DETERMINATION OF LEVELS OF TRACE ELEMENTS IN PASTURES IN SOME REGIONS IN KENYA

BY

JOSEPH MWANZIA NGUTA
BVM (University of Nairobi)

A THESIS SUBMITTED IN PARTIAL FULFILMENT FOR THE DEGREE OF MASTER OF SCIENCE IN PHARMACOLOGY AND TOXICOLOGY IN THE UNIVERSITY OF NAIROBI

Department of Public Health, Pharmacology and Toxicology,
Faculty of Veterinary Medicine,
College of Agriculture and Veterinary Sciences,
University of Nairobi

2007

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

……**.....**

Dr. JOSEPH MWANZIA NGUTA BVM, (Nairobi).

This thesis has been submitted for examination with our approval as university supervisors.

…….....

Prof. S.E.O. Mitema BVM, MS., PhD.

Department of Public Health, Pharmacology and Toxicology,

University of Nairobi.

……

Dr. James. M. Mbaria BVM, MSc., PhD.

Department of Public Health, Pharmacology and Toxicology,

University of Nairobi.

……

Dr. L. Kanja BSc, MSc., PhD.

Department of Public Health, Pharmacology and Toxicology,

University of Nairobi.

TABLE OF CONTENTS

CONTENTS **PAGE**

LIST OF TABLES

TABLES **PAGE**

LIST OF FIGURES

LIST OF ABBREVIATIONS

MKK1	Kikuyu grass from Gatuaba in Mt Kenya region.
MKN1	Napier grass from Naromoru in Mt Kenya region.
Mko1	Red oats grass from Kihato in Mt Kenya region.
MKS1	.Star grass from Nanyuki in Mt Kenya region.
MKSS1	Common setaria grass from Naromoru in Mt Kenya region.
CTK1	Kikuyu grass from Voi in Coastal region.
CTN1	Napier grasss from Voi in Coastal region.
CTO1	Red oats grass from Voi in Coastal region.
CTS1	Star grass from Voi in Coastal region.
CTR1	Rhodes grass from Voi in Coastal region.
CTK2	Kikuyu grass from Shanzu in Coastal region.
CTN2	.Napier grass from Shanzu in coastal region.
CTO2	Red oats grass from Shanzu in Coastal region.
CTS2	Star grass from Shanzu in Coastal region.
CTR2	Rhodes grass from Shanzu in Coastal region.
CTK3	Kikuyu grass from Bamburi in Coastal region.
CTN3	Napier grass from Bamburi in coastal region.
CTO3	Red-oats grass from Bamburi in Coastal region.
CTS3	Star grass from Bamburi in Coastal region.
CTR3	Rhodes grass from Bamburi in Coastal region.
CTK4	Kikuyu grass from Likoni in Coastal region.
CTN4	Napier grass from Likoni in Coastal region.
CTO4	Red oats grass from Likoni in Coastal region.
CTS4	Star grass from Likoni in Coastal region.

CTR4	Rhodes grass from Likoni in Coastal region.
CTK5	Kikuyu grass from Kilifi in Coastal region.
CTN5	Napier grass from Kilifi in Coastal region.
CTO5	Red oats grass from Kilifi in Coastal region.
CTS5	Star grass from Kilifi in Coastal region.
CTR5	Rhodes grass from Kilifi in Coastal region.
CTK6	Kikuyu grass from Kikambala in Coastal region.
CTN6	Napier grass from Kikambala in Coastal region.
CTO6	Red-oats grass from Kikambala in Coastal region.
CTS6	Star grass from Kikambala in Coastal region.
CTR6	Rhodes grass from Kikambala in Coastal region.
UGN1	Napier grass from Marula in Uasin-Gishu/Eldoret region.
UGR1	Rhodes grass from Marula in Uasin-Gishu/Eldoret region.
UGO1	Red-oats grass from Marula in Uasin-gishu/Eldoret region.
UGS1	Star-grass from Marula in Uasin-gishu/Eldoret region.
UGK1	Kikuyu grass from Marula in Uasin-gishu/Eldoret region.
UGN2	Napier-grass from Kiplombe in Uasin-gishu/Eldoret region.
UGR2	Rhodes-grass from Kiplombe in Uasin-gishu/Eldoret region.
UGO2	Red-oats grass from Kiplombe in Uasin-gishu/Eldoret region.
UGS2	Star-grass from Kiplombe in Uasin-gishu/Eldoret region.
UGK2	Kikuyu grass from Kiplombe in Uasin-gishu/Eldoret region.
UGN3	Napier-grass from Kuinet in Uasin-gishu/Eldoret region.
UGR3	Rhodes-grass from Kuinet in Uasin-gishu/Eldoret region.
UGO3	Red-oats-grass from Kuinet in Uasin-gishu/Eldoret region.
UGS3	Star-grass from Kuinet in Uasin-gishu/Eldoret region.

UGK3	Kikuyu-grass from Kuinet in Uasin-gishu/Eldoret region.
UGN4	Napier-grass from Kamukunji in Uasin-gishu/Eldoret region.
UGR4	Rhodes-grass from Kamukunji in Uasin-gishu/Eldoret region.
UGO4	Red-oats-grass from Kamukunji in Uasin-gishu/Eldoret region.
UGS4	Star-grass from Kamukunji in Uasin-gishu/Eldoret region.
UGK4	Kikuyu-grass from Kamukunji in Uasin-gishu region.
UGN5	Napier-grass from Serogoit in Uasin-gishu region.
UGR5	Rhodes-grass from Serogoit in Uasin-gishu region.
UGO5	Red-oats-grass from Serogoit in Uasin-gishu/Eldoret region.
UGS5	Star-grass from Serogoit in Uasin-gishu/Eldoret region.
UGK5	Kikuyu-grass from Serogoit in Uasin-gishu/Eldoret region.
UGN6	Napier-grass from Eldoret town in Uasin-gishu region.
UGR6	Rhodes-grass from Eldoret town in Uasin-gishu region.
UGO6	Red-oats-grass from Eldoret town in Uasin-gishu region..
UGS6	Star-grass from Eldoret town in Uasin-gishu region.
UGK6	Kikuyu-grass from Eldoret town in Uasin-gishu region.
NYR1	Rhodes-grass from Homabay town in Nyanza region.
NYS1	Star-grass from Homabay town in Nyanza region.
NYSS1	Common-Setaria-grass from Homabay town in Nyanza region.
NYBI1	Bothriocloa-Insculpta grass from Homabay town in Nyanza
NYN1	Napier-grass from Homabay in Nyanza region.
NYR2	Rhodes grass from Olalo in Nyanza region.
NYS2	Star-grass from Olalo in Nyanza region.
NYSS2	Common-Setaria grass from Olalo in Nyanza region.
NYBI2	Bothriocloa Insculpta grass from Olalo in Nyanza region.

NYN2	Napier-grass from Olalo in Nyanza region.
NYR3	Rhodes grass from Wikondiek in Nyanza region.
NYS3	Star-grass from Wikondiek in Nyanza region.
NYSS3	Common Setaria from Wikondiek in Nyanza region.
NYBI3	Bothriocloa Insculpta from Wikondiek in Nyanza region.
NYN3	Napier grass from Wikondiek in Nyanza region.
NYR4	Rhodes grass from Wikondiek in Nyanza region.
KBN2	Napier grass from Kanyariri in Kiambu region.
KBO2	Red-Oats grass from Kanyariri in Kiambu region.
KBK2	Kikuyu grass from Kanyariri in Kiambu region.
KBS2	Star grass from Kanyariri in Kiambu region.
KBR2	Red-Oats grass Kanyariri in Kiambu region.
KBN3	Napier grass from Loresho in Nairobi region.
KBO3	Red-Oats grass from Loresho in Nairobi region.
KBK3	Kikuyu grass from Loresho in Nairobi region.
NYR4	Rhodes grass from Achuth in Nyanza region.
NYS5	Star grass from Achuth in Nyanza region
NYSS4	Common Setaria from Achuth in Nyanza region.
NYBI4	Bothriocloa Insculpta from Achuth in Nyanza region.
NYN4	Napier-grass from Achuth in Nyanza Region.
NYR5	Rhodes grass from Achuth in Nyanza region.
NYS5	Star grass from Achuth in Nyanza region.
NYSS5	Common Setaria from Achuth in Nyanza region.
NYHR5	Hyperrhenia rufa from Achuth in Nyanza region.
NYN5	Napier-grass from Migingo in Nyanza region.

NYR6	Rhodes grass from K-bay in Nyanza region.
NYS6	Star grass from K-bay in Nyanza region.
NYSS6	Common Setaria from K-bay in Nyanza region.
NYBI6	Bothriocloa Insculpta from K-bay in Nyanza region.
NYN6	Napier grass from K-bay in Nyanza region
NYN7	Napier grass from K-bay in Nyanza region.
NKS1	Star grass from Nakuru region.
NKK1	Kikuyu grass from Nakuru region
NKBI1	Bothriocloa Insculpta from Nakuru region.
NKSS1	Common Setaria from Nakuru region.
NKSD1	Sudan grass from Nakuru region.
NKN1	Napier grass from Nakuru region.
NKS2	Star grass from Njoro in Nakuru region.
NKSS2	Common Setaria from Njoro in Nakuru region.
NKN2	Napier grass from Njoro in Nakuru region.
KBN1	Napier grass from Kenyatta University in Kiambu region.
KBO1	Red-Oats grass from Kenyatta University in Kiambu region.
KBK1	Kikuyu grass from Kenyatta University in Kiambu region.
KBS1	Star grass from Kenyatta University in Kiambu region.
KBR1	Rhodes grass from Kenyatta university in Kiambu
KBS3	Star grass from Loresho in Nairobi region.
KBR3	Rhodes grass from Loresho in Nairobi region.
KBN4	Napier grass from Field-Station/Kabete in Nairobi region.
KBO4	Red-Oats grass from Field-Station/Kabete in Nairobi region.
KBS4	Star grass from Field-Station/Kabete in Nairobi region.

KBK4	Kikuyu grass from Field-Station/Kabete in Nairobi region.
KBR4	Rhodes grass from Field-Station/Kabete in Nairobi region.
NBO1	Red-Oats grass from Karen in Nairobi region.
NBS1	Star grass from Karen in Nairobi region.
NBK1	Kikuyu grass from Karen in Nairobi region.
NBBI1	Bothriocloa Insculpta from Karen in Nairobi region.
NBN1	Napier grass from Karen in Nairobi region.
NBR1	Rhodes grass from Karen in Nairobi region.
NBO2	Red-Oats from Ngong in Kajiado region.
NBS2	Star grass from Ngong in Kajiado region.
NBK2	Kikuyu grass from Ngong in Kajiado region.
NBBI2	Bothriocloa Insculpta from Ngong in Kajiado region.
NBN2	Napier grass from Ngong in Kajiado region.
NBR2	Rhodes grass from Ngong in Kajiado region.
NBO3	Red-Oats grass from Ruai in Nairobi region.
NBS3	Star grass from Ruai in Nairobi region.
NBBI3	Bothriocloa Insculpta from Ruai in Nairobi region.
NBN3	Napier grass Ruai in Nairobi region.
NBR3	Rhodes grass from Ruai in Nairobi region.
NBO4	Red-Oats grass from Kantafu in Machakos region.
NBS4	Star grass from Kantafu in Machakos region.
NBBI4	Bothriocloa Insculpta from Kantafu in Machakos region.
NBN4	Napier grass from Kantafu in Machakos region.
NBR4	Rhodes grass from Kantafu in Machakos region.
MSN1	Napier grass from Athi-River in Machakos region.

MSS1	Star grass from Athi-River in Machakos region.
MSR1	Rhodes grass from Athi-River in Machakos region.
MSO1	Red-Oats grass Athi-River in Machakos region.
MSBI1	Bothriocloa Insculpta from Athi-River in Machakos region.
MSSS1	Common Setaria from Athi-River in Machakos region.
MSN2	Napier grass from Makutano in Machakos region.
MSS2	Star grass from Makutano in Machakos region.
MSR2	Rhodes grass Makutano in Machakos region.
MSO2	Red-Oats grass from Makutano in Machakos region.
MSBI2	Bothriocloa Insculpta from Makutano in Machakos region.
MSSS2	Common Setaria from Makutano in Machakos region.
MSN3	Napier grass from Kangundo in Machakos region.
MSK3	Kikuyu grass from Kangundo in Machakos region.
MSS3	Star grass from Kangundo in Machakos region.
MSO3	Red-Oats grass Kangundo in Machakos region.
MSR3	Rhodes grass Kangundo in Machakos region.
MSBI3	Bothriocloa Insculpta from Kangundo in Machakos region.
MSN4	Napier grass Mwala in Machakos region.
MSK4	Kikuyu grass from Mwala in Machakos region.
MSS4	Star grass from Mwala in Machakos region.
MSO4	Red-Oats grass from Mwala in Machakos region.
MSR4	Rhodes grass from Mwala in Machakos region.
MSBI4	Bothriocloa Insculpta from Mwala in Machakos region.
DM	Dry matter.
p^H	Hydrogen ion concentration.

P.P.M	Parts per million.
R	Correlation coefficient.
HGA	High Graphite Absorption.
AA	Atomic Absorption.
ICP	Inductively Coupled Plasma.
RF	Radio Frequency.
ICP-MS	Inductively Coupled Plasma Mass Spectrometry.
EDLs	Electrodeless discharge lamp.
MHS	Mercury high concentration spectrophotometer.
.>	Greater than.
<	Less than.
RNA	Ribonucleic acid.
EDTA	Ethylene diaminetetra-acetic acid.
COA	Coenzyme A.
Ado cbl	Adenosylcobalamin.
FDA	Food and Drug Administration.
(-)	< 0 or trace values (Below detection limit).

