet and dry season.

	!	Mn(ppm)		Zn(ppm)	C	Cu(ppm)		gm)
Season	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Goodhope	64.17±3.47**	32.94±2.83by	113.20±9.22*7	32.51±6.29 ^{by}	2.97±0.54**	3.67±0.10 ^{b7}	305.98±32.39**	352.89±36.22 ^{by}
Morale	72.0±3.47**	94.16±3.47 ²²	139.4 ± 9.22	312.70 ±9.22**	2.43 ±0.54*2	4.60±0.54**	346.11± 32.39**	197.53±32.39 ⁵⁴

^{*}Numbers in the same row for each mineral concentration with different letters are significantly different at P<0.05.

"Numbers in the same column for each mineral concentration with different letters are significantly different at P<0.05.

Values for minerals are got from (LSM ± SE)

^{*}Numbers in the same row for each mineral concentration with different letters are significantly different at P<0.05
"Numbers in the same column for each mineral concentration with different letters are significantly different at P<0.05

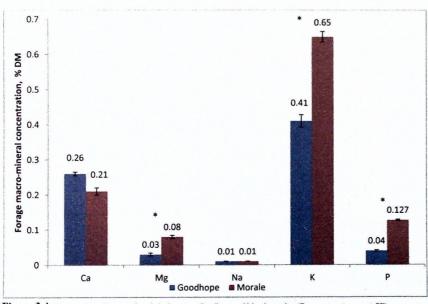


Figure 3.4 Concentration of macro-minerals in forages at Goodhope and Morale ranches (Bars represent means + SE)
Mineral bar graphs with an asterix above them are significantly different at P<0.05

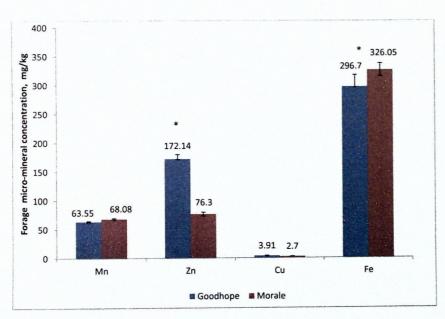


Figure 3.5 Concentration of micro-minerals in the forages at Goodhope and Morale ranches (Bars represent means + SE) Mineral bar graphs with an asterix above them are significantly different at P<0.05)

3.5.3 Water Mineral Concentration

Water mineral concentrations are presented in table 3.7. Water concentrations of Ca, Mg, Na and K were similar for both sites during the wet season but water Na was higher (P<0.05) at Goodhope compared to Morale, and K concentration was higher (P<0.05) at Morale compared with Goodhope. Because of these differences, water Na concentrations remained higher (P<0.05) at Goodhope than at Morale, whilst water K concentration remained higher (P<0.05) at Morale compared with Goodhope when averaged across season at each site, or average across sites within each season.

Table 3.7 Mineral concentrations (mg/l) for water samples from Goodhope and Morale tested during the wet and dry season.

Site	Season	Ca	Mg	Na	K
Goodhope	Wet	42.26 ± 18.97	27.32 ± 1.12	84.41 ± 4.76	18.72 ± 1.68
Morale	Wet	52.78 ± 18.97	27.47 ± 1.12	74.72 ± 4.76	21.81 ± 1.68
Goodhope	Dry	8.43 ± 2.41	29.15 ± 1.12	107.28 ± 4.76*	13.28 ± 1.68
Morale	Dry	55.78 ± 18.97	28.22 ± 1.12	73.32 ± 4.76^{b}	21.58 ± 1.68*
Goodhope	Average	25.44 ± 10.93	28.24 ± 0.30	95.84 ± 5.55°	16.0 ± 1.49
Morale	Average	54.28 ± 10.93	27.84 ± 0.30	74.02 ± 5.55 ^b	21.69 ± 1.49*
Average across sites	Wet	47.52 ± 9.76	27.40 ± 0.70	79.65 ± 2.41 ^b	20.26 ± 1.26°
Average across sites	Dry	32.20 ± 9.76	28.68 ± 0.70	90.30 ± 2.41°	17.43 ± 1.26 ^b

^{*}Numbers in the same part of a column for each mineral concentration that are followed by different letters are significantly different at P<0.05 Values for minerals are got from (LSM ± SE)

3.6 DISCUSSION

3.6.1 Calcium content of soil, forage and water samples from the study sites

The concentration of Ca in the soil in both locations was higher than the critical levels proposed by Adams and Hartzog (1980) of 250mg/kg. Higher levels of Ca in the soil may increase forage Ca, but no crop yield response would be expected for soil Ca above 250 mg/kg (Adams and Hartzog, 1980). Ca was mostly higher (P<0.05) during the dry season figure 3.3. The mineral was low during wet season because as moisture increases the level of minerals goes down as influence by several factors including leaching. When minerals leach they tend to go deeper into the ground, and sometimes they may also go far from the grass roots thereby being unavailable. These values of soil Ca were higher than those reported by Tiffany et al. (2000) in North Florida averaged at 430mg/kg and lower than those reported by Tejada et al. (1987) in Guatemala and Ogebe et al. (1995) in North Florida at 1160mg/kg. The concentrations are almost similar to those reported by Prabowo et al. (1991); Khan, (2003); Ignacio et al. (2008). The soil content of an element is one of the most important limitations for plant growth; however availability factors including soil pH, texture, moisture content and organic matter are probably more often the limiting factors rather than mineral content. With increasing acidity of soils there is impaired absorption of Ca (McDowell and Valle 2000).

The investigated pastures had concentration of Ca in forage that was lower than the critical level of 0.35%, (NRC,1996) for both locations. The issue of mineral adequacy remains debatable because the requirement is influenced by animal type and level of production, age, and weight. Calcium requirement for growing and finishing animals expected to grow at 0.89 kg d⁻¹ is 0.35% DM (Khan *et al.*, 2004a). Since the concentration of Ca in the soil was adequate for forage or plant growth, the concentration of Ca in forage had a negative relationship with the concentration of Ca in the soil. The poor uptake of Ca by forage showed that the soil Ca was unavailable to the forage. Unavailability of Ca could be attributed to among other factors minerals binding to each other hence becoming unavailable.

Most range in the semi arid regions is found to be deficient in Ca hence livestock is always supplemented with di-calcium phosphate (lundu, 2012). Forage samples from Goodhope ranch in

the wet season had Ca concentrations that were similar to Ca concentrations reported by Espinosa et al. (1991); Cuesta et al. (1993); Ogebe et al. (1995); Gizachew et al. (2009) and Khan et al. (2004b) at 0.30%. The concentration of Ca in forage sample from Goodhope ranch in dry season and for Morale ranch in both season were similar to the one found by Ignacio et al. (2008); Khan et al. (2004b) at 0.20%.

Critical value of Ca requirements in blood for sheep is 105 mg/l (Khan *et al.*, (2003). The concentration of Ca in water together with that in forage would not be enough to meet the critical nutritional value of 0.35% NRC (1996). High concentrations may indicate hardness, but no negative animal impacts have been reported in the scientific literature. Naturally salt concentration of drinking water decreases mineral supplementation intake. Calcium deficiency is rare in grazing animals with the exception of lactating animals, or those animals grazing on acid, sandy or organic soil in humid areas where herbage consist of mainly of quick growing grass devoid of legume species (Underwood 1981).

3.6.2 Magnesium content of soil, forage and water samples from the study sites

The concentrations of Mg in the soil for both locations were higher than the critical value of 30 mg/kg suggested by Hanlon *et al.* (1990); Khan *et al.* (2004a); Khan *et al.* (2008a). Soil Mg concentration of 30 mg/kg is considered adequate in Mg for forage growth requirement. The concentration of soil Mg at Morale ranch was higher (P<0.05) than that of Goodhope ranch figure 3.1, these difference was caused by difference in the parent rock Mg concentration between the two sites. Mg may be deficient in acidic soils becoming more available with reduced soil acidity (Landon 1984). However at very high pH the minerals become less available in soils, hence further studies should be conducted on the soil ph so as to relate ph to availability of Mg. The concentration of Mg in the soil was lower than that reported by Khan *et al.* (2008b) and Khan *et al.* (2006) at 240mg/kg but higher than that reported by Khan *et al.*, (2004a) and Fordous *et al.*, (2010) in winter averaged 38.5g/kg.

Forage samples from both study sites were far below the critical value of 0.20% (McDowell et al., 1984). It was also observed that forage from Morale ranch had Mg that was higher (P<0.05) figure 3.4 than those at Goodhope ranch. This was an indication that Mg was absorbed by

forages proportional to its concentration in the soil. Forage samples from Goodhope ranch had Mg concentration lower than those found by Khan et al. (2008a) at 0.18. In Morale ranch concentrations of Mg in the forage were almost similar to those found by Khan et al. (2005c) at 0.10%. The concentration of Mg in forage samples from Goodhope ranch was almost similar to those recorded by Khan et al. (2009) in a study conducted in Punjab Pakistan. A high dietary level of protein, Ca, and P increases the requirements of Mg due to their depression of Mg absorption in ruminants. Mg availability is improved with increasing the maturity of grasses (Wilkinson and Staedman, 1979; Khan, 2003).the concentration of Mg which was adequate for forage requirements was not absorbed by the sampled forages, these may be as a results of soil-plant minerals interactions which might have inhibited the uptake of Mg by forage.

The concentration of Mg in water concentration and that in forage would not be able to reach the critical value of Mg for sheep (0.20%) (McDowell et al., 1984). If the Mg concentration are found to be high it would only indicate hardness, but no negative animal impacts have been reported in the scientific literature. It is uncertain if all Mg in water is available. High dietary levels of K and Nitrogen (N) will inhibit Mg absorption. Calcium and soluble carbohydrates may respectively increase and decrease dietary Mg requirements of livestock (Khan, 2003).

3.6.3 Sodium content of soil, forage and water samples from the study sites

The mineral concentration of soil and plants differs from species to species and varies from place to place in the soil and pasture Tolsma et al., (1987); Ben-Shahar and Coe, (1992); Fardous et al. (2010) and they are subjected to seasonal variation (Khan et al., 2006). Sodium deficiency in ruminants is documented in the semi-arid regions and can be overcome by regular supplementation throughout the year (Khan et al., 2005c and 2006). The concentrations of Na in the soil were severely lower than the critical value of 62mg/kg (Rhue and Kidder 1983; Khan et al., 2004a) in both locations. The concentration of Na in the soil during the wet season was severely lower than that of the dry season. To increase the fertility of soil in the studied site Na containing fertilizers should be used in this area for soil amendment.

The concentrations of Na in forage from both sites were deficient from the critical values of 0.08% as proposed by McDowell *et al.* (1984) and Anon, (1984). The deficiency in the concentration of Na is in agreement with McDowell *et al.* (1993) that one of the most prevalent mineral deficiencies for grazing animals in the world is Na. In addition deficiency of this element has been reported in many developing countries, e.g. Nigeria (Ogebe *et al.*, 1995), Colombia (Pastrana *et al.*, 1991) and in Pakistan (Khan *et al.*, 2005b, 2007a). Natural forages low in Na has been reported in numerous tropical countries throughout the world (McDowell, 1985a). The content of Na in the ration is important in determining the adequacy of the minerals. To meet the need of highly productive animals, forage should contain more than 0.15% DM sodium (Anon., 1973). Sodium deficiency is more likely to occur in animal grazing tropical pasture species and these plants generally accumulate less Na than temperate species (Morris *et al.*, 1980; Khan *et al.*, 2004a). The deficiency of Na in forage is well in accordance with the concentration of Na in soil which had shown to be inadequate for plant growth.

The concentration of Na in water together with that of forage would be adequate to satisfy the critical value of 0.08% as stated by McDowell (1984) hence supplementation of Na would only be necessary in times when forage Na concentration goes down especially during dry season. Since water which was used to water sheep was underground water, high water Na was an indication of the high concentration of Na in the deep of the parent rock were the water was being drawn from. Care should be considered when supplementing to avoid over engaging. The exposure to salt in drinking water has a more dramatic effect on the reproductive success of ewes than ingestion of salt in the diet. A salt concentration of 2% or greater ingested from drinking salty water alone is associated with a severe reduction in food intake and possibly death (Peirce, 1968; Potter, 1963 and 1968; Wilson, 1966; Wilson and Dudzinski, 1973; Hamilton and Webster, 1987). Feed intake is depressed when animals consume around 60 g of salt per day, and as salt concentrations increase further, the intake of feed further decreases (Masters et al., 2005; Blache et al., 2007).

3.6.4 Potassium content of soil, forage and water samples from the study sites

Potassium plays a vital role in a variety of processes in plants (Johnston et al., 2001). The concentrations of K in the soil for both sites were above the critical level of 60mg/kg proposed by (Hanlon et al., 1990). Soil samples from Morale ranch had K concentration that was higher (P<0.05) than those at Goodhope ranch. Higher concentration of K in Morale showed the difference in parent rock between the two sites. Soil samples from Goodhope ranch had K concentration that was similar to those found by Khan et al. (2010) at 140mg/kg. These values were also higher than the values reported by Merkel et al. (1990) in north central Florida, Espinoza et al. (1991) in central Florida averaged at100mg/kg. Soil samples from Morale ranch had concentration of K in the soil that was evidently higher than those from Goodhope ranch hence higher than the critical value proposed by (Hanlon et al., 1990). This soil K concentration is adequate for plant growth requirements of 60 mg/kg (Hanlon et al., 1990). A semi-arid climatic condition like that of the present study certainly influences the mineral composition of soils and forages as well as the animal's metabolism. Therefore, the results presented here can be used with some caution, particularly with reference to hot semi-arid conditions.

In both sites the concentrations of K in forage were below the critical value of 0,80% that was suggested by McDowell *et al.* (1984). Forage K from Morale ranch was higher than those at Goodhope ranch just as it was in soils. The level of K absorbed by forage was proportional to K in the soils for both sites. The soils from which these forage grew had adequate K concentration for plant growth in both sites. This was an indication that several soil-plant factors have played a part in denying the uptake of the mineral by forage from the soil. The concentration of K in forage was marginally deficient from the critical values proposed by NRC (1996) of 0.60%. In dry season concentrations of K in forage for both sites were extremely below the critical value. The concentration of K in forage sample from Goodhope ranch during wet season was marginally deficient.

Gizachew et al. (2002) in the upland native pasture of Ethiopia reported concentration of K in forage similar to the one found at Goodhope ranch. The concentration of K in forage in the dry season for both sites were similar to those found by Gizachew et al. (2002) in the bottom land native pasture in Ethiopia at 0.31%. The concentration of K in forage in the present study is

consistent to McDowell (1992); Tiffany et al. (2000) and (2001) whose finding suggested that plant age generally has greater influence on forage mineral concentration than the soil K concentration. In dry season forage gets older and loses moisture hence low concentration of K in forage at that particular time. Such changes often reflect increase in the proportion of stem to leaf ratio and old to new leaves, with stems and old leaves having lower mineral concentrations than young leaves (Minson, 1990).

Water together with forage would not meet the critical value of 0.8 % (McDowell, 1984) required by sheep, since water provide a very insignificant amount of K concentration. It is therefore recommended that sheep be supplemented with feed higher in Potassium.

3.6.5 Phosphorus content of soil and forage samples from the study sites

The greatest environmental concern with many grazing areas is the level of soil P due to P accumulation in soil, and the subsequent loss of sediment-bound and soluble P in runoff (Sigua and Tweedale, 2003). The ability to estimate the levels and changes of soil P and other crop nutrients in subtropical beef cattle and sheep pastures has the potential to improve the understanding of P dynamics and nutrient cycling in the soil system (Yerokun, 2008). Grazing animals affect the movement and utilization of nutrients through the soil and plant system, and thus the fertility of pasture soils (Haynes and Williams, 1993). In the present study the concentration of P in soils from Goodhope ranch was below the critical value of 17mg/kg (Prabowa et al., 1991) while that from Morale ranch was marginally deficient. The concentration of P in soils from Morale ranch were higher (P<0.05) than those at Goodhope ranch, these is an indication of difference in the parent rocks of these sites. Soils from both sites though different could not satisfy forage P requirement. Soil samples from Goodhope ranch had concentrations of P in soil that were almost similar to those reported by Prabowa et al. (1991) in the western region of South Salawes Indonesia at 9mg/kg. The concentration of P in soil samples from Morale ranch were almost similar to those found by Prabowa et al. (1991) in the central region of south Salawes Indonesi at 20mg/kg. Both sites soil samples had P concentrations that were lower than the concentration reported by Areghore et al.(2007).

Phosphorus deficiency is the most widespread and economically important mineral deficiency of grazing livestock. This is attributed to the fact that most Southern Africa soils are reported to be deficient in Phosphorus (Yerokun, 2008). The presence of high amounts of oxides and the rate of soil weathering due to high temperatures and moisture in part explain the low availability of P in Botswana soils (Yerokun, 2008). Low soil moisture is said to reduce P uptake by plants (Jones, 1998). Phosphorus is also affected by soil pH. At pH values lower than 5.5, phosphate ions combine with iron and aluminum to form compounds which are not readily available to plants (Landon, 1984). The situation changes at pH value greater than 8.0. In the presence of Calcium, Phosphate tends to be converted to Calcium phosphate, and availability of P to plants is reduced (Lundu et al., 2012).

Forage samples from both sites had P concentrations that were below the critical value 0.25% proposed by McDowell (1997). The concentration of forage P was higher (P<0.05) at Morale ranch than at Goodhope ranch. This is consistent to the findings of soil P which was also higher at Morale ranch. Forage samples from Morale ranch had P concentrations that were almost similar to the concentration found by Bhat et al. (2011) and Chelliah et al. (2008) at 0.05%. Forage sampled from Goodhope ranch had P concentration that was almost similar to the concentration found by Prabowa et al. (1991) at 0.09%. The concentration P in forage samples from both site were less than the concentrations found by Gizachew et al. (2002) except for forage sample from Goodhope ranch in wet season. Studies done in Ethiopia and Zambia have shown that most grasses were marginal to deficient in P content (Lundu et al., 2012). Hence it is not surprising for Botswana forage to be deficient in forage P concentration since places ecologically similar to it had experienced similar deficiency. There was a consistent relationship between the soils and forage, as the low levels of P in the soils were reflected in the forage as well.

3.6.6 Manganese content of soil, forage and water samples from the study sites

In the present study concentration of Mn in soil sample from both sites was well above the critical value of 19ppm that was suggested by (McDowell et al., 1984) and critical level of 5 mg/kg as established by Viets and Lindsey (1973). Difference between the parent rock for two

sites was observed as the soil Mn at Morale was higher than those at Goodhope. Both sites had concentration of Mn in the soil that was higher than the concentration reported by Khan et al. (2007b). Soil samples from Morale ranch had Mn concentration that was in agreement to the concentration recorded by Ahmad et al. (2010) at 64ppm. These values were sufficiently higher than the requirements of forage plants. These findings are in agreement with that of Aregheore et al. (2007) and lower than the values reported by Miles et al. (2001) at 93ppm. Soil samples from Goodhope ranch had concentration of Mn higher than the values reported by Espanoza et al. (1982). Manganese in the soil is known for its rapid oxidizing and reduction under variable soil environment. Oxidizing conditions may reduce Mn availability and reducing conditions may increase its availability (Khan, 2003). It had been reported that soil pH and organic matter may cause increased solubility of Mn for plants (Espinoza et al., 1991; Pastrana, 1991), which might have been the major factors for maintaining the levels of Mn in both sites.

The minimum dietary Mn requirements of ruminants are not precisely known but ranges between 15-25 mg/kg (NRC, 1980). Manganese is poorly absorbed (1% or less) from ruminant diets. Manganese requirements are substantially lower for growth than for reproduction performance and these are increased by high intake of Ca and P (Khan, 2003). Manganese has received little attention, probably because manganese deficiency is not considered to be a major problem in ruminants. Limited evidence suggests that high dietary Calcium and Phosphorus may reduce Manganese bioavailability (Spears, 2003)

The present study has shown that concentrations of Mn in forage from both sites were well above the critical value (20ppm) McDowell (1997). The forage levels of Mn in wet season were lower than those in dry season in both sites though adequate. The concentrations of Mn in forage for both sites were lower than the concentration found by Areghore *et al.* (2007) at 105ppm and above those found by Khan *et al.* (2004b); Ahmad *et al.* (2010) at 10ppm. Studies conducted by Khan (2003); Mathis and Sawyer (2004) had consistent concentration of Mn in forage as those found in the present study. The concentration of Mn in forage had shown consistent relationship with the concentration of Mn in the soil. Manganese requirements were substantially lower for growth than for optimal reproductive performance (Mcdowell and Arthington, 2005). In this

study Mn levels were found to be sufficient for all requirements of different categories of livestock, which precludes a need for supplementation.