LIST OF APPENDICES

ACKNOWLEDGEMENTS

Were it not for the Almighty God, I would have given up. Praise and honour be to him! For me to have arrived in my current level in academia has been a journey characterised by all kinds of twists and turns. I can say with absolute certainty that there have been thrills and pitfalls on the way.

I would like to express my sincere gratitude to my supervisors for their unfailing encouragement, inspiration, guidance and positive criticism. I would like to express my appreciation to Professor Eric Mitema for introducing me to the field of Nutritional Pharmacology and Toxicology and for his good intentions and unfailing support in my professional growth. I would like to thank Dr. James Mbaria for his encouragement, guidance, and useful criticism. Dr. L. Kanja is acknowledged for assistance in analytical techniques and instrumentation, her incessant spiritual support and for working closely with me in all my academic endeavours. I would also like to thank Dr. Kanja for availing herself for constant consultations, kindness and guidance. Last but not least, I would like to thank Brigid McDermott for introducing me to the world of statistics. Her constant guidance especially in data analysis made it possible for this work to be completed as scheduled.

This work was supported by the Higher Education Loans Board (HELB) and CEVA SANTE ANIMALE, LIBOURNE, FRANCE. Their financial support is highly acknowledged.

I express my gratitude to the technical staff, department of animal production, University of Nairobi, for their friendship, kindness and unfailing assistance.

I express my sincere gratitude to the chairman, department of Public Health, Pharmacology and Toxicology, Dr. William Ogara, for his continued encouragement and inspiration throughout the study.

I would also want to express my gratitude to those other people, though their names are not listed, who supported me in one way or another.

Behind my academic achievement, are men whom I consider to be my role models. Talk of pursuit for academic excellence and you will find that these gentlemen have two key characteristics in common, namely; **A PASSION FOR SUCCESS and AN EYE FOR DETAIL.** These men are none other than Professor ERIC MITEMA and Dr. JAMES MBARIA both of the University of Nairobi. Both supervised me for my MSc. My other great role models have been my parents; Mzee DAVID NGUTA KIETI and TABITHA MUKULU. Both are paragons of hardwork and sensitivity to detail in all that they set out to do.

Everything that came to pass in and around this work was enabled by the **LORD JESUS** who has been given all authority in heaven and on earth **(MATTHEW 28:18). PRAISE BE UNTO HIM!**

DEDICATION

This work is dedicated to my dear mum, Mukulu, for all her love for education

GOD IS GOOD ALL THE TIME AND ALL THE TIME GOD IS GOOD.

HE IS MY SHEPHARD, I SHALL NOT WANT.

ABSTRACT

Trace elements are needed by ruminant animals for physiological and biochemical processes. The trace elements at optimum levels make animals to maintain desired production performance parameters. Excesses of some of these trace elements can also lead to toxicosis. The important trace elements are copper, cobalt, zinc and selenium.

The present study was conducted to determine levels of copper, cobalt, zinc, and selenium in known pasture grasses from eight regions of Kenya; to establish whether the named trace elements are deficient or in toxic quantities; to relate the trace element levels from the pasture grasses from different regions of Kenya with known recommended values in mg/kg of dry matter (mg/kg of DM) for optimum livestock production, and finally to recommend any intervention measures.

Forage samples (n = 171) were collected from eight regions in Kenya during months of July and August 2005. These regions were: Coast province, Machakos district, Nairobi province, Kiambu district, Mount Kenya region, Nakuru district, Eldoret region and Homabay district. The pasture grasses collected were: kikuyu grass, napier grass, red oats grass, star grass, sweet pitted grass, common setaria, brown hood grass and sudan grass. The samples were then transported to the laboratory.

The grass samples (20 grams) were chopped into one-centimetre pieces and oven dried for 48 hours at 60^0C. They were then ground using Willey mill no. 20 stainless steel sieve and allowed to equilibrate with the atmospheric pressure for 12 hours and mixed properly pending assay for trace elements. Nine validation assay techniques were conducted, correlation coefficients determined, and the technique that gave the highest positive correlation, (R^2) value of 0.997, with a regression equation of, $Y=0.046 + 0.021X$, was adopted in the isolation and quantification of trace

elements from the sample matrix. A linear regression curve was fitted for the technique. Constituent trace elements (Co, Cu, Zn, and Se) were separated from the organic matrix using wet oxidation, with the digestion chemicals being nitric acid and perchloric acid at the ratio of 3:1 respectively. The trace elements were assessed using Perkin-Elmer model 2380 flame atomic absorption spectrophotometer at the following wavelengths: 327.4 nm, 240.7 nm, 213.9 nm, and 196.0 nm for copper, cobalt, zinc, and selenium respectively. Mean (mg/kg of DM) ± SD national values for zinc, copper, selenium and cobalt were 44.1 ± 20.5, 4.2 ± 1.6, < 0 and 4.4 ± 6.3 respectively. These values were found to be below the nutritional requirements for optimum livestock production for copper, zinc and selenium and suggestive of a deficiency, while fifty percent (50%) of all the regions investigated had cobalt levels that were observed to be below the normal recommended values for optimum livestock production, and were found to be deficient of the trace element. Deficiency was shown in 68% of pastures for copper, 62% of pastures for zinc, in 61% of pastures for cobalt and in 42% of pastures for selenium.

The results of the current study indicate that more than fifty eight percent of grass in the studied areas are deficient of all the trace elements investigated, and intervention measures, such as trace element supplementation is required. The pasture grasses from the eight regions studied do not accumulate toxic levels of the trace elements studied.

CHAPTER ONE

1. INTRODUCTION

Mineral deficiencies, imbalances and toxicities severely inhibit livestock production in many developing countries (McDowell 1985). Grazing livestock in the tropics may not receive mineral supplementation, except for common salt (Nacl), and must depend almost exclusively upon forages for their requirements (McDowell *et al* 1984). In Kenya, as in many developing countries, natural pastures are the main source of nutrients for cattle. Early indications of mineral deficiency and toxicity in Kenyan pastures followed observations of earth eating and osteofagia by cattle and wild ruminants (Todd, 1954; French, 1955; Howard, 1963). The availability of mineral elements from earth licks is also probably low since only up to 6% of the organic matter is soluble in dilute acid (French, 1955). Cattle owners saltlicks as essential to the well being of their animals (Hudson, 1944).

Poor animal performance has usually been interpreted in terms of low dry matter (DM) intake and inadequacy of energy and protein in the dry matter (Grover and Dougall, 1961; Abate *et al.*, 1981; Abate, 1985). Little mention is made on the effect micro minerals have on production mainly because under controlled experimental conditions, mineral supplements are normally fed. Previous studies carried out in Kenyan pastures revealed deficiencies and as well as excessive levels of trace elements and toxicity with deficiency states in animals (Burdin and Howard, 1963; Howard, 1969; Mwakatundu, 1977) with obvious consequences on animal development and reproduction (Todd, 1954,

Howard *et al.*,1962). The trace elements associated with patho physiological states in animals include copper, cobalt, zinc and selenium.

Some of the elements are required by all biological systems. Hart *et al*. (1928) reported the importance of copper for the growth and hemoglobin formation in the rats. There are extensive copper deficient areas throughout the world which has adverse effects on the crops and livestock (Underwood and Suttle, 1999). Copper plays various important biological roles in the body of the animals through several copper dependent enzymes. Some of important copper dependent enzymes are cytochrome oxidase, widely involved in many oxidative reactions, monoamine oxidase, associated with maturation of collagen and elastin; and ceruloplasmin, involved in mobilization and utilization of iron stored in the liver.

Disorders such as stilted gait, rusty coat colour and fragility of bones in calves have been reported in Kenya and are associated with copper deficiency (French, 1955; Howard, 1963). Copper concentrations in Kenyan pastures seem to lie between 4.0 mg/kg of dry matter (DM) and 12.2 mg/kg of dry matter (DM) (Howard *et al.,* 1962). Copper deficiency symptoms in animals manifest at levels lower than 5.0 mg/kg of DM, and this has been shown in about 35% of pastures analyzed in Kenya (Howard, 1963). Shortage of copper has been reported in animals grazing in the Rift Valley province of Kenya because of copper deficiency in the soil (Howard, 1963, Pinkerton *et al.,* 1965). Soils in which the underlying rock is ash and pumice may be expected to be deficient in copper (Nyandat and Ochieng, 1976). Mwakatundu (1977) reported sub clinical copper deficiency widespread in Kenya as a result of copper deficiency either in the soil or pasture or bovine plasma and is more pronounced in dry periods (Nyandat and Ochieng, 1976). The functioning of dietary copper can be inhibited

by excess molybdenum (Mo) resulting in a conditioned copper deficiency. Animals consuming forages with molybdenum concentrations above 15 mg/kg to 20 mg/kg of DM showed copper deficiency symptoms even though the copper levels in the forage were higher than 5 mg/kg of DM (Hodgson *et al.*, 1962). Conditioned copper deficiency is not a problem in Kenya because molybdenum was found to be deficient in several areas surveyed (Mwakatundu, 1977).

Recent data on copper levels and status in Kenyan pastures is scanty. Despite the fact that copper plays an important physiological role, information on its status in Kenya was lastly reported nearly two decades by Mwakatundu (1977), even though agricultural practices have greatly changed today, and increased agricultural activities have been shown to have a great effect on copper status in grass pastures (Mitchell, 1954).

Cobalt was first shown to be an essential nutrient for sheep and cattle as an outcome of Australian investigations of two naturally occurring diseases, "coast disease" of sheep (Lines and Marston, 1935) and "wasting disease" or enzootic marasmus, of cattle (Underwood and Filmer, 1935). The findings were confirmed in sheep grazing similar calcareous soils of Aeolian origin on the West Coast of Scotland in the same decade (Suttle, 1988). Progress in understanding the mode of action of cobalt in the animal organism was slow until 1948, when two groups of workers independently discovered that the antipernicious anaemia factor, subsequently designated vitamin B_{12}, contained cobalt (Rickes *et al.*, 1948; Smith *et al.*, 1951). The essentiality of cobalt for mammals is linked to two distinct forms of vitamin B_{12}, with contrasting enzyme functions.

As methylcobalamin (Mecbl), cobalt assists a number of methyltransferase enzymes by acting as a donor of methyl groups and is thus involved in one-carbon metabolism. Methylcobalamin is important for microbes as well as mammals and is needed for methane, acetate and methionine synthesis by rumen bacteria (Poston and Stadman, 1975). In mammals, Mecbl enables methionine synthatase to supply methyl groups to a wider range of molecules, including formate, noradrenaline, myeline and phosphatidyl ethanolamine (PE). Failure of methylation in vitamin B_{12} deficient sheep also inhibits folate uptake by the liver (Gawthorne and Smith, 1974). As adenosylcobalamin (Adocbl), cobalt influences energy metabolism, facilitating the formation of glucose by assisting methylmalonyl-coenzyme A (CoA) mutase to form succinate from propionate, chiefly in the liver. The enzyme 2,3 mutase is also Adocbl-dependent and a breakdown in this pathway has been implicated in pernicious anaemia in humans (Poston, 1980). The identification of rate limiting pathways, has, however, long been a matter of controversy (Chanarin *et al*., 1981., Scott, 1992) and it has been suggested that the myelopathy which accompanies pernicious anemia may be attributable to Mecbl rather than Adocbl deficiency (Small and Carnegie, 1981; Scott, 1992).

Cobalt deficiency leads to chronic starvation or wasting which is often indistinguishable from energy and protein malnutrition (French, 1952; Howard, 1963; McDowell *et al.,* 1984). It is rare for grasses to contain cobalt in concentrations that meet the demands of grazing animals (Hodgson *et al.,* 1962). When the content in the pastures herbage is 0.10 mg/kg of DM or less, grazing animals are likely to suffer from cobalt deficiency (Hodgson *et al.,* 1962). In Kenya, the condition is known as “Nakuruitis”(French, 1952; Howard, 1963) or

as “Narurasha” among Maasai herdsmen (Hudson, 1944; French, 1952). Cobalt deficiency is common along the Rift Valley and is seasonal with symptoms usually appearing after the rains when grazing is plentiful and green (Hudson, 1944). Mwakatundu (1977) also confirmed cobalt deficiency in the Egerton, Kabete and Molo areas of Kenya. Animals suffering from cobalt deficiency lose appetite and condition, may abort if in calf or may have difficulty to conceive again. The condition seems to affect lactating cows more than any other type of stock (Hudson, 1944; French, 1952).

Cobalt status in Kenyan pastures is not well understood, yet it is a major component of cobalamins. Recent data on cobalt levels in grass pastures in Kenya is scanty. Agricultural practices have greatly changed over years, and this has been shown to play an important role in influencing the status of cobalt in grass pastures (Mitchell, 1954).

Todd *et al.* (1934) obtained the first unequivocal evidence that zinc is necessary for growth and health in rats and mice and shortly thereafter zinc deficiency was produced experimentally in pigs, poultry, lambs and calves. The deficiency was associated in all species with severe inappetance and growth depression, impaired reproductive performance and abnormalities of the skin and its appendages.

Zinc plays various important biological roles in the body of the animals through several zinc dependent enzymes. Some of important zinc dependent enzymes are alcohol dehydrogenase, involved in NAD+ linked interconversion of alcohol and aldehyde, alkaline phosphatase, involved in freeing phosphate from bound forms, for example, monoesters, carbonic anhydrase, involved in facilitation of carbon dioxide transport, carboxypeptidase A and B, involved in

hydrolysis of mannose, and super oxide dismutase, involved in destruction of the free radical oxygen (O^{-2}), (NAD+, nicotinamide adenine dinucleotide).