3.6.7 Zinc content of soil and forage samples from the study sites

Most soils contain sufficient amounts of Zinc (Zn) to support growth of many forage species. Still, Zn deficiency is a constraint to forage production in one third of the world's agricultural soils (Khan *et al.*, 2005b). Present study shows that across the two sites concentrations of Zn in the soil were almost similar to each other. These soil Zn concentration were well above critical level of 0.5 mg/kg which was stipulated by Sanchez. (1976); Khan *et al.*, (2004b). Soil texture has been reported to have a significant effect on Zn distribution in soils, particularly in semi-arid region, with heavier textured soils having more zinc than the lighter textured sandy soils (Khan *et al.*, 2004a, 2005b, 2006, 2007a and 2007b). Soil texture should be investigated in feature, so as to determine if it relates to Zn concentration. This soil Zn values were almost similar to values reported by Areghoere *et al.*, (2007) in Apia Samoa Brazil averaged at 110ppm.

Based on dietary requirements of 30 ppm which was suggested by McDowell *et al.* (1982) the concentration of Zn in forages for both sites in this study was adequate for sheep requirement. The Zn content in these forages could be sufficient for recommended requirement for sheep, however efficiency of Zn utilization of these forages would depend on zinc bioavailability, and its interaction with other mineral elements. Plant maturity has also been reported to affect Zn concentration of forage and it also depends upon the tissue type of plants (Underwood, 1981; Kabata-Pendias and Pendias, 1992; Church and Pond, 1988).

3.6.8 Copper content of soil, forage and water samples from the study sites

Copper is essential for the normal healthy growth and reproduction of all higher plants and animals. The forage and animal losses caused by shortages of Copper can, at one extreme, be total, for example lambs can die of sway back and forage on newly reclaimed peats or sandy heath lands can fail completely; but fortunately, such occurrences are fairly rare. Pasture growth requires adequate soil copper; however excessive iron, molybdenum and sulphur levels will reduce the amount of Cu available to ruminants. Across the two sites the concentrations of Cu in the soil were all above the critical level of 0.3 ppm (Rojas et al., 1993) for normal plant growth.

Soil samples from Goodhope ranch had Cu concentrations that were almost similar to values reported by Areghoere et al. (2007) valued at 1.1ppm in Samoa and lower than those reported by Ndebele et al. (2005); Khan et al. (2007c). The concentrations of Cu in the soil samples from Morale ranch were almost similar to the values reported by Tejada et al. (1985); Khan et al.(2007b) and lower than those reported by Tiffany et al. (2001) at 2.5ppm Consistent to Kabata-Pendias and Pendias (1992) report that Cu binding capacity of any soil and Cu solubility are highly dependent on the amount and kind of organic matter. In this study the Cu levels were above critical concentrations. These indicate the presence of higher organic matter in the soil.

Copper requirements vary greatly in ruminants depending on concentrations of other dietary components, especially sulphur and molybdenum (Khan, 2003). All forage samples from both locations had Cu concentrations that were below the critical value of 8 ppm (McDowell et al., 1984; Rojas et al., 1993). Evaluation of copper in the forage or other diet for ruminants has a limited diagnostic value and can in fact be misleading. Other elements with which Cu interacts, particularly molybdenum and sulphur, should be determined, for assessing the ruminant requirements in pastures (McDowell et al., 1983; Ndebele et al., 2005; Khan et al., 2005c). The concentrations of Cu in forage were found to be low in pastures to meet the demand of animals in both sites during both seasons. Soils from which forage grew from had Cu that was adequate in Cu, since these Cu was not up taken by forage, it's a sign of other minerals interfering with the uptake of Cu.

The concentration of Cu in forages samples from Goodhope ranch were almost similar to values reported by Khan et al. (2007c) at 4.1 ppm while forage samples from Morale ranch had Cu concentration that was consistent with those reported by Ignacio et al.(2008). Both sites had concentrations of Cu in forage that were lower than those reported by, Tejada et al. (1985; 1987) at 18.4ppm in Guatemala Khan et al. (2006, 2007b) in Pakistan and Kuria et al. (2004) in the western Masabat Kenya. The concentrations of Cu in the soil samples were inconsistent with the forage Cu concentration as the Cu found in the soil did not translate into forage Cu.

3.6.9 Iron content of soil and forage samples from the study sites

The concentrations Fe in the soil were well above the critical value of 2.5 ppm (Viet and Lindsay, 1973) for both locations. Soils from Morale ranch were higher in Fe concentration than those at Goodhope ranch. The difference is an indication of parent rock difference as it has been with the other minerals. These values were consistent with those found by (Moosa, 1982) and (Merkel, 1990). McDowell *et al.* (1984) reported that soil Fe concentration were rarely deficient. However Beker *et al.* (1965) reported soil Fe deficiency. Iron availability to plants is also highly dependent on soil pH; as the soil pH decreases the availability of Fe to plant increases (Kabata-Pendias and Pendias 1992). Iron in its reduced form (Fe2+) is available to plants, and if the soil pH is acidic, more Fe3+ is reduced to Fe2+, thus, more availability to plants.

All mean forage values for Fe were above the recommended value of 50 ppm (Jones, 1972). It was observed that there was a positive correlation between the soil and forage as minerals were absorbed proportionally to their concentrations in the soil. The absorption of the Fe from soil to the forage shows that Fe was not inhibited in the soil. Similar concentration Fe in forage was recorded by Cuesta *et al.* (1993) and (Velasquez-Pereira et al 1997). Previously relatively lower concentrations of Fe in forage than those of the present study had been reported by McDowell *et al.* (1982); Prabowo *et al.* (1991) at 90ppm in Indonesia and by Orden *et al.* (1999) in the Philippines. The concentrations of Fe in forage were extremely higher than the critical value for the grazing animals which may cause toxicity to the animals. High concentration of iron in the forage coincides with zero incident of Fe deficiency found in soils.

(1995年) [1995] [1995

3.7 CONCLUSION

The findings of this study showed that there was a significant (P<0.05) difference between the soil sampled from Goodhope and Morale ranches in their content of macro minerals during wet and dry season. Soils sampled from Morale ranch were higher (P<0.05) in mineral content than those sampled from Goodhope ranch. A similar trend was observed in the content of forage minerals. There was a significant (P<0.05) difference between the forage samples from Goodhope and Morale ranches during the wet and dry season on the content of macro and micro minerals. In water only Na was influenced (P<0.05) by interaction of site and season while Ca, Mg and K were not. The concentration of Ca, Mg, K, Mn, Zn, Cu and Fe in the soil for both locations was found to be within forage requirement while Na and P concentrations were below the forage requirements. The concentration of K, Mn, Zn and Fe in forage was within the nutritional requirement for sheep while Ca, Mg, Na, P, Cu were below the necessary requirement. Water Mg, Na and K concentration together with that of forages would be adequate for animal requirement.

3.8 RECOMMENDATIONS

Mineral intake by animals depends more on the type of plant and level of consumption than on the parent rock from which the soil was derived and on which forage grows on. Since the concentration of Na and P in soil was deficient there is need for direct methods to counter this condition such as soil fertility amendment. It is recommended that animals that graze in these areas where Ca, Na, Mg and P are deficient should be provided with mineral licks. A single mineral supplementation is not necessarily the solution to a particular mineral deficiency because there are several complexities associated with minerals supplementation. It is therefore suggested that supplementation studies be conducted to determine the need and economic benefits of mineral supplementation for ruminants. Minerals requirement depends on the production status, this includes age, breed of animal and state of current production and the intended purpose an animal is fed. Further studies needs to be done to clarify the phenomena like interactions, digestibility and bio availability of minerals, to eliminate unnecessary supplementation of minerals that might have been attached to each other. To formulate free choice mineral

...

supplement for various soil types and ecological regions, information on mineral supplement consumption is required.

CHAPTER 4

EFFECT OF SEASONAL VARIATION ON THE MINERALS STATUS OF TSWANA SHEEP GRAZING NATURAL PASTURES AT MORALE AND GOODHOPE RANCHES IN BOTSWANA

ABSTRACT

This study was conducted at Goodhope and Morale ranches to determine the effect of site, age, season and sampling interval on the mineral (Ca, Mg, K, Na, Mn, Zn, Cu and Fe) status of grazing Tswana sheep. Twenty five yearling sheep and 25 adult sheep from each location were randomly selected. Blood samples were collected from each sheep every sampling interval for mineral analysis. There was a significant difference (P<0.05) on the concentration of Ca, Na, K, Mn, Zn and Cu for yearling sheep at Goodhope ranch between wet and dry season. At Morale ranch only K, Mn, Zn and Cu showed a noteworthy difference (P<0.05) on yearling sheep between wet and dry season. During the wet season there was a significant difference (P<0.05) in Na and K between the yearlings at Morale ranch and Goodhope ranch. In the dry season the only difference observed between the yearling at Morale and Goodhope ranch was on Ca, Na and Cu. In Goodhope ranch Ca, Na, K, Mn, Zn and Cu minerals concentration for adult sheep were significantly different (P<0.05) between wet and dry season, while at Morale ranch the difference (P<0.05) was observed on Mn, Cu and Fe. During the dry season adult sheep from Morale and Goodhope ranch had Ca, Na and K that were noteworthy (P<0.05), while during the wet season the only difference (P<0.05) was observed on the concentration of K. Blood Ca and Mg were not within the adult and yearling sheep requirement of (80mg/l and 20 mg/l respectively) hence supplementation necessary. The blood K, Na, Mn, Zn, Fe and Cu were within the yearling and adult sheep blood requirement (200 mg/l, 3200mg/l, 0.015 to 0.05 mg/l, 0.60ppm, 1 to 2mg/l and 0.65mg/l respectively) in both study sites.

4.1 INTRODUCTION

Sheep are economically important livestock to the ordinary people in Botswana. The feeding of these animals mostly depends largely on available grasses for their minerals requirements. The quantitative control of mineral elements in animals depends directly on their concentrations in various environmental factors such as soil, water and plants (Khan *et al.*, 2009). Animal tissue or fluid mineral concentrations reflect better availability of minerals than the forage mineral analyses. Furthermore, blood is considered as the most important bio-substrate for the estimation of mineral status of an animal (Khan *et al.*, 2006).

There is wide spread deficiencies of macro and micro nutrients elements in grazing ruminants in the tropics including Botswana because grazing ruminants depend largely upon forage to fulfill their requirements (Aregheore et al., 2007). The presence of minerals in higher concentration in a diet does not ensure full availability to the animals. Khan et al. (2008a) concluded that this happens due to various dietary interactions in the metabolic processes. The levels of minerals in blood and other body fluids also depend on various factors such as physiology of the guts, metabolic usage, homeostasis and excretion.

Mineral blood concentration for naturally grazing sheep may be altered by fodder species, stage of harvesting, and season of the year. The mineral contents of plants may vary widely among plant species and different places and these may be pronounced seasonal changes (Khan, 2003; Khan et al., 2004a). Thus feeding places and seasons may affect the mineral status. In Botswana the extent of mineral deficiency and borderline deficiency is not known in most of the livestock farms. Under such situation it is difficult to suggest some preventive measures to overcome the deficiencies or excess of mineral nutrient. Knowledge of mineral concentration in the body fluid or blood is usually used as a diagnostic tool for assessing a variety of disorders.

4.2 OBJECTIVES

The main objective of this study was to determine the effect of season (wet and dry seasons) and sampling periods on the mineral status of grazing Tswana sheep at Morale and Goodhope ranches.

4.2.1 Specific objectives

To determine the macro (Ca, Mg, Na, and K) and micro (Mn, Zn, Cu and Fe) minerals content in the blood of Tswana sheep during the dry and wet season at Morale and Goodhope ranches.

To compare the concentrations of the macro (Ca, Mg, Na, and K) and micro (Mn, Zn, Cu and Fe) minerals of sheep in the respective ranches with the normal concentration in healthy sheep.

4.3 HYPOTHESIS

- Ho: The concentration of minerals in the blood of sheep is not influenced by interaction of site, season and age.
- H_A The concentration of minerals in the blood of sheep is influenced by interaction of site, season and age.

4.4 MATERIALS AND METHODS

4.4.1 Site description

This study was conducted during 2010-2011 using the Tswana breed of sheep, grazing on two different ranches. Morale ranch, which is in the central district of Botswana of coordinates 23" 06° 32, 30" south and 26° 49, 33 26" east and Goodhope ranch located in the southern district of Botswana coordinates 25° 06° 32, 30 South and 26° 49°33, 26 East. (Google earth 2010) as described on page 18 of this thesis. The animals were allowed to graze freely on enclosed paddocks. Animals were vaccinated for Pulpy kidney and Pasteurella, thereafter they were dewormed and dipped. Drinking water was readily available on the drinking troughs.

In each ranch fifty female animals were sampled, these animals were divided into two groups of yearlings (11 to 18 months) and adults (above 24 months) which comprised of 25 animals each. Each animal had an identification tag. Blood samples were collected once every two months during the dry and wet season. December, February and April represented the wet season while June, August and October represented the dry season.

4.4.2 Blood sample collection

Approximately 4 ml of blood was collected from each sheep sampled Disposable blood needles for sheep were used to collect the blood through a jugular vein puncture. Sheep was held down. The jugular vein was raised by pressing the posterior side of the neck with the thumb. The needle was then inserted into the vein. Blood was then drawn into the clean sterile anticoagulant free test tube. Coagulant was removed in order to remain with the serum which was frozen at -20° C until analysis (Fick *et al.*, 1979).

4.4.3 Blood sample preparation

Blood serum (1.5ml) was digested with 8ml of Nitric acid (65%) and 2ml of hydrogen peroxide (30%). After digestion the volume was made up to 100ml with distilled water into a 100ml volumetric flask. The digested samples were kept at room temperature in the caped volumetric flask until mineral analysis (Kamada *et al.*, 2000).

4.4.4 Analytical procedure for determination of minerals

Combinations of standards were prepared (Na and K), (Mg and Ca), (Mn, Fe, Zn and Cu) to calibrate their respective curves on a inductively coupled plasma atomic emission spectrometry (ICP-AES). Prepared samples were analyzed for their respective elements using ICP-AES. To ensure quality, a check sample was run every time at the beginning of the analyses.

4.4.5 Statistical analysis

A split-split design was used to analyze the data collected in the study. The whole plot was the site (Morale and Goodhope ranches). The sub-plot was the seasons (dry season and wet season), and the sub-sub-plots were the sampling time (beginning of the season, mid-season and end of the season). Means were separated using the P-diff means separation method.

4.4.5.1 Experimental model

 $Y_{ijkl} = \mu + S.t_i + A_j + (S.t*A)_{ij} + S_k + (S*S.t)_{ik} + (S*A)_{jk} + (S.t*A*S)_{ijk} + T_E + T_{l(ijk)} + E_{ijkl}$

Where:

Y_{nkl} = Mineral observation from sheep as influenced by site, age, season, and sampling time

 μ = overall mean

 $S.t_i = effect of i^{th} type of site.$

 A_1 = effect of j^{th} age group

 $(S.t \times A)_{ij}$ = interaction between site and age

 S_k = effect of k^{th} season type

 $(S.t \times S)_{ik}$ = interaction between site and season

 $(S \times A)_{jk}$ = interaction between season and age

 $(S.t \times S \times A)_{ijk}$ = interaction between site, season and age

T₁-effect of lth sampling time

 $T_{I(\eta k)}$ - sampling time nested in site, age and season.

 \mathcal{E}_{ijkl} = random error

4.5 RESULTS

4.5.1 Sheep minerals concentration

The concentration of minerals in the blood samples of Tswana sheep was not different in most of the minerals (Table 4.1) except for Ca, Fe and Na. Seasonal effect was observed only on Na. Zn and Cu. Additionally there was a numerical decrease of sheep Fe (P=0.08) from wet season to dry season (Table 4.2)

4.5.1.1 Yearlings sheep minerals concentration

The yearling sheep macro and micro minerals are presented on table 4.3 and table 4.4 respectively for the two sites during wet and dry season. There were some interaction on site x season in most of the yearlings macro and micro minerals concentrations table 4.3 and 4.4 respectively. There was a significant difference (P<0.05) on the concentration of Ca, Na, K, Mn, Zn and Cu for yearling sheep at Goodhope ranch between wet and dry season. At Morale ranch only K, Mn, Zn and Cu showed a noteworthy difference (P<0.05) on yearling sheep between wet and dry season. During the wet season there was a significant difference (P<0.05) in Na and K between the yearlings at Morale ranch and Goodhope ranch. In the dry season the only difference observed between the yearling at Morale and Goodhope ranch was on Ca, Na and Cu. The changes in mineral concentration from wet to dry season at the two sites were 5% vs 12% for Ca,1% vs 7% for Mg,7%vs 41% for Na,155% vs 9% for K, 40% vs 16% for Mn, 93% vs 46% for Fe, 9% vs 56% Cu and 4% vs 13% between Goodhope and Morale ranch respectively.

Table 4.1 Concentration (mg/l) of Ca, Na, and Fe in sheep blood sampled at Goodhope and Morale ranches

Site	Ca	Na	Fe
Goodhope	115.09±3.11 ^y	7276.84±443.94 ^y	1.62±0.20 ^y
Morale	102.95±2.69 ^z	5376.70±443.20 ^z	2.39±0.20 ^z

[&]quot;Numbers in the same column for each mineral concentration followed by different letters are significantly different at P<0.05

Table 4.2 Concentration (mg/l) of Na, Fe, Zn, and Cu in sheep blood sampled at Goodhope and Morale ranches during the wet and dry season

season	Na	Fe	Zn	Cu
Wet	5561.15±336.25 ⁷	2.64±0.40 ^y	2.80±0.247	2.71±0.12*
Dry	7092.48±339.44°	1.36±0.40 ^z	1.40±0.24*	8.50±0.12²

Numbers in the same column for each mineral concentration followed by different letters are significantly different at P<0.05

Table 4.3 Interaction for the concentration (mg/l) of macro minerals in the blood of yearling sheep at Goodhope and Morale during the wet and dry season.

	1	Ca		Mg		Na	K	
Season	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Goodhope	61.20±5.33	67.36±4.21	16.61±1.41 ^{bz}	13.06±1.52 ^{by}	8601.44±912.03b7	5407.88±97868**	402.29±51.16 ^{bt}	207.43±54.47"
Morale	64.83±4.28	75.81±4.20	15.43±1.36 ⁴²	12.89±1.30*7	5060.39±878.58 ^{bz}	5812,55±840.06 ^{by}	365.62±48.9 ^{bs}	530.09±46.76 ¹⁴

Numbers in the same row for each mineral concentration followed by different letters are significantly different at P<0.05.

Values for minerals are got from (LSM ± SE)

Table 4.4. Interaction for the concentration (mg/l) of micro minerals in the blood of yearling sheep at Goodhope and Morale tested during the wet and dry season

		Mn		Zn		Cu		Fe	
Season	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	
Goodhope	2.05±0.19 ^{bz}	1.20±0.16*7	1.46±0.21 ^{bz}	2.73±0.22*y	6.90±0.11 ^{bx}	2.71±0.12**	1.13±0.41 ^{bz}	1.86±0.44 ^{ba}	
Morale	1.87±0.19 ^{bz}	1.10±0.19 ^{ay}	1.65±0.20 ^{bx}	2.84±0.19*y	10.83±0.11 ^{by}	2.98±0.10**	1.66±0.39 ^{b2}	3,59±0.39bs	

^{*}Numbers in the same row for each mineral concentration followed by different letters are significantly different at P<0.05.

"Numbers in the same column for each mineral concentration followed by different letters are significantly different at P<0.05.

Values for minerals are got from (LSM ± SE)

[&]quot;Numbers in the same column for each mineral concentration followed by different letters are significantly different at P<0.05

4.5.1.2 Adult sheep minerals concentration

The adult sheep macro and micro minerals are presented on table 4.5 and table 4.6 respectively. There was some season x site interactions in some of the adult sheep minerals concentration. In Goodhope ranch Ca, Na, K, Mn, Zn and Cu minerals concentration for adult sheep were significantly different (P<0.05) between wet and dry season, while in Morale ranch the difference (P<0.05) was observed on Mn, Cu and Fe. During the dry season adult sheep from Morale and Goodhope ranch had Ca, Na and K that were significantly different (P<0.05), while during the wet season the only difference (P<0.05) was observed on the concentration of K. The changes in minerals concentration from wet to dry season at the two sites were 10%vs 2% for Ca, 0.8% vs 16% for Mg, 5% vs 44% for Na, 20% vs 33% for K, 8%vs 3% for Mn, 31% vs 9% for Fe, 9% vs 13% for Cu, 5% vs 41% for Zn between Goodhope and Morale ranch respectively.