Data on zinc levels in Kenyan pastures is scarce. Kariuki *et al*. (1999) found zinc levels to lie between 7.5 mg/kg of DM and 8.4 mg/kg of DM in napier grass collected from Naivasha. In New Zealand, mixed pastures was found to contain more zinc in the North Island than in the South Island (38 Vs 22 mg/kg of DM); tussock grasslands was found to contain 8-48 mg/kg of DM of zinc (Grace, 1972). Uncontaminated pastures in Scotland had from 25 to 35 mg/kg of DM (Mills and Dalgarno, 1972), but values five to fifty times higher were obtained for herbage exposed to industrial contamination. One study of North American forages revealed contrasting ranges of 20 to 60 mg/kg of DM for legumes and 10 to 30 mg/kg of DM for the grasses (French *et al*., 1957), but in another study levels were lower and the contrast much less (11 to 18 Vs. 8 to 17 mg/kg of DM): Price and Hardison (1963). Similar low values have been found to occur in Western Australia, where herbage yield is increased by zinc fertilizers (Masters and Fels, 1985). Differences between grass species contribute little to reported variation in forage zinc (Minson, 1990); although studies of rhodes grass in Kenya reported poor zinc levels (Jumba *et al.,* 1995b). Stage of maturity of pastures is more important, with concentrations falling by almost 50%, irrespective of level of zinc fertilizer used, for successive cuts in one study (Gladstones and Loneragen, 1967). Hays, therefore, tend to be low in zinc (13 to 25 mg/kg of DM) and silages slightly richer (12 to 45 mg/kg of DM).

For many years, biological interest in selenium has been its toxic effects in animals. Two naturally occurring diseases of livestock, “blind staggers” and “alkali disease”, occurring in parts of the Great plains of North America, were

identified as manifestations of acute and chronic selenium poisoning, respectively (Moxon, 1937).

Selenium is necessary for growth and fertility in animals and for the prevention of a variety of disease conditions, which show a variable response to vitamin E. The four known peroxidases utilize glutathione as reducing substrate, and other multiplicity and ubiquity reflect the importance of controlling peroxidation, an essential biochemical reaction which, when unconstrained, can lead to chain reactions of free radical generation and tissue damage. The task of terminating such reactions and protecting against peroxidation are shared by other tissue enzymes (the super oxide dismutases, copper-zinc- and manganese (Mn) SOD-, catalase, glutathione sulphur) and by non-enzyme scavengers, such as vitamin E (Macpherson, 1994).

Data on selenium status in the Kenyan pastures is not available. Selenium levels in Kenyan pastures are not well known. The mountainous countries of Northern Europe (Finland, Sweden and Scotland) are low in selenium, and geochemical mapping confirm this observation (Selinus, 1988). Selenium plays an integral role in lipid peroxidation (as an antioxidant) thus preventing cellular damage. Excesses of selenium in grass pastures can also lead to selenosis (blind staggers), whereas its deficiency causes muscular dystrophy in animals. The present study was thus designed with a broad objective of examining the trace element status in Kenyan pastures.

1.1. SPECIFIC OBJECTIVES OF THE STUDY.

1.1.1 To determine levels of copper, cobalt, zinc and selenium in pasture grasses in various regions of Kenya with sand, clay, limestone and volcanic soils.

1.1.2 To compare the trace element levels from the various pasture grasses from different parts of Kenya with known recommended values.

1.1.3 To recommend intervention measures for areas with deficient or excess levels of the trace elements.

CHAPTER TWO

2: LITERATURE REVIEW

2.1: Introduction

Trace minerals are needed for vitamin synthesis, hormone production, enzyme activity, collagen formation, tissue synthesis, oxygen transport, energy production, and other physiological processes related to growth, reproduction and health. The susceptibility for these physiological processes to deficiencies or excess of trace elements varies. For example, growth, feed intake, and feed efficiency may not be altered during sub clinical deficient states, although impairment of reproduction or immune competence may occur. The requirement of trace minerals is often based upon the ability of the animal to maintain desired production performance parameters. Requirements for finishing cattle and lactating dairy cows are shown in Table 1.

Table 1: Trace mineral requirements (mg/kg of DM) for finishing and lactating dairy cows (Adapted from NRC, 1996).

Mineral	***Finishing cattle***	***Lactating dairy cows***
Cobalt	*4.2*	*0.1*
Copper	*10*	*10*
Selenium	*0.1*	*0.1*
Zinc	*30*	*30*

These requirements are based upon average cattle consuming average diets. Because copper utilization can be low in ruminant diets, and especially when the antagonists molybdenum and sulphur are present in moderate to high levels, the National Research Council (NRC) recommendations may require adjustment. Molybdenum and sulphate form thiomolybdates in the rumen when fed in excess. Thiomolybdates complexes copper at both the gastrointestinal and tissue level rendering it unavailable to the animal (Allen and Gawthorne, 1987; Gooneratne *et al.,* 1989; Suttle, 1991). Disorders associated with a simple or induced (high molybdenum and sulphur) copper deficiency include anaemia, diarrhoea, depressed growth, change of hair colour, neonatal ataxia, temporary infertility and weakness, fragile long bones which break easily (Underwood, 1981). Herd (1997) indicated that there is concern that trace elements may be limiting production in better managed herds to a much greater extent than previously recognised. Sub clinical trace mineral deficiencies in cattle may be a larger problem than an acute deficiency, because specific clinical symptoms are not evident to allow the producer to recognise the deficiency (Wikse, 1992). Animals with a sub clinical status can continue to reproduce or grow, but at a reduced rate, with decreased feed efficiency, and a depressed immune system (Nockles, 1994). Correcting sub clinical mineral deficiencies in animals that have been nutritionally stressed may have a positive economic impact on cattle production efficiency. The trace elements are involved as component parts of many tissues, and one or more enzyme activities and their deficiency leads to a wide variety of pathological consequences and metabolic defects as shown in Table 2.

Table 2: Principle pathological and metabolic defects in essential trace element deficiencies (Adapted from McDonald, I.W. 1993).

Deficiency:	***Pathological consequences***	***Associated metabolic defect***
Copper	*Defective melanin production*	*Tyrosine/DOPA oxidation*
	Defective keratinisation; hair; wool	*-SH oxidation to S-S*
	Connective tissue defects	*Lysol oxidase*
	Ataxia, myelin aplasia	*Cytochrome oxidase*
	Growth failure	
	Anaemia	
	Uraemia	*Ureate oxidase.*
Cobalt	*Anorexia*	
	Impaired oxidation of propionate	*Methyl malonyl COA mutase*
	Anaemia	*Tetrahydrofolate methyl-transferase*
Selenium	*Myopathy; cardial/skeletal*	*Peroxide/hydrogen peroxide destruction.*
	Liver necrosis	*Glutathione Peroxidase*
	Defective neutrophil function	*OH; O_2 generation.*
Zinc	*Anorexia, growth failure*	
	Parakeratosis	
	Peri-natal mortality	*Polynucleotide synthesis, transcription, translation?*
	Thymic involution	
	Defective cell mediated immunity	

2.2. Copper

2.2.1: Metabolic function and requirements

Copper is an essential trace mineral which is useful for growth and prevents a wide range of clinical and pathological disorders in all farm animals. Copper is a metallo-component of many biochemical enzymes. Some of these enzymes include ceruloplasmin, cytochrome C, dopamine beta-monoxygenase, lysyl oxidase, copper-zinc super-oxidase dismutase, tyrosinase (Underwood and Suttle, 1999).

Copper levels ranges from 1.1-2 mg/kg of dry matter (DM) with normal contents in pastures usually 2-15 mg/kg DM. Fattening and milking cows require 5 and 10 mg /kg DM of copper in their forage respectively. Copper is present in several feeds with fresh grass usually being poor, whereas brassicas and cereals are excellent sources for sheep. Absorption of copper from forage is determined by events in the rumen, notably by synchronicity of release and its potential antagonists-Mo, S, and iron from diet (Suttie, 1991). Absorbability of Cu reported for various feeds vary for instance- leafy brassica 13 % , cereals 9%, root brassicas 6.7 %, hay 7.3%, silage 4.9% , grazed herbage during wet season 2.5 %, grazed herbage during dry period 1.4% (Underwood and Suttie, 1999). Copper is primarily stored in the liver and is released in circulation if the levels reach maximum and this can lead to toxicosis.

Copper's main function physiologically is in the aid of erythropoeisis where copper is thought to influence absorption, mobilization and utilization in heme synthesis. Copper is also known to protect tissue from oxidant stress compounds. Copper is also important in central nervous system development and deficiency may lead to "sway back" or neonatal ataxia in young lambs. Copper is also involved in the normal functions of immune system and deficiency may lead to

immune incompetence. A copper deficiency is also considered as molybdenum toxicosis.

2.2.2: Assessment of copper levels in ruminants.

The concentration of copper in the liver of ruminants is correlated to the bio-available copper in the liver of ruminants (McDowell, 1992). In sheep, liver contains approximately half of the total copper in the carcass (Langlands, *et al*., 1984). Concentration of copper in the liver is affected by physiological needs (such as pregnancy). In pregnant cows fed 5.5 ppm of copper, liver copper declined continuously until parturition during the 8-week non lactating period. However, dietary supplementation of 10 ppm cu prevents the decline in liver cu (Xin *et al.*, 1993). Liver of newborn ruminants normally contains high concentrations of copper (more than 200 mg of Cu/kg of liver DM (Hidiroglou and Williams, 1982; Branum *et al.*, 1998). Lambs with swayback disease had 17mg cu/kg liver, compared to normal lambs with 109 mg cu/kg liver (McC.Howell and Davison, 1959). When copper intakes of animals are less than physiological needs, concentrations of copper and activities of ceruloplasmin (Cp) in plasma are not consistently reduced until liver copper is <40mg/kg (Claypool *et al.*, 1975; Mills, 1987). Engle *et al*., (1964) found a significant correlation between concentrations of copper in liver and plasma. Claypool *et al.*, (1975) suggested that plasma copper values of 0.5 µg/mL or less are indicative of low stores of cu in liver.

Factors other than copper intake affect the concentrations of copper in plasma. Copper in serum was higher at oestrus than at day 21 in nulliparous heifers (Small *et al.*, 1997) and depressed in beef cows on the day of calving (Small, 1997). Xin *et al.*, (1993) found plasma cu was lowest at 5 week prior to parturition. Serum copper values are increased by infection (as it is an acute phase

protein) in all species (Etzel *et al*., 1982). Copper metabolism is also influenced by genetics, and significant differences exist among breeds of sheep in concentrations of cu in plasma and liver (Woolliams *et al*.,1985).

2.2.3: Interactions of copper with other elements.

Intakes of zinc, iron, molybdenum and sulphur affect copper utilization (McDowell, 1992). Large intakes of zinc reduce concentrations of copper in plasma and liver of cattle and sheep (Ott *et al*.,1966a; Kincaid *et al*., 1976; Kellog *et al*., 1989). Dietary molybdenum can inhibit uptake and utilization of copper. In the rumen, molybdenum combines with reduced sulphur to form tetrathiomolybdate that binds copper and prevents its absorption. Other thiomolybdates and molybdate are absorbed into blood and bind endogenous copper to render it unavailable for metabolic purposes (Mason, 1982). In plasma, molybdenum is bound to protein, removes copper from liver, and exacerbates urinary copper loss, although some of the Cu-Mo complex accumulates in the kidney (Kincaid and White, 1988). The presence of a Cu-Mo complex in plasma explains why plasma cu has been reported to increase in ruminants consuming Mo (Suttle and Field, 1968) even though liver copper is being depleted and ceruloplasmin activity is reduced (Kincaid, 1980). The Cu-Mo complex in plasma is precipitated by trichloroacetic acid (TCA; Paynter, 1982) thus plasma samples should be treated with TCA prior to copper determination to prevent an overestimate of the copper status.

In plasma, 70 to 90% of the copper is associated with ceruloplasmin. Accordingly, the activity of ceruloplasmin is closely correlated with serum copper in cattle ($r = 0.83$) and sheep ($r = 0.92$); Blakely and Hamilton, 1985). Ceruloplasmin is very stable and retains activity in samples during shipment and

handling. Activities of ceruloplasmin increase sharply around parturition (Kincaid and White, 1988) and as an acute phase protein, ceruloplasmin increases during infection (Etzel *et al*.,1982) unless the cows have a marginal or low copper status (Erskine and Bartlett, 1993). Activities of ceruloplasmin are 18 to 35% lower in serum than in plasma and concentrations of copper are approximately 14% lower in serum than in plasma (Kincaid *et al.,* 1986). Erythrocytes have a labile fraction of copper that is loosely bound to protein and a more stable copper fraction that includes super oxide dismutase (SOD). Approximately, 60% of the copper in erythrocytes is associated with SOD. Activity of SOD is not a sensitive measure of copper status because SOD activity does not fall with deficient intakes of copper until after plasma copper and ceruloplasmin are reduced (Andrewartha and Caple, 1980; Ward and Spears, 1997; Gengelbach and Spears, 1998). Cytochrome oxidase activity in neutrophils and other tissues have been suggested as a potentially useful marker of copper status. However, cytochrome oxidase activity in circulating leucocytes of sheep and cattle declined more slowly than plasma copper or ceruloplasmin and therefore was less sensitive to cu status (Boyne, 1978). Neutrophils isolated from heifers fed approximately 7 ppm copper were less effective at killing ingested bacteria than neutrophils of heifers supplemented with 20 ppm cu, although phagocytosis and super oxide production were not affected (Torre *et al.*,1996). Thus, the killing ability of neutrophils may have a useful role in copper assessment.

2.2.4: Copper deficiency.