Table 4.5 Interaction for the concentration (mg/l) of macro minerals in the blood of adult sheep at Goodhope and Morale tested during the wet and dry season.

		Ca		Mg		Na		К	
Season	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	
Goodhope	60.73±5.13	64.61 ±6.88	16.91±1.38 ^{to}	14.29±1.18 ⁶²	9441.71±892.56	5656 32±765.96*	476.73±49.6	300 30±42,46°	
Morale	67.21±4.50	62.87±6.11	14 12±1.38 ^{to}	14.41±1.43 ^{bz}	5266.38±891.95	5367.48±924.10 ^{kz}	319.56±49.60°	362.7(±51.43*	

[&]quot;Numbers in the same column for each mineral concentration followed by different letters are significantly different at P<0.05 "Numbers in the same row for each mineral concentration followed by different letters are significantly different at P<0.05 Values for minerals are got from (LSM ± SE)

Table 4.6 Interaction for the concentration (mg/l) of micro minerals in the blood of adult sheep at Goodhope and Morale tested during the wet and dry season

		Mn		Žn		Cu		Fe	
Season	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	
Goodhope	1.93±0.19**	1.20±0.16**	1.12±0.20°y	2.90±0.17**	7.70±0.11 ^{bt}	2.71±0.17**	1.27±0.40**	2.21±0.347	
Morale	1.87±0.19 ¹¹	1.10±0.19**	1.58±0.21by	2.73±0.11**	8.76±0.11**	2.64±0.11*7	1.39±0.40=	2.91±0.417	

^{*}Numbers in the same row for each mineral concentration followed by different letters are significantly different at P<0.05.

"Numbers in the same column for each mineral concentration followed by different letters are significantly different at P<0.05.

Values for minerals are got from (LSM ± SE)

4.6 DISCUSSION

4.6.1. Concentration of Calcium in the blood of adult and yearling sheep

Calcium constitutes about 2% of body weight and its nutritional adequacy depends not only on sufficient dietary supplies but also on the chemical forms in which it is presented in the diet (McDowell, 1997). Ca availability depends on other nutritional constituents like P and vitamin D. Dietary Ca: P ratio can be very important. Ruminants can tolerate wide range of Ca: P ratio particularly when tissue vitamin D is high. Excess intake of calcium may reduce absorption of Mg, Fe, I, Mn, Zn and Cu. In the present study, site had a major influence (P<0.05) on concentration of Ca in blood for adult and yearling sheep during the dry season. Both adult and yearling sheep from Goodhope had Ca concentration that was higher (P<0.05) than of adult and yearling sheep raised at Morale ranch by 22% and 19% respectively during the dry season. Though the forage Ca concentration was the same in both sites the difference was probably caused by the forage species variation in these two areas and since these animals are known for being highly selective, it could have also led to the difference.

The concentration Ca in the blood for yearling sheep was significantly higher than that of adult sheep. Since the yearling and adults were both on the growth stage as far as their age difference is concerned Ca difference was probably caused by the fact that yearlings were on the stage of rapid growth as compared to adult sheep. This is consistent to the finding reported by (Schneider et al., 1985; Bronner, 1987) that Ca is absorbed from the diet according to need by a hormonally regulated process in the small intestine up to limits set by the diet and by the net movement of Ca into or out of the skeleton. Since the concentrations of Ca in forage in figure 4.1 were below the animal's requirement in both season, both adults and yearling sheep Ca concentrations were below the critical value of 80mg/l as proposed by Pamela et al. (2001) and McDowell, (1985). The concentration of blood Ca were similar to those found by Khan et al. (2009) on lactating goats and above those found by Khan et al., (2003) during the dry season. The concentration of blood Ca for both yearling and adult sheep were both below the concentration found by Orden et al. (1999); Ndebele et al. (2005)

4.6.2 Concentration of Magnesium in the blood of adult and yearling sheep

Magnesium availability to stock is markedly affected by several dietary components, especially Potassium. High dietary levels of Potassium and Nitrogen has the ability to inhibit Mg absorption (Dua and Care, 1995). Calcium and soluble carbohydrates may respectively increase and decrease dietary Mg requirements of livestock, whereas raised dietary P levels appears to lower the requirements for both Ca and Mg (Judson and McFarlane, 1998). In the present study site and season had no significant effect (P>0.05) on the concentration of blood Mg for both adults and yearling sheep. The concentration of Mg in the blood for adult sheep was not significantly different (P>0.05) from that of yearling sheep, probably because they had almost similar metabolic activities since their age difference was minimal. The concentration of Mg in the blood for both adult and yearling sheep was marginally below the critical value of 20 mg/l (Pamela et al., 2001) in both sites and season.

The concentration of Mg was deficient during both seasons, although the source of Mg (forage and water) had marginally higher Mg level than the requirements of ruminants. The depression in blood Mg may have been due to minerals dietary interactions that might have rendered Mg unavailable (Baumgurtel and Judson, 1998). Concentrations of Mg in the blood were similar to those found by Khan *et al.* (2008a, 2003) for non-lactating goats. Mean blood Mg values were lower than those reported in the other studies conducted in Venezuela (Rojas *et al.*, 1993) and in Indonesia (Prabowo *et al.*, 1990). Dietary amendment is necessary by supplementing the stock with feedstuffs that have got bioavailable Mg to correct the deficiency.

4.6.3 Concentration of Sodium in the blood of adult and yearling sheep

In the present study the interaction of site and season had a noticeable (P<0.05) effect on the concentration of Na in the blood for both yearlings and adults sheep. There was no significant difference (P>0.05) between the two classes of animals in the dry season at Morale and Goodhope ranches. The concentration of blood Na for both yearling sheep and adult sheep was way above the critical value of 3200 mg/l (Georgievski et al., 1982). High level of Na in blood was an indication that the animal had excess quantity of salt. These excess did not come from the forage that they consumed since forage was deficient in Na, hence probably from the supplement salt that was occasionally given to these pastured sheep. Some of the salt could have been

consumed from the soil, as sheep have got a tendency of licking the soils in search of Na and other minerals. These happens mostly when the salt are deficient in forages. These Na concentrations were consistent with those that were found by Kumaresan *et al.*, (2010). Blood Na concentration for both adult and yearling were more than those found by Khan, (2003); Khan *et al.* (2003); Khan et al. (2009); Talat *et al.* (2009).

4.6.4 Concentration of Potassium in blood of adult and yearling sheep

The present study showed that the interaction of site and season had a significant effect on both yearling and adult sheep. Both classes of animals had concentration of K in blood that was above the critical value of 200mg/l (Pamela et al 2001). These blood K concentrations were consistent to the findings of (Ignacio et al., 2008). There were also higher than the blood concentration reported by Khan et al. (2003); Areghere et al. (2007); Talat et al. (2009). In table 3.5 forage consumed by sheep was marginally deficient in K concentration hence its adequacy may be due to the body homocostatic processes. Some other components that could have contributed to the Potassium could be soil, bucks and other non-feed objects.

4.6.5 Concentration of Manganese in the blood of adult and yearling sheep

The present study showed that blood Mn for both adult and yearlings sheep were high (P<0.05) during dry season and low during wet season for both locations. The concentration of Mn in the blood was well above the critical values of 0.015 to 0.05 mg/L proposed by McDowell *et al.* (1984); Underwood (1981) for both yearling and adult sheep. The concentration of blood Mn had a positive relationship with the concentration of Mn in forage and soils. Concentration of forage Mn was high during dry season this trend was observed on the blood Mn as well. The higher blood Mn concentrations absorbed in the blood stream indicate that there was no interference from the concentration of Zn and P in the forage, which badly affect the utilization of Mn (Aregheore *et al.*, 2007.). These blood concentrations were consistent to the finding by Kumaresan *et al.* (2010) and were lower higher than those reported by Fardous *et al.* (2011) and Khan *et al.* (2004b).

4.6.6 Concentration of Zinc in the blood of adult and yearling sheep

There was a significant difference (P<0.05) between wet and dry season for both yearling and adult sheep Zn concentration in both Goodhope and Morale ranches. Site difference that was observed on sheep blood was consistent with the trend observed on forages Zn. Blood Zn concentration for both adult and yearling sheep in both site and season were well above the critical values of 0.60 ppm (McDowell et al., (1984). The forage that was consumed by sheep had sufficient Zn and it reflected on the sheep blood. This was a sign of Zn availability for digestion and absorption, hence there was a positive relationship between the forage Zn and blood Zn. Blood Zn concentrations were higher than blood Zn reported by Khan et al. (2008b); Vilallonga et al. (2012). Blood Zn status is a reasonable status criterion; however values are susceptible to animals stress during sampling and can fluctuate rapidly (Underwood, 1981). According to Cornrad, (1978) the blood Zn is rapidly and markedly reduced with severely deficient forages and increased by forages with sufficient Zn concentration.

4.6.7 Concentration of Copper in the blood of adult and yearling sheep

There was a significant difference (P<0.05) between yearling at Goodhope and those at Morale ranch in dry season. A similar trend was observed on the soils and the forages, all of which showed that samples from morale had higher Cu concentration. The absorption of minerals at Morale during dry season was proportional to the minerals available. The findings of this study showed that the concentration of blood Cu for both adults and yearlings sheep were well above the critical level of 0.65 ppm (McDowell, (1985); Pamela et al. 2001). Blood Cu in this study for both adult and yearling sheep was consistent with the blood Cu found by Vilallonga et al. (2012). Higher blood Cu for sheep has been reported by Pastrana et al., (1991) and in cattle by (Velasquez-pereira et al., 1997). Since the forage associated with these animals were marginally low in Cu as shown in table 3.4 adequacy of Cu in the blood was inconsistent with the forage Cu concentration, and may be attributed to animals homeostatic effect and other factors like soil, bucks and non-fed material.