Copper deficiency results in a depletion of circulating copper and hepatic stores. In most cases anaemia is present and iron absorption is limited. It has been associated with a decrease in cytochrome oxidase and impaired phospholipid synthesis. All animals

except pig show greater or lower degree of decreased hair pigmentation but sheep seem particularly sensitive. In addition wool loses its crimp during periods of copper depletion. Copper plays a role in connective tissue metabolism and both collagen in bone and elastin in arteries are defective when there is a copper deficiency. The defect is associated with a decrease in cross- linking between molecules so that the polymers do not have the requisite structural function (Hill et *al.,* 1967).

In beef calves, low copper status has been associated with the development of abomasal ulcers. Calves with abomasal ulcers had 45 to 48 µg of cu/g of liver compared to control calves with 245 µg of cu/g (Lilley *et al.,* 1985). These investigators suggested the effect could be mediated through impaired immunity in copper deficient calves or structural weakness in abomasums of copper deficient calves. Lysyl oxidase (LO) is a copper dependent enzyme involved in the formation of cross-linkages in collagen and elastin that gives structural strength to these tissues (Gallop *et al*., 1972). Reduced growth rate and anaemia is particularly associated with copper deficiency secondary to high intakes of dietary molybdenum and sulphur (file://A:/VEIN Sheep Health and Production.htm). Copper deficient animals are listless, show depigmentation of skin, stiff gait, anaemia and diarrhoea (Mills *et al.,* 1976). The serum haemoglobin values in copper deficient animals are significantly lower than that of animals on copper rich diet.

Copper is essential for erythrocyte production (Radostits *et al.,* 2000). Sharma et *al.* (2003) reported low haemoglobin in copper deficient animals. Cordoso *et al*. (2001) reported hypochromic anaemia related to copper deficiency. Saba *et al.* (1999) reported significant increase in haemoglobin level after mineral supplementation. The anaemia of copper deficiency is caused by breakdown in the intracellular iron metabolism

in the liver (Williams *et al.,* 1983). Haemolysis may contribute to the development of anaemia. Signs of oxidative stress inform of Heinz bodies have been reported in copper deficient lambs (Suttle *et al.,* 1987). The survival time of erythrocyte is shorter than normal in the copper deficient pigs (Bush *et al.,* 1956). Sharma *et al*. (2003) have reported a correlation of vitamin E with copper and zinc serum status of the animals. Carvens and Vaden (1994) reported deficiencies of vitamins E in cattle associated with the deficiency of copper. A significant deficiency of hormones tri-iodothyronine and thyroxine (T_3 and T_4) have been observed in copper deficient animals (Bush *et al.,*1956). Copper deficiency impairs secretion of tyrosine hydroxylase and dopamine β-enzyme systems which are both copper containing in hypothalamic neurons. This causes inhibition of synthesis of thyroid hormone releasing factor. The significant reduction in serum copper gives an idea regarding metabolic regulation of the thyroxin hormone (Singh *et al*., 2002). The status of vitamins and thyroid hormones can give the indication of the severity of copper deficiency; these parameters should also be monitored.

The enzymes whose activities are known to be reduced by a low copper status and thus associated with copper deficiency are cytochrome oxidase through its wide involvement in oxidative reactions; monoamine oxidase through its involvement in maturation of collagen and elastin; and ceruloplasmin, an enzyme involved in the utilization of hepatic stores of iron. Ceruloplasmin also promotes the incorporation of iron into the storage protein, ferritin (Saenko *et al.,* 1994). Copper deficiency has been found to cause disturbances in iron metabolism resulting in sequestration of iron by liver (Mills *et al*., 1976). Liver is the main storage organ for copper, hence the first biochemical change is the decline in liver copper concentration (Underwood and Suttle, 1999). Significant reduction in

the candidacidal activity is observed in copper depleted animals (Humphires *et al.*, 1983). In the copper deficiency, the ability of neutrophils to kill the ingested organisms is usually compromised (Jones and Suttle, 1981; Boyne and Auther, 1986; Babu and Failla, 1990). The lack of lysyl super oxide dismutase (LSOD) and consequent increase in cellular oxygen associated with copper deficiency is thought to enhance oxidative damage, sufficiently impairing normal cellular function and decreasing the candidacidal activity.

2.2.5: Therapeutics and Prophylaxis of copper deficiency.

In deficient areas, copper in the form of cupric sulfate can be added to the salt at a rate of 0.5 %. Soil fertilization with copper has been used in Australia and New Zealand but was effective in a high molybdenum area in Nevada in either reducing molybdenum or increasing copper content of feeds (Spencer *et al.*,1958). Treatment of pasture or crops with copper has not been extensively used in Kenya. It is likely that animals that are receiving concentrated diet and are not restricted to feed produced in copper deficient soils will receive adequate copper. For this reason poultry feeds rarely require supplemental copper. If cattle and sheep on copper deficient range or pasture are not receiving adequate copper, oral drenching with a soluble copper form will provide copper for several months. Single parenteral injections of organic copper forms such as Cu-Ca ethylene diaminatetra–acetic acid (EDTA) or cu methionate will alleviate deficiency signs. Oral dosing of copper as oxidised copper wire particles is the most effective method for prophylaxis of copper deficiency in sheep. The particles, administered in a gelatine capsule (cuprax, Pitman-Moore), are retained in the abomasum and release copper slowly through acid solubilisation of the copper. Up to 2.5 g cupric oxide raise liver

copper levels for 10 weeks and develop sufficient stores to maintain adequate copper status for a further 20 weeks (Judson *et al*., 1982).

2.2.6: Copper toxicity.

Copper toxicity is depended on dietary copper levels as well as the concentration of molybdenum, sulphur, iron and zinc in the diet. Therefore, a single toxic level under field conditions cannot be determined. Sheep, however, are more susceptible to copper toxicity and chronic copper poisoning occurs in several high copper regions. It is characterized by a continuing accumulation of copper in the liver until a "breaking point" is reached. Copper is swiftly mobilized. A haemolytic crisis with icterus develops and there are hepatic necrosis and renal dysfunctions (Judson *et al*., 1982).

2.2.7: Treatment and control of copper toxicosis.

Toxic copper effects have been reported when 250 mg/kg of dry matter (DM) of copper is used as an oral drench. The harmful effects are probably due to a lack of zinc and iron in the body. The addition of 130 mg/kg DM iron has prevented harmful effects when using 250 mg/kg DM of copper in the diet. However, in piglet, 170 mg/kg DM, is needed to counteract 250 mg/kg DM of copper in the diet. Diets high in calcium may reduce zinc availability, which in turn could reduce the levels at which copper is toxic. Copper toxicity can be controlled by restricting the use of 250 mg/kg DM of copper to 100-125 mg/kg DM, or by using a sulphide in the diet. Based on the present knowledge, the safest procedure would be to use copper at a level no higher than 125-150 ppm in the diet, since it gives the same effect as 250 mg/kg DM (file://A:/Copper for Animals. htm,2006).

2.2.8: Treatment of chronic copper poisoning

This can be achieved by increasing the levels of molybdenum, iron and sulphur in the diet. Chronic copper poisoning can also be treated by parenteral or oral administration of high levels (30 mg/kg DM) of either molybdenum, zinc, iron or sulphur (Humphires *et al*., 1983).

2.3. Zinc

2.3.1: Metabolic function and requirements

Zinc is distributed throughout the body with high concentration in muscle, hair, wool, male reproductive fluids and the tapetum lucidum. Apart from copper, zinc is one metal which is more heavily used in metalloenzymes. It is a component of several enzymes such as carbonic anhydrase and alkaline phosphatase. Zinc is also essential for many normal functions in the body. Perhaps the most basic is RNA synthesis. Zinc is also important in the improvement of appetite of farm animals as well as reproductive performance. It is also known to protect pigs from "dermal parakeratosis". Therefore, normal growth and repair are dependent on adequate zinc. Mean zinc concentration levels in pastures are 36 mg /kg of dry matter (DM) (Judson et *al.*, 1982). Values between 7-100 mg/kg are common in most pastures and a good proportion of pastures have between 25-50 mg /kg DM (Minson, 1990). Zinc in cereal has low absorbability for pigs and poultry (Judson et *al.*, 1982). Pagot (1992) reports levels of zinc in forage to range from 1-112 mg /kg DM with normal pasture content of 15-60 mg /kg DM. Fattening and milking cows require in each case 30 mg/kg DM of zinc in pasture (Judson *et al*., 1982). Dietary calcium interacts with zinc and influences its absorption. Cattle consuming diets with less than 1.2 ppm dietary zinc content have depressed concentrations of zinc in plasma within 36 hours (Mills *et al*., 1976).

2.3.2: Assessment of zinc

Neathery *et al*., (1963a) found plasma zinc was reduced (0.79 Versus 0.96 ppm Zn) after 6 weeks in cows fed 17 versus 40 ppm Zn. Sheep deficient in zinc had serum zinc values of 0.44 µg/mL, and serum zinc increased to 0.78 µg/mL when they were given a zinc oxide supplement (Suleiman *et al.*,1998). The zinc deficient sheep also had reduced alkaline phosphatase activity and increased lactate dehydrogenase and displayed wool biting. McDowell *et al*. (1991) surveyed 11 dairy goat herds in Florida and found plasma zinc was lower in goats with seasonal dermatosis (0.54 Versus 0.83 µg/mL). Previously, Neathery *et al*., (1973b) reported that goats fed 4 ppm zinc had 0.62 µg of Zn/mL of plasma and showed signs of zinc deficiency. Dietary concentrations of 600 ppm Zn nearly doubled the concentration of zinc in plasma of calves (Ott *et al*., 1966b; Stake *et al*., 1975). Heifers responded with higher plasma zinc than steers (Ott *et al*.,1966b). Lambs fed diet with 500 ppm supplemented zinc had 1.22µg of Zn/mL of plasma, compared to 0.95µg of Zn/mL of plasma in control lambs (Ott *et al.*, 1966a). Concentrations of zinc in plasma fluctuate with age, stress, infection, and feed restriction. Plasma zinc is very high (2.3 µg/mL) in newborn calves and drops to 1.2 µg/mL by 12 week of age (Kincaid and Hodgson, 1989). Plasma zinc, as part of an acute phase response, is initially reduced by infection (Wellinghausen and Rink, 1998), only to become elevated within a few days. Serum zinc also is decreased by hyper thermal stress and ketosis in cows and is increased in cows with mastitis and in older cows (Wegner *et al.*, 1973).

The relationship between zinc intake and concentrations of zinc in liver is affected by age of the ruminant (Kincaid *et al.*, 1976). Calves readily absorb and bind large amounts of zinc as metallothionein (MT) in liver in response to

elevated zinc intakes (Kincaid *et al.,* 1976). For example, diets supplemented with 600 ppm zinc fed to young calves caused zinc in liver to increase by 600% but did not affect zinc in liver of mature cows (Kincaid *et al.,*1976). Once the added zinc is removed from the diet of calves, concentrations of zinc in liver return to normal within a few weeks (Kincaid and Conrath, 1979). There are four isoforms of metallothionein (MT), and increased dietary zinc increases induction of both MT-1a and MT-11 mRNA in liver and kidney tissue but not in the duodenum, muscle or skin. Increased concentrations of MT-1A protein account for most of the increased zinc in liver (Lee *et al*., 1994). Concentrations of MT in serum and erythrocytes may be useful as indicators of zinc status that are less affected by infection. Another potential measure of zinc status includes the unused capacity of plasma to bind zinc. In calves fed 20 or 70 ppm Zn, there was no difference in plasma zinc concentrations, whereas the percentage of unsaturated plasma zinc binding capacity reflected zinc intakes (Kincaid and Conrath, 1979).

Other potential measures in blood for determining zinc status include zinc concentrations in lymphocytes, granulocytes, and platelets. In human, these measures were shown to be more responsive than plasma zinc to zinc status (Prasad, 1998). Among the various roles for zinc in immunity are gene expression, mitosis and apoptosis of lymphoid cells because DNA polymerase, the major enzyme regulating DNA replication, is Zn-dependent (Shanker and Prasad, 1998). Proliferation responses of macrophages, T-cells, or B cells may have use as early indicators of zinc status. Engle *et al*.,(1997) found the cell mediated immune response to phytohemagglutinin was reduced in calves fed 17 ppm zinc compared to calves fed 40 ppm zinc, even though zinc in plasma and liver were not affected. Radioactive zinc given orally or intravenously reach peak

concentrations in the liver within a few days, but concentrations in red blood cells, muscle, bone and hair do not peak for several weeks. Zinc is present in many enzyme systems which are concerned with the metabolism of feed constituents. For example, zinc is a constituent of carbonic anhydrase, carboxypeptidase A and B, several dehydrogenases, alkaline phosphatase, ribonuclease and DNA polymerase. Zinc is required for normal protein synthesis and metabolism, and it is also a component of insulin so that it functions in carbohydrate metabolism. Because zinc plays so many important roles in the body, it is required by all livestock and poultry (file://A:/Zinc for Animals.htm, 2006).

2.3.3: Interactions

Zinc interacts with several metallic ions. Both iron and copper reduce zinc absorption. Cadmium and zinc compete with each other. Zinc is not well absorbed, but this depends on body needs. Excess levels of zinc are excreted via the pancreatic juice into the intestine. Zinc is present in most plant products. It is fairly high in milk and animal by-products, especially fish meal. Colostrum has high zinc content. There are not wide geographic deficiencies of zinc as those that occur with selenium and iodine. Most concentrates contain marginal levels: sugar beet products are particularly low (Jones, 1977).

2.3.4: Deficiency

Depressed growth and altered epithelial cell metabolism are the main signs of deficiency (less than 30 mg/kg DM). Also anorexia, skin abnormalities, skeletal and reproductive disorders. Parakeratosis occurs in pigs and cattle on zinc deficient diets. Parakeratosis is a hyperkeratinisation of epithelial cells of skin and oesophagus. Other

keratinized tissues i.e. hoofs, wool, feathers and horns are malformed. Wound healing is impaired in zinc deficiency as in reproduction. Both of this latter signs are indicative of basic role of zinc in RNA synthesis (Jones, 1977).

2.3.5: Prophylaxis and Therapeutics of zinc deficiency.

Zinc should be added to concentrate rations as a precautionary measure. It can be accomplished by zinc salts in the salt mixture as well as incorporation in pelleted concentrates. Parakeratotic lesions may be treated superficially with ointment such as zinc oxide (Jones, 1977).

2.3.6: Toxicity

Zinc is relatively non-toxic. Dietary concentrations must be greater than 100 mg/kg DM of feed to cause toxicity. Feeds however, are generally in palatable when zinc concentrations are this high: most cases have been experimentally induced. Animals show inappetance and anaemia. Changing the diet will remedy the condition (file://A:/Zinc for Animals.htm). Allen *et al.*, (1986) identified three therapeutic uses of zinc which could lead to intoxication of sheep with this element. These are the use of zinc against facial eczema, against lupinosis and in the treatment of foot rot in sheep. They reported the death of 19 of 100 treated weaners, 14 within 24 hours of oral treatment with 3 mg of zinc. At necropsy, there was marked necrosis and a lime green discolouration of the mucosa of the abomasum and duodenum.

2.4. Cobalt

2.4.1: Metabolic function and requirements

Cobalt is a core element of vitamin B_{12} or cyanocobalamin, which was isolated in 1948 and was recognised as the reason why liver consumptions could

cure pernicious anaemia in humans. (file://A:/Cobalt for Soil and Animal Health.htm, 2006). In Australia and Scotland, it had been associated with coast disease and wasting disease of sheep respectively (file://A:/Cobalt for Soil and Animal Health.htm). Cobalt is a constituent of vitamin B_{12} which has 4.4% of cobalt (Underwood and Suttie, 1999). Vitamin B_{12} is synthesized by ruminants in their rumen by micro-organisms. It is poorly stored in the liver and exists as two distinct forms, methylcobalamin and adenosylcobalamin. Methylcobalamin (MeCbl) where cobalt assists a number of methyl transferase enzymes by donating methyl groups and thus involved in one carbon metabolism i.e. building of carbon chains. Methylcobalamin is important for microbes, as well as for mammals and is needed for methane, acetate and methionine synthesis by rumen bacteria.

As adenosylcobalamin (Ado cbl), cobalt influences energy metabolism facilitating the formation of glucose by assisting methylmalonyl co-enzymes A (COA) mutase to form succinate from propionate in the liver. Cobalt is also important for proper growth of animals and those pastures deficient can lead to poor growth (Young, 1979). Legumes are usually richer in cobalt than grasses grown in the same conditions (Pagot, 1992). Non- ruminant animals have to ingest food with cobalt to supply vitamin B_{12} even though cobalt deficiency is rare in these animals. Cobalt in the other hand ranges from 0.02 -4.2 mg /kg DM, with normal pasture content of 0.05 -0.3 mg/kg DM. Fattening and milking cows require 0.10 mg /kg DM in pasture (Pagot, 1992). Levels of cobalt below 0.07 mg /kg DM in pastures exposed to ruminants for several months may require intervention by supplementation (Underwood and Suttie, 1999).

2.4.2: Assessment of cobalt

Because the metabolic role of cobalt is as a component of vitamin B_{12} (Smith, 1997), assessment of cobalt nutriture often centres on measures of vitamin

B_{12} status, although concentrations of cobalt in liver and performance response of ruminants to cobalt supplementation can also be used in assessment (McDowell, 1992). Vitamin B_{12} is a cofactor for the enzyme methylmalonyl-CoA mutase that catalyzes the conversion of methylmalonyl-CoA to succinyl-CoA (Smith, 1997). In a vitamin B_{12} deficiency, methylmalonic acid (MMA) accumulates, and the elevated concentrations of MMA in plasma (Rice *et al*., 1989; Kennedy *et al.*, 1991) and urine (Quirk and Norton, 1988) may be used for differential diagnosis. Sheep are more sensitive to the effects of a cobalt deficiency than are cattle (Kennedy *et al*., 1995). The upper limit for normal MMA in plasma is 2.0 µmol/L in cattle (Paterson and Macpherson, 1990) and 5.0 µmol/L in sheep (Rice *et al.*, 1989). Concentrations of MMA in plasma are elevated in the early stages of a cobalt deficiency in sheep, preceding the onset of loss of production and clinical signs of the disease (Rice *et al*., (1989). Although some MMA may arise from the rumen, increased MMA still indicates a functional B_{12} deficiency (Rice *et al.*, 1989; Paterson and MacPherson, 1990).

Concentrations of B_{12} in the liver, but not in serum, reflects the body's reserve. Serum B_{12} concentrations are not a good measure of B_{12} status because they change very rapidly with cobalt intake and they also are increased by liver disease, stress and starvation (Paterson and MacPherson, 1990). Thus, the correlation between vitamin B_{12} in serum and liver is often low unless the sheep are severely deficient. Vitamin B_{12} concentrations in plasma are a passive marker of cobalt and vitamin B_{12} status, because vitamin B_{12} in plasma is bound to transcobalamins that have no active role. During the assay for vitamin B_{12} in plasma of cattle, a large proportion of total plasma B_{12} is not released from transcobalamin 1, the principal B_{12} carrier protein in bovine plasma (Price *et al*.,

1993). Another measure of vitamin B_{12} status arises from the enzyme methionine synthetase, which transfers methyl groups in the folic acid cycle (Smith, 1997). A deficiency of vitamin B_{12} subsequently impairs conversion of formiminoglutamic acid (FIGLU) to glutamic acid (Smith, 1997). Hence, FIGLU accumulates and the increased concentrations of FIGLU in urine is an indicator of cobalt deficiency (Quirk and Norton, 1988). Finally, in a vitamin B_{12} deficiency, there are increased concentrations of branched chain fatty acids in the carcass (Kennedy *et al*., 1994). No suggestion has been made concerning how to incorporate this change in fat deposition into the assessment of cobalt status.

2.4.3: Source and occurrence of cobalt.

Cobalt deficiency is common along the rift valley in Kenya and is seasonal in character with symptoms usually appearing after the rains when grazing is plentiful and green (Hudson, 1944). Mwakatundu (1977) also confirmed cobalt deficiency in the Egerton, Kabete and Molo areas of Kenya. Australia, New Zealand and portion of Africa have cobalt deficient areas. In North America cobalt deficiencies have been reported around the great lakes and in new England and Florida. In regions where cobalt is plentiful in the soil, it is provided to animals upon plant ingestion (Jones, 1977).

2.4.4: Deficiency

It is rare for grasses to contain cobalt in concentrations that meet the demands of grazing animals (Hodgson *et al.*, 1962). When the content in the pastures is 0.10 mg/kg of DM or less (Hodgson *et al*., 1962) grazing animals are likely to suffer from cobalt deficiency. In Kenya, the condition is known as "Nakuruitis" (French, 1952; Howard, 1963) or as "Narurasha" among maasai

herdsmen (Hudson, 1944; French, 1952). In Great Britain, "pine", "bush sickness" in New Zealand and "grand traverse disease" in the United States (Brander, 1982).

Cobalt deficiency can lead to increased susceptibility of the gastro-intestinal nematode, *Ostertagia circumcita* in cattle (MacPherson *et al* 1987). Ruminants in cobalt deficient areas become weak and emaciated and show progressive inappetance and anaemia. Under marginal deficiency conditions, there is a decrease in production that is most noticeable in young animals in the spring when plant growth is most rapid. Cobalt is poorly absorbed from the digestive tract. Ovine white liver disease (OWLD) occurs in New Zealand (Sutherland *et a*l., 1979), Victoria (Mitchell *et al.*, 1982), Western Australia (Richards *et al.*, 1981), and Tasmania (Mason *et a*l., 1983). The disease appears to be a vitamin B_{12} deficiency complicated by liver damage. It principally affects lambs 2 to 6 months of age causing severe ill-thrift, hepatopathy, depression, serous ocular discharge, crusty lesions on the ears probably as a result of photosensitization, high morbidity with mortality rates of 10 to 15% or, rarely, over 80% (Richards *et al*., 1981). At necropsy, the liver is pale and, in some cases, dusty white, and very swollen. Lipidosis is usually present.

2.4.5: Prophylaxis and Therapeutics

Marston, (1969) found out that heavy cobalt pellets devised by Dewey, Lee, and Marston (1948) introduced into the rumen of sheep, either singly or accompanied by an abrasive steel grinder or by a second pellet were equally as effective, over a period of more than five years, as a supplement of 1mg co/day per os in maintaining health, body weight, and adequate concentrations of vitamin B_{12} in liver and serum of sheep fed on a cobalt deficient ration in pens.

Cobalt deficiency can be prevented by a number of methods using cobalt salts or oxides in the fertilizers. Oral supplementation or drenching is possible with dilute cobalt solutions. Sheep can be dozed twice each week with 2 mg of co or once each week with 7 mg of co. Manufacturers of anthelmintic drenches commonly add co to their products but the amount added range widely (for lambs) and give short lived increases of plasma vitamin B_{12} concentration (Field *et al*, 1988). Amounts in anthelmintic preparations cannot be relied upon to alleviate very severe clinical deficiency when given every month as part of worm control program (Field et *al*, 1988).

2.4.6: Toxicity

Cobalt is relatively non-toxic and toxicity is not recognized under natural conditions. Overdoses may result in depressed appetite, weight loss, and anaemia (due to hemolysis), which are strangely analogous to the description of deficiency (Jones, 1977).

2.5. Selenium

2.5.1: Metabolic function and requirements

Selenium is a dietary essential with a bad name. For years selenium toxic manifestations have drawn more interest than its role in nutrition. Selenium is absorbed from the upper gastro –intestinal tract and is distributed throughout the body. Non-ruminants absorbs a greater percentage of dietary selenium than ruminant, apparently because a portion of selenium is reduced to an insoluble form in the rumen. Following absorption, excess selenium is excreted via the kidneys. Selenium has been shown to be effective in prevention and treatment of a number of necrotizing diseases of domestic animals. It is necessary for growth and fertility and has an interaction with vitamin E that allows each to partially but not completely substitute for the other. Since both selenium and vitamin E have anti-oxidant properties scientists have tried to

relate their protective functions to this ability to prevent membrane damage. Hoekstra (1974) summarized work that established one function of selenium as a components of the enzyme glutathione reductase. He further postulated that selenium and vitamins E work together to decrease lipid peroxidation. Glutathione peroxidase functions to enhance the reaction of reduced glutathione(GSH) with hydrogen peroxide, which oxidizes the glutathione to oxidised glutathione (GSSG) and forms water. The result is less hydrogen peroxide available to cause lipid peroxidation. A decreased rate of lipid peroxidation lengthens the life of cellular membranes.

There may be other specific actions of selenium in the body but this function (ant oxidation) can adequately explain many of the symptoms seen during deficiency states. Selenium concentrations vary in feeds depending on many factors in the forage. Areas with low selenium deficiency have < 0.05 mg /kg DM of Se in forage and can be as low as 0.02 mg /kg of DM even though pasture concentrations may not be the best index of risk (Whelan *et al* 1994). Legumes contain less selenium than grasses but the difference diminishes as the soil selenium status declines (Minson, 1990.) Levels in cereals can be as low as 0.006 mg/kg DM of Se in some plants. Pagot (1992) reports selenium levels in forage from 0.01-400 mg/kg DM of Se with normal content in pasture between 0.03 -0.15 mg/k DM. Fattening and milking cows require >0.03 mg /kg of DM of Se in pasture.

2.5.2: Assessment of selenium

Measures for estimating the selenium status of livestock include concentrations of selenium in the liver, serum, and whole blood; glutathione peroxidase (GPx) activities in erythrocytes and liver; and mRNA levels for GPx or hydro peroxide glutathione peroxidase (Kincaid, 1995). In serum of cows, selenium is associated with albumin, GPx, and selenoprotein P (Awadeh *et al*.,

1998). These various measures can lead to different interpretations unless the level and chemical form of the dietary selenium are considered. Whole blood has a selenium concentration that is approximately three times higher than that in serum (Scholz and Hutchinson, 1979) and is often better for selenium determination because any hemolysis of the erythrocytes will cause serum to have a fake high value for selenium (Maas *et al.,* 1992). Concentrations of selenium in whole blood are responsive to selenium intake (Levander, 1986). Clinical deficiencies in ruminants are associated with values of <30 ng of Se/mL of whole blood (Sheppard *et al.,* 1984; Pherson *et al*., 1986). Based on mastitis resistance, Smith *et al*., (1988) recommended that whole blood contain at least 200 ng of Se/mL. For plasma, most researchers (Pehrson *et al.,* 1986; Smith *et al.,* 1988; Gerloff, 1992) recommend that selenium exceed 70 ng/mL

Concentrations of selenium in whole blood of newborn calves and their dams are highly correlated ($r = 0.74$, $P < 0.05$; Kincaid and Hodgson, 1989). Cows in late pregnancy need 3 to 5 mg of Se/day to ensure adequate selenium reserves in tissues of newborns (Abdurrahman and Kincaid, 1995). Relatively large amounts of selenium are transferred from the dam to the foetus during the last trimester of pregnancy; therefore, selenium levels in maternal blood are reduced unless selenium intakes of cows exceed 3 mg/day. The efficiency of maternal transfer of selenium to the foetus is affected by the chemical form of selenium in the diet. Compared to the selenite, more selenium from selenomethionine is transferred from the dam to the foetus and into milk (Kincaid and Rock, 1999; Knowles *et al.,* 1999).