.6.8 Concentration of Iron in the blood of adult and yearling sheep

here was a noteworthy difference (P<0.05) between wet and dry season at Morale ranch in the dult sheep blood Fe. This difference was a result of a similar trend that was observed on forages the same time and site. Blood Fe concentrations for both yearling and adult sheep were within the critical value of 1.0-2 mg/L as established by Miles et al., (2001). Blood Fe deficiency is ery rare in ruminants grazing the pastures due to adequate levels in forages and soils and ontamination of forage plants in soil particles (Aregheore et al., 2007; Khan et al., 2005), unless here is considerable blood loss by the attack of parasites or any disease. Values for wet season here in agreement with values reported by Khan, (2003). Dry season values were lower than hose reported by Khan et al., (2003; 2008).

.7 CONCLUSION

here were some interaction on site x season in most of the yearlings sheep and those of adult neep macro and micro minerals concentrations. During the dry season there was a significant ifference (P<0.05) of 41% and 44% for Na, 32% and 9% for K in yearling and adult sheep spectively. Sheep from Goodhope had higher (P<0.05) blood mineral concentration than that at lorale ranch. Notable changes in the blood concentration of minerals in yearling sheep from wet odry season at the two sites were 7% vs. 41% for Na, 155% vs. 9% for K, 40% vs. 16% for Mn, 3% vs. 46% for Fe, 9% vs. 56% Cu. A similar trend was observed in the adult sheep minerals everal soil-plant- animal mineral interaction influenced the uptake of minerals especially where he minerals were adequate in forage but taken up by the animals. The findings of this study idicate that the concentration of Ca and Mg were sub-optimal in the blood of adult and yearling neep, while K, Na, Mn, Zn, Fe and Cu were within the normal range.

4.8 RECOMMENDATIONS

The findings of this study have shown that Ca and Mg were deficient in both adult and yearling from Goodhope and Morale ranches in the wet and dry season. These mineral deficiencies may be the limiting factors to livestock production, since minerals are involved in very important metabolic reactions, depending on the animal's production status. Supplementation with fortified mixtures containing these elements in appropriate proportion and high bioavailability during both seasons is highly recommended. The supplementation of mineral elements should, however, be preceded by animal response studies. Specific minerals should not be seen to be as a cure-all or scapegoat for any particular problem without through investigations. Further studies should be conducted with esophageal fistulated animals in order to compare the mineral content of what animals have consumed with that in the soil and sheep's blood. Studies such as these one should be done on various ecological regions so that famers can be advised on appropriate mineral supplementation programs in their respective areas.

CHAPTER 5

4.9REFERENCES

- Adams, F. and Hartzog, D. L. (1980). The nature of yield responses of Florunner peanuts to lime. Peanut Science., 7: 120-123.
- Adogla-Bessa T, and Aganga, A A (2000). Responses of Tswana goats to various length of water deprivation. S Afric. J. Anim Sci., 30 (1): 87-91.
- Ahmad K., Khan Z. I., Ashraf M. Y., Ashraf M and Valeem, E.E, (2008). Forage evaluation for some trace elements: A case study in the soone valley, Pakistan *Pak. J. Bot.*, 40(3): 999-1004
- Aganga A.A., Tsopito C. M and More K, (1997). Water as a source of minerals for grazing ruminants in Kweneng west, *Archs zootec.*, 46:89-94
- Allen, S.E., Grimshaw, H. M., Parkinson, J. A. and Quarmby, C, (1974). *Chemical analysis of ecological materials*. Oxford: Blackwell Scientific.
- Animal Production and Range Research, (2001). Pg 20
- Anonymous. (1973). Tracing and Treating Mineral Disorders in Dairy Cattle. Netherlands Committee on Mineral Nutrition, Center for Agriculture Publishing and Documentation, Wageningen, Netherlands.
- Anonymous. (1984). Nutrient Requirements of Domestic Animals. No. 4: Nutrient Requirements of Beef Cattle. 6th revised ed. (National Academy of Sciences, National Research Council: Washington DC.).
- AOAC, (1996). Official Methods of Analysis (16th edition), Association of Official Analytical Chemists, Arlington, Virginia.
- Aregheore, A., Hunter, D., Perera, D and Mauotaotasi M.T, (2007). The soil-plant-animal phenomena: Serum minerals status of Fiji fantastic sheep grazing Batiki grass (*Ischaemum aristatum Var.indicum*) and Pangola grass(*Digitaria Decumbens*) in Samoa, J Anim and Vet Adv., 6 (3): 349-357)
- Baumgurtel, K. L. and Judson, G. J, (1998). Evaluation of a sustained release bolus to supply trace elements and vitamins to beef cattle. Animal Production of Australia., 22: 133-136.
- Becker, R. M., Henderson J. R and Leighty, R.B, (1965). Mineral malnutrition in cattle, Bulletin 699 Agricultural experimental station, Gainesville, Florida

- Belesky, D. P., Turner, K. E., Fedders J.M, and Ruckle J.M, (2001). Mineral composition of swards containing forage chicory. *Agro. J.*, 93: 468-475.
- Ben-Shahar, B. and Coe M. J. (1992). The relationships between soil factors, grass nutrients, and the foraging behavior of wildebeest and zebra. *Oecologia*., 90: 422-428.
- Bhat M. S., Shaheen M., Zaman R and Muhee (2011). Mineral Inter-relationship among Soil, forage and Dairy cattle in Kashmir, India, *Vet. World*, 4(12):550-553
- Blache D, Chagas L. M and Martin G. B., (2007). Nutritional inputs into the reproductive neuroendocrine control system a multidimensional perspective. In Reproduction in domestic ruminants VI (ed. J. I Juengel, J. F Murray and M. F Smith), pp. 123–139. Nottingham University Press, Nottingham, UK.
- Blincoe C., Pespperance A. L and Borhman, V. R. (1973). Bone Magnesium, Calcium and strontium concentration in range cattle, *J. Anim Sci.*, 36:971-975
- Bosman H. G., Ademosun A. A and Koper-Limbourg H. A. G, (1996). Goat feeding practices and options for improvement in six villages in south-western Nigeria, *Small Ruminant Res*, 19:201-211
- Bray R. H and Kurtz. L. T, (1945). Determination of total, organic, and available forms of phosphorus in soils. *Soil Sci.* 59: 39-45
- Bronner, F. (1987) Intestinal calcium absorption: mechanisms and applications. *J Nutri.*, 117, 1347–1352.
- Central Statistics Office, (2008), 2004 Agricultural census, Issue No 5 of 2008/09
- Chelliah G. R., Myer, R. O., Carter J. N., McDowell, L. R. and Wilkinson N, (2008), Mineral Concentrations of Cool Season Pasture Forages in North Florida During Winter-Spring Grazing Season. 1. Macrominerals and Forage Organic Constituents, *J Plant Nutri.*, 31: 1756–1773
- Chicco, C. F., Ammerman C. B., Hillis W.G. and Arrington. L. R.(1972). Utilization of dietary magnesium by sheep, *American J. Physi.*, 222: 1469-1472.
- Church, D.C. and Pond W.G, (1988). Basic Animal Nutrition and Feeding. 3rd Edition. Published by John Wiley and Sons, New York, NY. pp. 196-199.
- Coe, F. R. (1973). Micronutrients. In: A Review of Soil research in tropical Latin America. pp. 182-189. North Carolina Agricultural Experimental Station, Raleigh.

- Cornrad, J. H. (1978). Soil, plant animal tissue as predictors of the mineral status of ruminants. In: Latin American symposium on mineral nutrition research with grazing ruminants. Univ. Florida, Gainesville (J. H. Cornrad and L. R McDowell eds) pp (143-148)
- Cuesta, P. A., McDowell L. R., Kunkle W. E., Bullock F., Drew A., Wilkinson N.S and Martin F.G. (1993). Seasonal variation of soil and forage mineral concentrations in North Florida. Commun in Soil Sci Plan., 24: 335-347.
- Desalegn, T and Mohammed Y.K. (2012). Physical properties and critical mineral concentration of mineral waters commonly consumed by camels (*Camelus dromedarius*) in Jijiga District, Eastern Ethiopia, *Livestock Res. Rural. Dev.*, 24 (3)
- De Carvalho, P. R. (2009). Mineral imbalances in the complex interaction soil-plant-animal in the grassland of center west region of the Sau-Paulo state Brazil, *Pak J. Nutri.*, 8 (10): 1603-1612.
- Devendra, C, (2001) Small ruminants: Imperatives for productivity enhancement improved livelihoods and rural growth. A review. *Asi-Austra. J Anim Sci.*, 14, 1483-1496.
- Dua, K and A. D. Care. 1995). Impaired absorption of magnesium in the etiology of grass tetany.

 Brit. Vet. J., 151:413-26.
- Espinoza, J.E., McDowell L. R., Rodriguez J., Loosli J. K., Conrad J. H and Martin F.G.(
 1982). Mineral Status of Llamas and Sheep in the Bolivian Altiplano Florida Agricultural
 Experiment Station J. Serie No. 3901
- Espinoza, J. E., McDowell. L. R., Wilkinson, N. S, Conrad, J. H and Martin. F. G, (1991). Forage and soil mineral concentrations over a three-year period in a warm climate region of central Florida. II. Trace minerals, *J. Seri.*, No. R-01100
- Fardous, A., Gondal, S., Shah, A. Z., Ahmad, K., Khan, K. I., Ibrahim ,M, Ahmad ,A.E.W, Ullah,S and Valeem, E. E, (2010). Sodium, Potassium and Magnesium dynamics in soil-plant animal continuum *Pak J Bot.*, 42(4): 2411-2421
- Fardous A., Ahmad K., Gondal S, Khan I Z. I., Ejaz A., and Aaleem E. E. (2011). Assessment of Iron, Cobalt and Manganese in soil and forage: A case study at a rural livestock farm in Sargodha, Pakistan, *Pak. J. Bot.*, 43 (3): 1463-1465.
- Fick, K. R., McDowell L. R., Miles P. H., Wilkinson N.S., Funk J.D, and Conrad J.H. (1979), Methods of mineral analysis for plant and animal tissues (2nd edition.). Department of Animal Science, University of Florida, Gainesville.