Concentrations of selenium and activities of GPx are highly correlated ($r = 0.92$, $P<0.001$, in blood of sheep and cows ($r = 0.59$, $P<0.001$; Thompson *et al.,*

1976), but the correlation coefficients are reduced by selenium supplementation. Activities of GPx reported between laboratories are variable because the assay is difficult to standardize and the enzyme is subject to deterioration with shipment (Stowe and Herdt, 1992). There also is considerable inter-laboratory variation in selenium determinations; reported ratios of selenium in whole blood to serum range from 3.3:1 to 1.8:1 in replicate samples (Waldner *et al*., 1998). Between 4% and 9% of total body selenium is in he liver of sheep (Langlands *et al.*, 1984), and liver tends to have the highest concentration of selenium among tissues. Hence, liver selenium concentrations are used as a measure of selenium status in ruminants. However, the largest body burden of selenium is found in muscle.

2.5.3: Interactions

Sulphur seems to exacerbate marginal selenium deficiencies. The effect is not strong enough to be of value in preventing toxicity (Waldner et *al*., 1998). .

2.5.4: Source and Occurrence

There are major geographic regions where selenium toxicity or selenium deficiency occurs. Often they are adjoining areas (Muth and Allaway 1963). The occurrence of selenium in plant is a function of the concentration of selenium in the soil and the presence of selenium accumulators which are plants that make selenium more available. Confinement rearing of swine decreases their vitamin E intake and makes marginal selenium deficiency more critical (Ullrey, 1974).

2.5.5: Deficiency

Deficiency of selenium has been known to lead to muscular degeneration in lambs and calves in Oregon (Schubert *et al.*, 1961) and New Zealand. Glutathione peroxidase is a selenium protein (Rotruck *et al*., 1973). Several other seleno –protein containing selenium and systems are now known (Arthur and Beckett, 1994; Arthur, 1997).

Selenium is also known to interact physiogically with vitamin E. In young pigs diatetica is seen on selenium deficient diets. Necrotic liver lesions are present in addition to muscle degeneration in ruminants. In chicks the disease is called exudative diathesis and is characterized by oedema, subcutaneous haemorrhages, progressive weakness and death as well as the more common muscle lesions. Decreased fertility and birth of dead or weak offspring may occur in all species.

2.5.6: Prophylaxis and Therapeutics

Selenium can be supplied to animals in a number of forms. Oral drenches, as sodium selenite concentrate or addition to anthelmintic drenches (as organic or inorganic form) or for dilution with water and drenching, are effective short-term (up to 3 months) therapeutic and preventive treatments. Injectable selenium, usually included with clostridial vaccine is frequently used to protect lambs at marking. For adult sheep, intra-ruminal selenium pellets (5% elemental selenium, 95% iron) are effective in raising and maintaining tissues levels of selenium-containing enzymes, including glutathione peroxidase (GSHPx) (Paynter, 1979). Pellets will provide adequate selenium nutrition for 3 to 4 years (Judson *et al.,* 1991) although they may need to be accompanied by a steel grinder to prevent coating of the pellets. Selenium can be applied to deficient pastures as encapsulated selenium (Selcote, Mintech Nz Ltd). When added to phosphate fertilizers, and applied at the rate of 10 g/hectare of selenium, it is a safe and effective method of supplementation which lasts for over 12 months (Halpin *et al.,* 1987).

2.5.7: Toxicity

Selenium toxicity, which occurs primarily in cattle and sheep grazing on alkali soils in Australia, is called "blind staggers", alkali disease, or forage Poisoning

(file://A:/G 2081 Mineral Supplements for Beef Cattle, MU Extension.htm). Certain species of Astragalus growing on seleniferous soils contain 3000 to 5000 mg/kg of DM selenium (Underwood, 1981). While other species may contain only 10 to 20 ppm selenium when grown on the same soil (file://A:/Factors Affecting The Trace Mineral Composition Of Feedstuffs.htm).

Young lambs may be particularly susceptible to selenium poisoning due to incomplete rumen development (Lambourne and Mason, 1969). Signs of acute selenium toxicity include blindness, abdominal pain, excessive salivation, paralysis and death after 1 to 7 days. In a number of field trials, high doses of selenium (0.1 mg/kg live weight) or less resulted in negative responses while, in the same trials, doses of 0.1mg/kg or less resulted in positive responses (McDonald, 1975). Toxic manifestations are not recognised below 5 mg/kg DM Se of diet (Underwood, 1981). Selenium can also be toxic and poisoning can occur naturally by treating animals which already have high selenium stores or by overdosing. Doses of 15 mg have been fatal in lambs of 10 kg live weight. Young lambs may be particularly susceptible to selenium poisoning due to incomplete rumen development (Lambourne *et al*., 1969).

2.6. Analytical techniques for determination of copper, zinc, selenium and cobalt in pastures.

This technique is applicable to copper, cobalt, zinc, selenium, calcium, magnesium, manganese and potassium and is in accordance with the method of journal of Association of Analytical Chemists (JAOAC, 1975). This method involves weighing of one gram sample (dried and ground) into high form porcelain crucible which is then ashed for two hours at 500^0C and then left to cool. The ash is then made wet with 10 drops of de-ionized water. Three to four

millilitres of nitric acid (70%) are then carefully added. Excess nitric acid is then evaporated on an hot plate set at 100 to 120^{0}C. The crucible is then returned to furnace and ashed for an additional one hour at 500^{0}C, left to cool and then dissolved in 10 millilitres of hydrochloric acid (70%). The contents are then transferred quantitatively to a 50 ml volumetric flask, where 10 mls of 5% Lanthanum solution is then added and diluted to volume using de-ionized water. The silica is then left to settle, supernatant decanted or filtered and the samples analyzed using flame atomic absorption spectrophotometry. Necessary dilutions are usually made with 10% hydrochloric acid to obtain solutions within the detection range of the instrument.

Other techniques used for determination of trace elements in grass pastures are: Direct reading spectrographic method (J AOAC 36, 411 (1953); 58, 764 (1975), Direct current arc excitation method (J AOAC 36, 1953), Alternating current spark excitation method (J AOAC 36, 1953), Inductively coupled plasma spectroscopic method (J AOAC 68, 499, 1975), Nitro cresol method (J AOAC 34, 710, 1953), Colorimetric method (J AOAC 25, 520 (1942), Mixed and single colour methods (J AOAC 36, 397 (1953), Gravimetric and fluorometric methods (J AOAC 52, 627 (1969). Of all these techniques, only flame atomic absorption spectrophotometry can detect all the trace elements in the study.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1: Selection criteria of study regions and sites

Eight different representative ecological regions in Kenya were conveniently selected for sample collection. These agro-ecological zones were: Coast, Machakos, Nairobi, Kiambu, Nakuru, Eldoret/Uasin-Gishu, Mount Kenya and Homabay/Nyanza. These regions represent areas of either sand, clay, limestone and volcanic soils in which various forages grow. These regions have differences in soil type, mineral concentrations, rainfall amounts and altitude. In each sampling region, five to six sites were selected, with each site being approximately five to ten kilometers away from one another. A total of thirty seven sites were considered in the study.

3.2: Selection of natural pasture grasses

Nine different varieties of pasture grasses were collected for the study. Grass pastures collected were: Napier (n = 32), rhodes grass (n = 30), red oats grass (n = 25), star grass (n = 15), brown hood grass (n = 2), common setaria (n = 11), kikuyu grass (n = 22), sweet pitted grass (n = 15) and sudan grass (n = 1).

3.3: Sampling procedure

Pasture grasses were identified and cut three centimeters from the ground with surgical scissors. They were then packed in brown paper bags containing all the sample details which included the date of collection, name of the region and site of collection and name of grass. The paper bags were then stapled.

3.4: Sample preparation

The various grass samples were spread on thoroughly cleaned and dried benches containing all the sample details. During the preparatory stage of the study, all the windows in the laboratory remained closed and the lights on for 24 hours. This

ensured that the samples were not contaminated by dust particles from the outside environment. Any soil particles on the grass pastures were also removed by proper shaking. Grass samples were then concentrated by chopping them into small pieces less than a centimeter long using clean surgical scissors. They were then packed in brown paper bags containing all the sample details and stapled thrice. The samples were then oven dried for 48 hours at 60^0C. After oven drying, pasture grasses were ground in Wiley mill no. 20 stainless steel sieve. They were then stored in clearly labeled air-tight laboratory containers upon equilibration with the atmospheric pressure for 12 hours. The ground samples were then properly mixed pending assay.

3.5: Determination of copper, cobalt, zinc and selenium in samples

There are nine possible assay techniques used in isolation of trace elements from the sample matrix. These techniques were all validated. The trace elements were then determined using their respective wavelengths. A perkin-Elmer model 2380 flame atomic absorption spectrophotometer was used for this assay. The assay technique that gave the highest positive correlation (r), of 0.997, and a regression equation of $Y=0.046 + 0.021X$, was used for the separation of trace elements from the sample matrix. The technique involved the use of nitric acid and perchloric acid mixture at the ratio of three parts to one respectively.

3.5.1: Chemicals and Reagents used

The chemicals and reagents used were: Spectroscopic standards for copper, cobalt, zinc and selenium (BDH Chemicals, Poole, UK); Double de-ionized water (p^H 6.5), concentrated nitric acid (70%), (Kobian Kenya Limited); concentrated hydrochloric acid (70%), (Molecular Laboratory Chemical, Nairobi, Kenya); perchloric

acid (60%), (Loba Chemie P.V.T. Limited. Mumbai); Lanthanum Chloride (5%), app.6H2O (353.36) (Prolabo 12, rue pele'e F 75011 Paris).

3.5.2: Validation of assay techniques

This was done according to the method of Association of Analytical Chemists (JAOAC, 1975). Two samples, each in duplicate were prepared for isolation/separation and quantification of trace elements using nine different assay techniques. A blank was also included in each assay technique. Matrix similarity was observed in all the samples under validation. Concentration and absorbance, respectively, extrapolated using validation methods were recorded. These techniques were: dry ashing; nitric acid/perchloric acid (3:1); nitric acid/perchloric acid and 5% lanthanum solution; dry ashing/5% lanthanum chloride; dry ashing/5 millilitres of hydrochloric acid; nitric acid/perchloric acid (1:3); dry ashing/10 millilitres of nitric acid; wet oxidation with 5% lanthanum solution; nitric acid/perchloric acid/hydrochloric acid. All the above mentioned techniques were validated as explained below. Concentration in milligram per kilogram of dry matter (mg/kg of DM) and absorbance in per centimetre per gram litre (cm^{-1} g^{-1} litre) were obtained from the machine readings for each of the assay technique as shown below.

3.5.2.1: Dry ashing

One gram of the sample was dry ashed at 560^0C for 16 hours in a porcelain crucible. It was then made wet using 10 drops of double deionized water. Three milliliters of 70% nitric acid was added and the excess evaporated on an hot plate at 120^0C. The sample was then heated at 560^0C for one hour in a furnace, cooled and 10 milliliters of 70% hydrochloric acid added. It was then transferred using double de ionized water into a 50 ml volumetric flask, and tightly covered. Following filtration, trace elements were determined using atomic absorption spectrophotometer and results obtained are shown:

Concentration: 0 09, 0.11, 0.14, 0.16, 2.5, 2.0, 2.8, 2.1

Absorbance: 0.004, 0.005, 0.006, 0.007, 0.34, 0.28, 0.38, 0.29.

3.5.2.2: Nitric acid/Perchloric acid

A mixture of 70% nitric acid and 60% perchloric acid was used in this method at a ratio of 3:1. Twenty milliliters of this mixture was added to a two gram sample in a pyrex beaker. The sample was heated, slowly at first until frothing ceased. It was then heated to white fumes of perchloric acid at a temperature of 120^0C. The sample was then cooled and transferred into a 50 ml volumetric flask using double de-ionized water, filtered, tightly covered and determined using atomic absorption spectrophotometer. The following results were obtained.

Concentration: 0.71, 0.47, 0.96, 0.78, 0.20, 0.17, 0.17, 0.14.

Absorbance: 0.1, 0.067, 0.136, 0.11, 0.008, 0.007, 0.007, 0.006.

3.5.2.3: Nitric acid/Perchloric acid 5% Lanthanum solution

To a one gram sample in a pyrex beaker, 10 milliliters of nitric acid (70%) was added. The sample was then left to soak for 12 hours. Three milliliters of perchloric acid was then added. It was then heated on a sand bath slowly first at 100^0C until frothing ceased. The sample was then heated at 120^0C to produce white fumes of perchloric acid, cooled and 10 milliliters of 5% lanthanum solution added. The sample was then transferred into a 50ml volumetric flask using double de ionized water, filtered, tightly covered and determined using atomic absorption spectrophotometry. The following results were obtained:-

Concentration: 0.13, 0.12, 0.17, 0.17, 6.6, 0.03, 7.6, 7.6;

Absorbance: 0.005, 0.005, 0.007, 0.85, -0.006, -0.95, 0.95 0.95

3.5.2.4: Dry ashing/5% lanthanum chloride

One gram sample in a porcelain crucible was dry ashed at 560^0C in a furnace for 2 hours and then cooled. It was then made wet with 10 drops of double de-ionized water. Ten milliliters of 70% nitric acid was added and the sample heated on an hot plate at 120^0C to evaporate the excess nitric acid. The sample was then heated in a furnace at 560^0C for one hour, and cooled. Ten milliliters of 5% Lanthanum solution was added and the mixture transferred into a 50 ml volumetric flask using double de-ionized water, filtered and determined using atomic absorption spectrophotometer. The following results were obtained.

Concentration: 0.19, 0.13, 0.18, 0.14, 0.06, 0.6, 0.12, 0.11;

Absorbance: 0.008, 0.005, 0.007, 0.006, 0.009, 0.08, 0.017, -0.015

3.5.2.5: Dry ashing/5 millilitres of hydrochloric acid

One gram sample in a porcelain crucible was dry ashed in a furnace at 560^0C for 16 hours, and then cooled. Five milliliters of 70% hydrochloric acid was added and the contents transferred into a 50 ml volumetric flask using double de ionized water, filtered and determined using atomic absorption spectrophotometry. The following results were obtained.

Concentration: 0.04, 0.06, 0.09, 0.04, 0.09, 0.09, 0.3, 0.4;

Absorbance: 0.002, 0.002, 0.003, 0.002, 0.012, 0.013, -0.04, -0.06.

3.5.2.6: Nitric acid/perchloric acid (1:3)

A mixture of 70% nitric acid and 70% hydrochloric acid at the ratio of 1:3 was used in this method. Twenty milliliters of the prepared solution was added to a 2 gram sample in a pyrex beaker. It was then heated to produce white fumes of perchloric acid. The contents were then transferred into a 50ml volumetric flask using double de-ionized water, filtered and determined using atomic absorption spectrophotometry. The following results were obtained.

Concentration: -0.18, 0.03, 0.14, 0.12, 0.65, 0.72, 1.02, 1.02

Absorbance: 0.007, -0.001, 0.006, 0.005, 0.092, 0.105, 0.143, 0.144

3.5.2.7: Dry ashing/10 milliliters of Nitric acid

One gram sample in a porcelain crucible was dry ashed at 560^0C for 16 hours and then cooled on an asbestos plate. It was made wet with 10 drops of double de-ionized water. Ten milliliters of 70% nitric acid was added and the contents heated on an hot plate at 120^0C to evaporate the excess nitric acid. The sample was then heated in a furnace at 560^0C for one hour and cooled on an asbestos plate. Ten milliliters of 70% nitric acid was added. To the contents, 10milliliters of

5% lanthanum solution was added and the mixture transferred into a 50 ml volumetric flask using double de-ionized water, filtered and determined using atomic absorption spectrophotometry. The following results were obtained.

Concentration: 0.06, 0.07, 0.08, 0.08, 0.49, 0.74, 0.53, 1.00

Absorbance: 0.002, 0.003, 0.003, 0.003, 0.069, 0.104, 0.075, 0.140.

3.5.2.8: Wet oxidation (3:1) / 5% lanthanum solution

Ten milliliters of 70% nitric acid was added to one gram sample in a pyrex beaker, and left to soak for 12 hours. Three milliliters of 60% perchloric acid was added and then heated in a sand bath at 120^0C to produce white fumes of perchloric acid. Ten milliliters of 5% lanthanum solution was added to the mixture. The contents were then transferred into a 50 ml volumetric flask using double de ionized water, filtered and determined using atomic absorption spectrophotometry. The following results were obtained.

Concentration: -0.06, 0.06, 0.07, 0.07, 0.57, 0.59, 0.62, 0.63

Absorbance: 0.002, 0.002, 0.00, 0.03, -0.085, -0.083, -0.089, -0.089

3.5.2.9: Nitric acid/perchloric acid/hydrochloric acid

One gram sample was weighed into a pyrex beaker and 10 milliliters of 70% nitric acid added. The mixture was then left to soak for 12 hours. Three milliliters of 60% perchloric acid was added and the mixture heated on a sand bath at 120^0C to produce white fumes of perchloric acid. It was then cooled on an asbestos plate. Ten milliliters of 70% hydrochloric acid was added. The contents were then transferred into a 50 ml volumetric flask using double de-ionized water, filtered and determined using atomic absorption spectrophotometry. The following results were obtained.

Concentration: 0.06, 0.06, 0.08, 0.08, 0.16, 0.13, 1.09, 0.21

Absorbance: 0.002, 0.002, 0.003, 0.003, -0.022, -0.019, -0.156, -0.031

The assay technique that involved the use of nitric acid and perchloric acid at the ratio of 3:1 gave the highest positive correlation compared with the other techniques. It was therefore used for the separation of trace elements from the sample matrix. A calibration curve for the validation technique with the highest positive correlation (Nitric acid/Perchloric acid (3:1), r =0.997) was plotted. Each point on the curve represents three replicate readings. A line of best fit was generated (Figure 1).

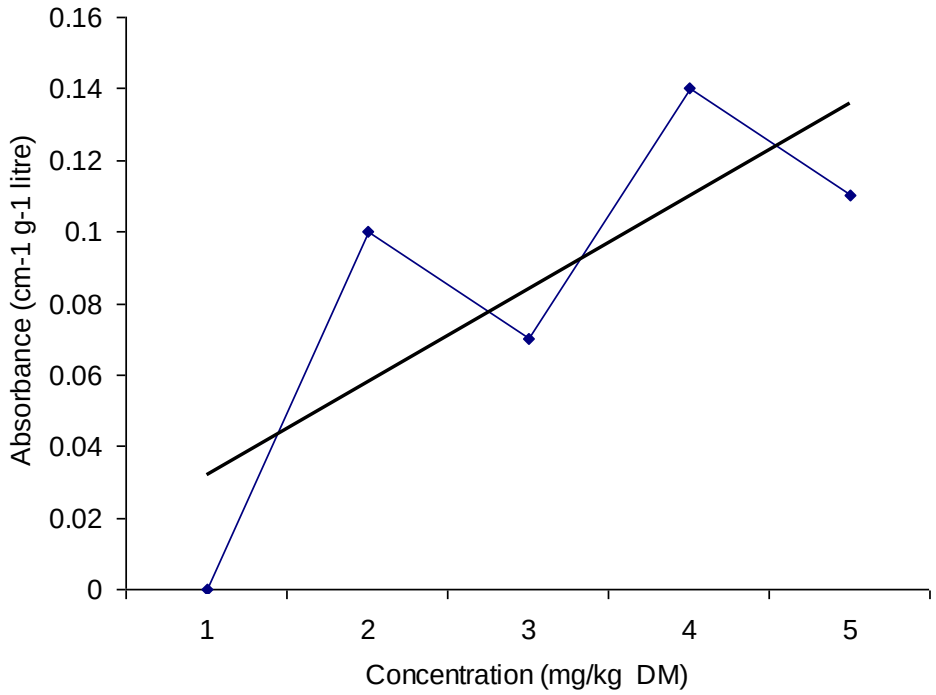

Figure 1: Calibration curve for Nitric acid/Perchloric acid (3:1). R^2=0.997.

3.6. Wet oxidation with some modifications

The technique used for the separation and isolation of the various trace elements was the method of the Journal of Association of Analytical Chemists

(JAOAC, 1975), which involved the use of nitric acid (70%) and perchloric acid (60%) at the ratio of 3:1. The technique was modified to exclude the following: 10 millilitres of 5% Lanthanum solution during the transfer of the cooled sample to a 50 ml volumetric flask; 20 mls of a mixture of nitric acid (70%) and 60% Perchloric acid in the ratio of 3:1 instead of dissolving the sample in 10 mls of 70% nitric acid until it soaks followed by addition of 3 mls of 60% Perchloric acid; two gram sample were used instead of one gram sample (J AOAC 58, 456, 1975).

All the 171 samples were assayed in duplicate using wet oxidation technique. Matrix similarity was observed in all the samples. Two grams of each sample being analysed were weighed using electronic weighing balance. The air-tight containers with the samples under investigation were thoroughly shaken to ensure an even mixture of the sample contents. The samples were then weighed into a 50 ml pyrex beaker. Twenty milliliters of the mixture containing three parts of nitric acid and one part of perchloric acid was then added to the sample. The sample contents in the 50 ml pyrex beaker were left to digest for 12 hours in the fume chamber. The digested sample contents were heated on a sand bath inside a fume chamber. The hot plate heating the sand bath was set at 100^0C at first, and the sample contents heated slowly until frothing ceased.

The sample was then heated at 120^0C until white fumes of perchloric acid were produced. At this point, the trace elements under investigation had been separated from the organic matter. The wet oxidized samples were left to cool for 12 hours on an asbestos plate inside a fume chamber. The sample contents were then transferred into a 50 ml volumetric flask using double deionised water. The

volumetric flasks were tightly covered awaiting spectrophotometric determination of copper, cobalt, zinc and selenium.

3.7: Criteria for selection of concentration of spectroscopic standards

A constant maximum relative error was required in the present study, and the results were to be accurate to within a specified percentage of the analyte concentration. The spectroscopic standards were selected, with the concentration such that:

i) The concentration of the first standard (S_1) was higher than that of the sample with the lowest concentration.

ii) The concentration of the second standard (S_2) was thrice the concentration of the first standard (S_1).

iii) The concentration of the third standard (S_3) was twice the concentration of the second standard (S_2). The most concentrated standard, S3, had greater concentration than the most concentrated sample. Single element standard solutions were used.

3.7.1: Preparation of standards

Single element standard solutions were prepared using spectroscopic standards for copper, cobalt, zinc and selenium. Two (S_1 and S_2) and three (S_1, S_2, S_3) standard solutions containing parts per million of each element were prepared.

3.7.2: Standard curve calibration

Four and three parameter logistic regression was used to calibrate the standard curves. A blank (auto-zero), S1, S2, S3, (0 ppm, 5 ppm, 15 ppm and 30 ppm) were used to calibrate standard curve for zinc. The standard curve for copper was calibrated using two standards: 5 ppm and 15 ppm; cobalt: 3 ppm and 9 ppm and

selenium curve was calibrated using three standards: 30 ppm, 90 ppm and 180 ppm. A specific blank for each element was used to auto-zero the standard curve. Matrix similarity was observed in all the blanks for copper, cobalt, zinc and selenium.

3.7.3: Standard calibration curves for zinc, copper, cobalt and selenium

Standard calibration curves for zinc, copper, cobalt and selenium were plotted. For all the curves, a line of best fit was generated. Each point on the curve represented three replicate readings.

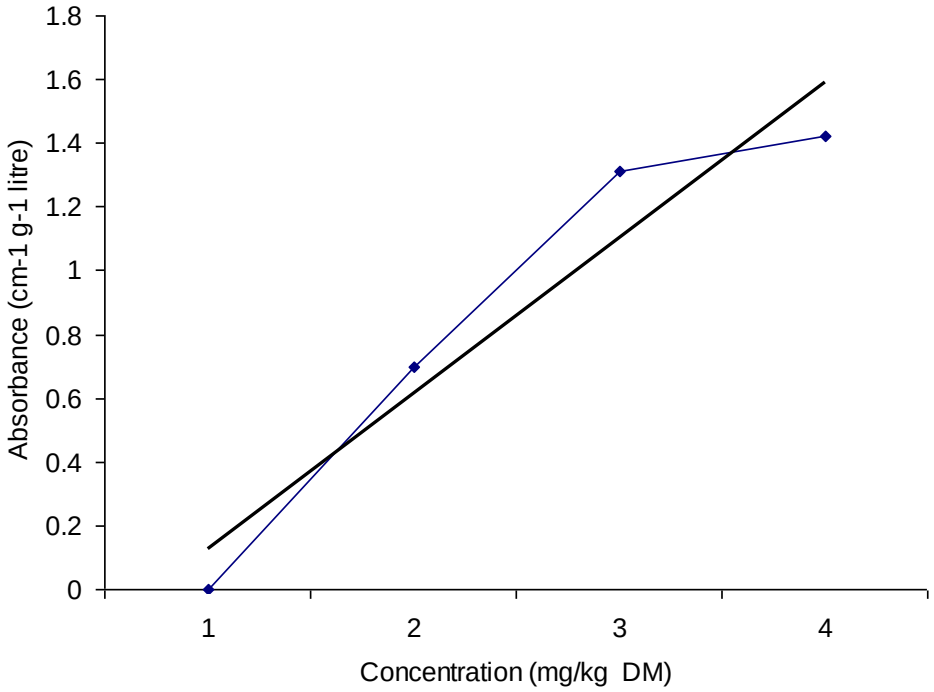

Figure 2: Standard calibration curve for zinc.

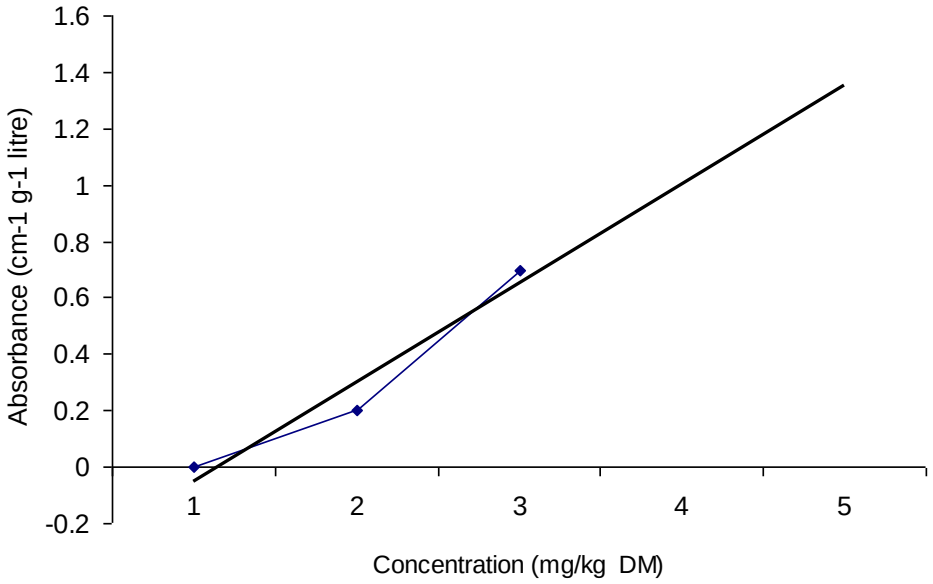

Figure 3: Standard calibration curve for copper.