- Georgievski, V. I., Annenkov, B. N., Samokhin, V. I., (1982). Mineral Nutrition of Animals. Mineral Nutrition of Animals, Butterworths, London, p 475.
- Gilbert C., Mary S. J., Samuel, W and Coleman, W., (2006). Long-Term Effects of Grazing and Haying on Soil Nutrient Dynamics in Forage-Based Beef Cattle Operations. J. Sustain Agr. Vol. 29 (3) 115-134
- Gizachew L., Hirpha A., Jalata F and Smit G.N (2002). Mineral element status of soils, native pastures and cattle blood serum in the mid-altitude of western Ethiopia. *African J. of Range and Forage Sci.*, 19:3, 147-155
- Gonzalez-V, E. A., Hussey M. A and Ortega-S. J. A (2006). Nutritive value of desmanthus associated with Klein grass during the establishment year. *Rangeland Eco. Manage.* 58: 308-314.
- Hamilton J. A and Webster M. E. D, (1987). Food intake, water intake, urine output, growth rate and wool growth of lambs accustomed to high or low intake of sodium chloride. Aust. J Agr. Res., 38, 187–194.
- Hanlon, E. A., Kidder G., and McNeal, B. L. (1990). Soil, Container Media and Water Testing:
 Interpretations and IFAS Standardized Fertilization Recommendations. Circular
 817.Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences,
 University of Florida, Gainesville, Flo.
- Haynes, R. J. and Williams, P. H, (1993). Nutrient cycling and soil fertility in grazed pasture ecosystem. *Adv Agron.*, 49:119-199.
- Ignacio A., Domínguez-Vara., Maximino Y and Huerta-Bravo, (2008). Mineral concentration and interrelationship in soil, forage and blood serum of sheep during two seasons in the Toluca valley, México, Publicado como XXXX en Agrociencia 42: 173-183.
- Johnston, A. E., Poulton P. R and Syers J. K, (2001). Phosphorus, potassium and sulphur cycles in agricultural soils. Proceedings No. 465, International Fertiliser Society, York, UK, London, UK: *Int, Fert, Soc.*, pp. 1-44.
- Jones J. B (1998). Plant nutrition manual. CRC Press, Boca Raton, Florida
- Jones, Jr. J. B. (1972). Plant tissue analysis for micronutrients, pp. 319. In: Micronutrients in Agriculture. (Eds.): J. J. Mortvedt, P. M. Giordano, W. L. Lindsay. Soil Sciences Society of America Inc., Madison, W.I.

- Judson, G. J and McFarlane J. D, (1998). Mineral disorders in grazing livestock and the usefulness of soil and plant analysis in the assessment of these disorders, *Aust J. Exp. Agr.*, 38:707-723.
- Kabata-Pendias, A. and Pendias H (1992), Trace Elements in Soils and Plants. CRC Press Inc., Boca Raton, Florida.
- Kafeel A., Zafar I.K., Muhammad Y.A., Muhammad A and Ehsan E. V (2008). Forage evaluation for some trace elements: A case study in the Soone valley, Pakistan, *Pak J. Bot.*, 40(3): 999-1004
- Kamada, H., Nishimura, H. K., Krongyuti, P., Sukkasame, P., Phoengpong N, and Intramanee. S., (2000). Selenium status of soil, herbage and beef cattle in Southern Thailand, *Asia-Aust*, *J Sci.*, 13:757-760
- Kaneko J. J (Editors.) (1980) Clinical Biochemistry of Domestic Animals, 3rd edition. Academic press inclusions United States of America: 649-668.
- Kayser, M and Isselstein J. (2005). Potassium cycling and losses in grassland systems: A review. Grass and Forage Sci., 60: 213-224.
- Khan Z. I (2003). Effect of seasonal variation on the availability of macro and micro nutrients to animals (sheep and goat) through forage from the soil (PHD thesis) Univ. Agric, Faisalabad, Pak.
- Khan Z.I., Ahmad K., Ashraf M., Naqvil S. A. H., Kashaf S., Ejaz A and Valeem E. E (2010). Evaluation of potassium status of rams: the use of pasture and blood as indicators under semi-arid environmental conditions in Punjab, Pakistan, *Pak. J. bot.*, 42(6): 3933-3938.
- Khan Z.I., Ashraf M., Ahmad K., Javed I, and Valeem E. E, (2008a), A comparative study on mineral status of blood plasma of small ruminants and pastures in Punjab, Pakistan, *Pak J. Bot.*, 40 (3): 1143-1151
- Khan, Z. I., Ashraf M., Ahmad K., McDowell L. R and Valeem E. E, (2008b). Transfer of Magnesium from soil and forage to goats grazing in a semiarid region of Pakistan: Influence of seasons and sampling periods, *Pakistan. Pak. J. Bot.*, 40 (5): 2127-2133
- Khan Z. I., Ashraf M., Ahmad K., Mustafa I and Danish M, (2007b). Evaluation of micro minerals composition of different grasses in relation to livestock requirements, *Pak. J. Bot.*, 39(3): 719-728

- Khan Z. I, Ashraf M Ahmad K, Valeem. E. E and Danish M, (2007a). Pasture concentration of minerals in relation to the nutrient requirements of farm livestock, *Pak. J. Bot.*, 39(6): 2183-2191
- Khan Z. I, Ashraf M, Ahmad K, Valeem. E. E and McDowell. L. R,(2009). Mineral status of forage and its relationship with that of plasma of farm animals In Southern Punjab, Pakistan, Pak. J Bot., 41(1): 67-72.
- Khan Z. I., Ashraf M., Altaf. H. L., Mcdowell. M, and Yaseen A, (2005a). Concentration of minerals in milk of sheep and goats grazing similar pastures in a semiarid region of Pakistan, Small Ruminant. Res., 65:274-278.
- Khan Z. I., Ashraf M., Altaf.H. L., Mcdowell.M, and Yaseen A, (2005b). Macromineral status of grazing sheep in a semi-arid region of Pakistan, *Small Ruminants Res*, 68:279-284
- Khan Z. I., Asharaf M., Asharaf M. Y., Zia-ur R and Hussain A, (2003). Minerals status of livestock (goats and sheep) based on soil, dietary components and animal tissue in relation to seasonal changes and sampling periods on specific regions of Pakistan, J. Anim. Vet. Adv., 2 (8): 478-495.
- Khan Z. I., Hussain A, Ashraf M., Ashraf M. Y., McDowell L. R and Huchzermeyer B (2007c). Copper nutrition of goats grazing native and improved pasture with the seasonal variation in a semiarid region of Pakistan, *Small Ruminant Res.*, 67 138-148,
- Khan, Z. I., A. Hussain, M. Ashraf, M. Y. Ashraf, E. E. Valeem and Ahmad M. S, (2004a). Soil and forage (Trace elements) status of a grazing pasture in the semiarid region of Pakistan. *Pak J. Bot.*, 36: 851-856.
- Khan, Z. I., Hussain A, Ashraf M and McDowell L. R, (2006). Mineral status of soils and forages in South Western Punjab-Pakistan: Micro-minerals. *Asia-Aust. J of Anim Sci.*, 19: 1139-1147.
- Khan Z. I., Hussain A., Ashraf M., Yas M., Ashraf I. N and Rahman Z (2004b). Seasonal Variation in Manganese Status of Soil, Dietary Components and Grazing Goats, *Pakistan J.Life .Sci.*, 2 (1): 9-15.
- Khan, Z. I., A. Hussain, M. Ashraf, E. E. Valeem and Javed I. (2005c). Evaluation of variation of soil and forage minerals in pasture in a semiarid region of Pakistan. *Pak J. Bot.*, 37: 921-

- Kumaresan. A., Bujarbaruah. K. M., Pathak. K. A., Brajendra P. And Ramesh .T (2009). Soil plant, animal continuum in relation to micro and macro minerals status of dairy cattle in subtropical hill agro ecosystem, *Trop. J. Anim, Health.*, 42: 569-577
- Kuria S. G., Wahome R. G., Gachulri C. K and Wanyoike M. M, (2004). Evaluation of forages as mineral sources for camels in western Marsabit, Kenya, S.Afr.J. Anim. Sci., 34 (3) 180-188
- Landon J R (Ed.) (1984). Booker Tropical Soil Manual. A handbook for soil survey and Agricultural evaluation in the tropics and subtropics. Booker Agriculture International limited: Longman, New York.
- Lundu T., Choongo K., Munyinda K., Walubita K and Siulapwa N. J, (2012). A survey of seasonal macro-mineral status of soil, plants and goats in Siavonga, Southern Zambia Livestock Res. Rural. Dev., 24 (5)
- Lindsay, W. L. and Norvell W. A (1978). Development of a DTPA soil test for zinc, iron, manganese, and copper. Am. j. Soil. Sci. Soc., 42: 421-428.
- Masters, D. G., D. I. Paynter, J. Briegel, S. K. Baker, and D. B. Purser. 1988. Influence of manganese intake on body, wool and testicular growth of young rams and on the concentration of manganese and the activity of manganese enzymes in tissues. *Aust. J. Agric. Res.*, 39: 517-524.
- Masters D. G., Rintoul A. J., Dynes R. A., Pearce K. L and Norman H. C (2005). Feed intake and production in sheep fed diets high in sodium and potassium. *Aust .J.Agri. Res.*, 56, 427-434.
- Mathis C. P and Sawyer J. E (2004). New Mexico forage mineral survey Proceedings, Western Section, Am. Soc., Anim, Sci., Vol. 55, p182-185
- McDowell, L. R. (1985b). In: Nutrition of Grazing Ruminants in Warm Climates. Academic Press New York, pp-443.
- McDowell, L. R. (1985a). Detection of minerals' status in grazing ruminants. In: Nutrition of grazing ruminants in warm climates (Ed.): L. R. McDowell. Academic Press: San Diego. pp. 291-315.
- McDowell, L. R. (1992). Minerals in animals and human nutrition 1st edition, Florida, United States of America

- McDowell L. R. (1997). Mineral for grazing ruminants in tropical regions, Extension bulletin, Animal Science department, Center for tropical Agriculture, University of Florida pp-81.
- McDowell, L. R. (2003). Minerals in animals and human nutrition. 2nd edition Amsterdam, The Netherlands
- McDowell, L. R and Arthington, J. D. (2005). Minerals for Grazing Ruminants in Tropical Regions, 5th Edition. pp86. University of Florida, Gainesville, Florida.
- McDowell, L. R., Conrad J. M and Ellis G. L, (1984). Mineral deficiencies and imbalances and their diagnosis, pp 67-88. In: Symposium on Herbivore Nutrition in Sub Tropics and Tropics. (Eds.):F. M. C. Gilchrist and R. I Mackie. University of Pretoria, Pretoria, South Africa.
- McDowell, L. R., Conrad, J. R., Ellis G. L and Loosli.L. K, (1983). Minerals for Grazing Ruminants in Tropical Regions. Extension Bulletin Animal Science Department, University of Florida. Gainesville, Florida.
- McDowell, L. R., Conrad J. H. and Hembry F. G. (1993). Minerals for grazing ruminants in tropical regions. University of Florida: Gainseville, Florida.
- McDowell, L. R., Kiatoko, M Bertrand, J. E. Chapman, H. L. Pate, F. M. Martin F. G. and Conrad J. H., (1982). Evaluating the nutritional status of beef cattle from four soil order regions of Florida: II. Trace Minerals. *J. Anim. Sci*, 55: 38-47.
- McDowell, L. R and Valle G, (2000). Major minerals in forages. In: Forage Evaluation in Ruminant Nutrition (Givens, D. I., Owen, E., Oxford, R. F. E. and Omed, H. M., editorSs). pp: 373. CAB International, Wallingford, UK.
- Merkel, R. C., McDowell, L. R., Popenoe H. L., and Wilkinson, N. S. (1990). Mineral status comparisons between water buffalo and charolais cattle in Florida. *Buffalo J.*, 1: 33-41.
- Miles, P. H., Wilkinson, N. S. and McDowell. L. R, (2001). Analysis of Minerals for Animal Nutrition Research. 3rd edition. Gainesville, Florida: Department of Animal Sciences, University of Florida, Gainesville, Florida.
- Minson, D. J. (1990) Forages in Ruminant Nutrition. Academic Press, San Diego, California, pp. 208–229.
- Morris, J. G., Delmas R. E. and Hull J. L., (1980). Sodium supplementation of range beef cows in California. J. Anim. Sci., 51: 722.

- Moosa G. D (1982) warm seasons grass and nutritive uptake with liquid and solid N. P. K fertelizers. M. S. Thesis. Brigham Young University. United Kingdom.
- Ndebele N., Mtimuni J. P., Mpofu I. D. T., Makuza S., Mumbu P. (2005). The status of selected minerals in soil, forage and beef cattle tissue in a semi-arid region of Zimbabwe, *Trop. Anim. Health .Pro.* 37 381-393
- Nsoso, S. J., Monkhei M and Thwaafalo B. E, (2004). A survey of traditional small stock farmers in Molelopole North, Kweneng district, Botswana: Demographic parameters, market practices and marketing channels, *Livestock Res. Rural. Dev.*, 16 (12)
- National Research Council. Mineral Tolerance of Domestic Animals (1980). National Academy of Science, Washington, D. C.
- NRC. 1996. Nutrient Requirements of Beef Cattle. 7th edition. National Academy Press, Washington, D.C.
- Ogebe, P. O., Ayoade J. A., McDowell L. R, Wilkinson N. S, and Martin F. G (1995). Mineral concentrations of forages and soils in Benue State, Nigeria Macrominerals and forage in vitro organic matter digestibility and crude protein concentrations, Commun. Soil.Sci. Plan., 26: 1989-2007.
- Orden, E. A., Serra A. B., Serra, S. D., Agenon C. P. Curz E. M., Curz C. L. Fujihara. (1999).

 Mineral concentration of blood of grazing goats and some forage in lahar, Lader area of central zozon, Phillipines, East Asia. *Aust. J. Anim Sci.*, 12: 422-428.
- Pamela H. M., Wilkinson N. S and McDowell L. R, (2001). Analysis of minerals for nutrition research. Department of Animal Science, University of Florida, pp 117.
- Pastrana, R., McDowell L. R., Conrad J. H and Wilkinson N. S. (1991). Mineral status of sheep in the Paramo region of Colombia. II. Trace minerals. *Small Ruminant Res.*, 5: 23-34.
- Peacock C, (1996). Improving Goat production in the tropics, 1stedition. Farm -Africa and)
 Oxfam (United Kingdom and Ireland)
- Peirce A. W. (1968). Studies on salt tolerance of sheep. VIII. The tolerance of grazing ewes and their lambs for drinking water of the types obtained from underground sources in Australia. Aust. J. of Agric Res 19, 589-595.
- Pope. A. L., (2000). A Review of Recent Mineral Research with Sheep, J. Anim. Sci., 33: 1332-1343.

- Potter B. J. (1963). The effect of saline water on kidney tubular function and electrolyte excretion in sheep. *Aust. J. Agric. Res.*, 14, 518–528.
- Potter B. J (1968). The influence of previous salt ingestion on the renal function of sheep subjected to intravenous hypertonic saline, *J. Physio.*, 194, 435–455.
- Prabowo A., Mcdowell L. R., Wilkson N. S (1991). Mineral status of grazing cattle in south Sulawes, Indonesia: Macrominerals, Animal Science Department, University of Florida Gainesville, Florida 32611-0691 USA.
- Rhue, R. D. and Kidder G (1983). Analytical procedures used by the I F A S extension soil laboratory and the interpretation of results. Soil Science. Department, University of Florida, Gainesville. Florida.
- Rojas L. X., McDowell L. R., Wilkinson N. S and Martin F. G (1993). Mineral status for soil, forage and beef cattle of southern Venezuela. *Int. J. of Anim. Sci.*, 8:175-181
- Sanchez P. A. (1976). Properties and management of soils in the tropics. John Wiley and Sons, New York.
- Schneider, K. M., Ternouth, J. H., Sevilla, C. C. and Boston, R. C. (1985), A short-term study of calcium and phosphorus absorption in sheep fed on diets high and low in calcium and phosphorus. *Aust. J. of Agr Res.*, 36, 91–105.
- Seleka T B (1999). Sustainability of Botswana's Small Ruminant Industry in the 21st Century. In Aganga A. A., Chabo R. G., Kgosimore M., Letso M and Omphile U. J. (editors). Proceedings of the Sheep and Goats Workshop held at the Centre for in-service and Continuing Education, Botswana College of Agriculture, Gaborone, Botswana April 19-23rd, 1999, 100-113
- Sigua, G. C. and Tweedale, W. A (2003). Watershed scale assessment of nitrogen and phosphorus loadings in the Indian River Lagoon Basin, FL. Jour. Environmental Mgt. 67(4):361-370.
- Singh, D., Chhonkar, P. K., Dwivedi, B. S. (2005). Manual for soil, water, plant and fertilizer analysis. Westville Pub, New Delhi, India. pp. 117-142
- Spears, J. W. (2003). Overview of mineral nutrition in cattle: the dairy and beef NRC.

 Proceedings of the 13th Annual Florida Nutrition Symposium. pp. 113-126.

- Suttle, N. F. (1991), Mineral supplementation of low quality roughages. In: *Proceedings of Symposium on Isotope and Related Techniques in Animal Production and Health*. International Atomic EnergyAgency, Vienna, pp. 101–104.
- Talat N., Muhammad P., Khan Z.I and Umer F (2009). Assessment of macro-minerals status in soils, water, feed resources and its influence on blood plasma of sheep and goats in central mix cropping zone of Punjab, Pakitan, *Trop. Sub-trop, Agro, eco.*,11:249 253 249
- Tejada, R., McDowell, L. R., Martin, F. G. and Conrad, J. H., (1985), Mineral element analyses of various tropical forages in Guatemala and their relationship to soil concentrations, *Nutri. Rep.*, 32, 313–323.
- Tejada, R., McDowell L. R., Martin F. G.and Conrad J. H. (1987). Evaluation of cattle trace mineral status in specific regions of Guatemala. *Trop. Agric.*, 64: 55-60.
- Tiffany, M. E., McDowell L. R., Connor G. A., Nguyen H, Martin F. G., Wilkinson N. S. and Cardoso E. C. (2000). Effects of pasture applied biosolids on forage and soil concentrations over agrazing season in north Florida. I. Macrominerals. Crude Protein and *In Vitro* Digestibility. *Commun. Soil Sci. Plan.*, 31: 201-203.
- Tiffany, M. E., L. R. McDowell, G. A. O'Connor, F. G. Martin, N. S. Wilkinsons, S. S. Percival and P. A. Rabiansky. (2001). Effect of residual and reapplied bio solids on performance and mineral status of grazing beef steers. *J. Anim. Sci.*, 80: 260-269.
- Thomas, L. B. (1982). Exchangeable cations. In: Methods of Soil Analysis. 2nd edition. Part 2 ed., Page A. L., Miller R. H and Keeney D. R (editors). Agronomy Monograph No.9. Mafison, W I: American Society of Agronomy., pp. 159-165
- Tolsma, D. J., Ernst W. H. O., Verweij R. A. and Vooijs. R. (1987). Seasonal variation of nutrient concentrations in a semi-arid savana ecosystem in Botswana. *J. eco.* 75: 755-770.
- Underwood, E. J. (1981). The mineral nutrition of livestock. p. 10. Commonwealth Agricultural Bureaux, Slough, England.
- Underwood, E. J., and N. F. Suttle. (2004), The Mineral Nutrition of Livestock, 4th ed. New York: CABI Publishing.
- Wilson A. D (1966). The tolerance of sheep to sodium chloride in food or drinking water, Aust. J. Agc. Res., 17, 503-514.

- Wilson A. D and Dudzinski M. L. (1973). Influence of the concentration and volume of saline water on the food intake of sheep and on their excretion of sodium and water in urine and faeces. Aus. J. Agr. Res., 17, 245–256.
- Wilkinson S. R, and Stuedemann J.A, (1979) In: symposium grass tetany (Reading V. V and Grunes eds) pp 93-117 American Society of Agronomy., Madison Wisconsin.
- Valasquez-Pereira J. B., McDowell L. R., Cornrad J. H., Wilkinson N. S and Martin F. G. (1997), mineral deficiency of cattle in Central America and the Carbean, emphasizing Costa Rica. International. Conference for Tropical livestock.
- Viets, F. G. and Lindsay W. L. (1973). Testing soils for zinc, copper, manganese and iron. In: Soil Testing and Plant Analysis. (Eds.): L. M. Walsh and J. Beaton. pp. 153-172. Soil Science Society of America, Madison.
- Yerokun O. A. (2008). Chemical characteristics of phosphorus in some representative benchmark soils of Zambia. Geoderma, 147: 63-68.