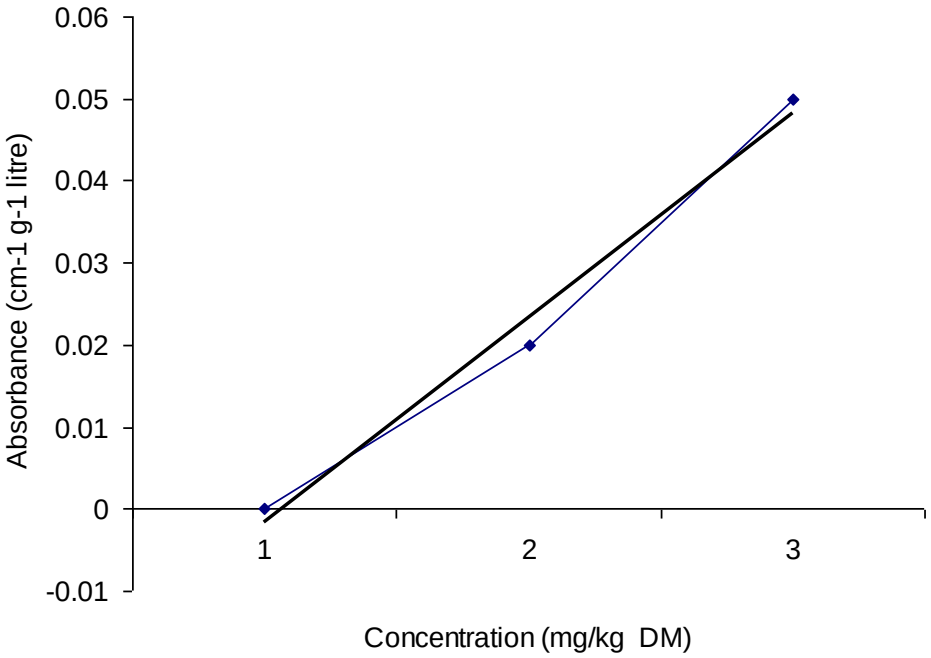

Figure 4: Standard calibration curve for cobalt.

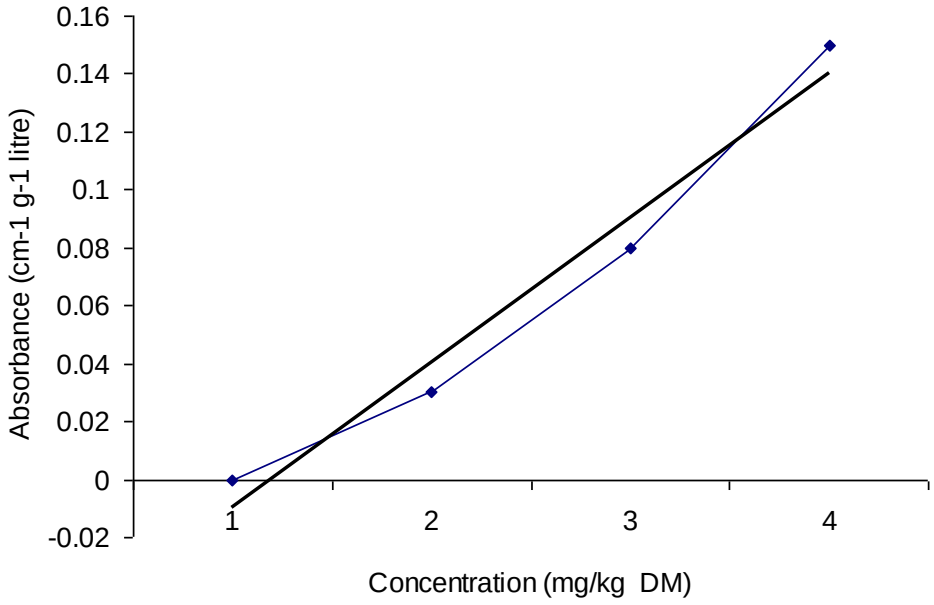

Figure 5: Standard calibration curve for selenium.

3.7.4: Reslope

The reslope facility of the model 2380 atomic absorption spectrophotometer enabled the slopes of the calibration curves to be adjusted, without the necessity of running all the calibration standards again. The system computes an equation where concentration is a function of absorbance:

$C=f(A)$.

Where C is the concentration, A is the absorbance and f represents the functional relationship between concentration and absorbance. In reslope the equation is modified to:

$$C=K.f(A).$$

Where K is the reslope factor necessary to modify the absorbance function in order to obtain correct concentration readings. The reslope facility is designed to compensate for small errors in sampling conditions, such as flow changes or nebulizer adjustments, or matrix differences between the standards used to set up the calibration curves and the samples. If larger changes occur, resloping will improve the results but a complete recalibration may be advisable. In the present study, recalibration was done following the analysis of every ten samples. The reslope facility may be used at any stage during sample analysis to ensure that the calibration curve is still applicable. For example, if for any reason the physical properties of the samples are thought to be changing (through evaporation or some other reason), leading to a slight general change in concentration, then the reslope facility may be useful. Before resloping, it is advisable to auto-zero incase the baseline has drifted.

3.7.5: Copper determination

Stock standard solution

About 1.000 g of copper metal was dissolved in a minimum volume of (1+1) HNO_3. The mixture was then diluted to 1 liter with 1% (v/v) HNO_3 to give a stock solution of 1000 mg/L.

Light sources

With multi-element lamps containing nickel or iron, a 0.2 nm spectral slit width was used with the copper 324.8 nm line.

Atomic absorption spectrophotometer working conditions for copper were 324.8 nm, 1.0 nm, 1.0 nm, 0.077 mg/L, 4.0 mg/L for wavelength, slit width, relative noise, sensitivity check and linear range respectively. Flame used was nitrous-oxide acetylene. The flame emission conditions for copper were 327.4 nm, 0.2 nm for wavelength and slit width respectively. Flame used was nitrous oxide acetylene.

3.7.6: Zinc determination

Stock standard solution

About 0.500 g of zinc metal was dissolved in a minimum volume of (1+1) HCL and diluted to 1 liter with 1% (v/v) HCL to give a solution of 500 mg/L.

Light sources

Hollow Cathode Lamps were used as light sources for zinc. Atomic absorption spectrophotometric working conditions for zinc were 213.9 nm, 0.7 nm, 1.0, 0.018 mg/L, 1.0 mg/L, 1.0 mg/L for wavelength, slit width, relative noise, sensitivity, sensitivity check and linear range respectively. Flame used was nitrous oxide-acetylene. Flame emission conditions for zinc were 213.9 nm, 0.2 nm for wavelength and slit width respectively. Nitrous oxide-acetylene flame was used.

3.7.7: Cobalt determination

Stock standard solution

About 1.000 g of cobalt metal was dissolved in a minimum volume of (1+1) HCL. It was then diluted to 1 liter with 1% (v/v) HCL to give a solution of 1000 mg/L.

Interferences

An excess of some transition and heavy metal could depress the cobalt signal, so matrix matching of standards was observed.

Atomic absorption spectrophotometric working conditions for cobalt were 240.7 nm, 0.2 nm, 1.0, 0.12 mg/L, 70 mg/L, 3.5 mg/L for wavelength, slit width, relative noise, sensitivity check and linear range respectively. Flame used was air acetylene. Flame emission conditions for cobalt were 345.4 nm, 0.2 nm for wavelength and slit width respectively. The flame used was nitrous oxide acetylene.

3.7.8: Selenium determination

Stock standard solution

About 1.000 g of selenium metal was dissolved in a minimum volume of concentrated HNO3 and evaporated to dryness. Up to two milliliters of water was added and evaporated to dryness. It was then dissolved in 10% (v/v) HCL and diluted to 1 liter with 10% (V/v) HCL to give a stock solution of 1000 mg/L.

Light sources

Hollow Cathode Lamps were used as light sources for selenium.

Interferences

The air-acetylene flame absorbed or scattered more than 50% of the radiation from the light source at the 196.1 nm selenium line. Due to this effect, a background corrector was used to improve the signal to noise ratio. Flame

absorption was reduced with the use of the nitrous oxide-acetylene flame, although sensitivity was reduced.

Atomic absorption spectrophotometric working conditions for selenium were 196.0 nm, 2.0 nm, 1.0, 0.59 nm, 30.0 mg/L, 200 mg/L for wavelength, slit width, relative noise, sensitivity, sensitivity check and linear range respectively. The flame used was air-acetylene.

3.7.9: Determination of trace elements using flame atomic absorption spectrophotometer

The digested samples in 50 ml volumetric flasks were filtered pending determination of the various trace elements. A Perkin-Elmer model 2380 atomic absorption spectrophotometer was used for this work. The machine settings used to obtain maximum sensitivity with pure aqueous standards differ somewhat from those suggested in the analytical methods book (1964). Optimum conditions vary slightly from one laboratory to another, since different instruments display minor individual differences.

The uptake rate was maintained at 3.5 ml per minute. Absorption for cobalt, copper, zinc and selenium was particularly sensitive to variations in burner height. The emission beam was focused as close to the burner top as possible. All the samples were analyzed in duplicate. An average of three readings was taken. As would be expected, absorption increased with decrease in source current (in amperes), and the values obtained in this study represented the best balance between sensitivity and signal noise.

3.8. Statistical analysis

Data was analyzed using MS Excel (descriptive statistics) and SPSS (ANOVA). MS Excel (2003) was used to analyze the data for the mean, standard deviation and

the 95% confidence interval for the national, regional and specific grass levels of copper, cobalt, zinc and selenium. It was also used to analyze descriptive statistics for the samples that were below, within and above the normal recommended range. SPSS was used to analyse the data for the true differences between the regional means of the four trace elements. Significant differences were tested at a 95 %($P<0.05$) confidence level. Because of unequal sampling, it was not possible to detect differences between forages of different regions. A comparison of sites within a region showed significant variation in forage content of copper, cobalt, selenium and zinc ($P<0.05$).

CHAPTER FOUR

4. RESULTS

4.1 Distribution of samples with levels of trace elements within normal, below normal and above normal in various parts of Kenya.

Deficiency was shown in 68% of pastures for copper, in 62% of pastures for zinc, 61% of pastures for cobalt and in 42% of pastures for selenium (Table 3a).

Table: 3a. Percentage of pastures deficient of copper, zinc, cobalt and selenium.

Element	Number deficient	Percentage deficiency
Copper	117	68.4
Zinc	107	62.6
Cobalt	104	61
Selenium	72	42.1

N= 171

Percentages of grass samples above the normal recommended values for copper (in parentheses) for livestock in Mount Kenya, Coast, Eldoret, Nakuru, Nyanza, Kiambu and Machakos, respectively, were as follows: copper (10 mg/kg of DM) 0, 0, 0, 0, 0, 0, 0 and 0 (Table 3b). Percentages of grass samples below the normal recommended values for copper (in parentheses) for livestock in Mount Kenya, Coast, Eldoret, Nakuru, Nyanza, Kiambu, Nairobi and Machakos, respectively, were as follows: copper (10 mg/kg of DM) 0, 3, 0, 11, 22, 0, 0 and 0 (Table 3b).

Table 3b: Percentage of grass samples below, within and above the normal recommended values in various regions in Kenya.

		Zinc	**Copper**	**Selenium**	**Cobalt**
Mt. Kenya	Above	20	0	0	100
	Within	80	100	0	0
	Below	0	0	100	0
Coast	Above	13	0	0	53
	Within	87	97	34	24
	Below	0	3	66	23
Eldoret	Above	30	0	0	53
	Within	70	100	30	21
	Below	0	0	70	16
Nakuru	Above	78	0	0	11
	Within	22	89	44	45
	Below	0	11	56	44
Nyanza	Above	39	0	0	52
	Within	58	78	84	25
	Below	3	22	16	23
Kiambu	Above	50	0	0	0
	Within	50	100	80	55
	Below	0	0	20	45
Nairobi	Above	9	0	0	40
	Within	86	100	60	30
	Below	5	0	40	30
Machakos	Above	0	0	0	24
	Within	100	100	80	36
	Below	0	0	20	40

n = 171

Percentages of grass samples above the normal recommended values for zinc (in parentheses) for livestock in Mount Kenya, Coast, Eldoret, Nakuru, Nyanza, Kiambu, Nairobi and Machakos, respectively, were as follows: zinc (50 mg/kg of DM) 20, 13, 30, 78, 39, 50, 9 and 0 (Table 3b). Percentages of grass samples

below the normal recommended values for zinc (in parentheses) for livestock in Mount Kenya, Coast, Eldoret, Nakuru, Nyanza, Kiambu, Nairobi and Machakos, respectively, were as follows: zinc (50 mg/kg of DM) 0, 0, 0, 0, 3, 0, 5 and 0 (Table 3b).

Percentages of grass samples observed to be above the normal recommended values for selenium (in parentheses) for livestock in Mount Kenya, Coast, Eldoret, Nakuru, Nyanza, Kiambu, Nairobi and Machakos, respectively, were as follows: selenium (5 mg/kg of DM) 0, 0, 0, 0, 0, 0, 0 and 0 (Table 3b). Percentage of grass samples observed from the present study to be below the normal recommended values for selenium (in parentheses) for livestock in Mount Kenya, Coast, Eldoret, Nakuru, Nyanza, Kiambu, Nairobi and Machakos, respectively, were as follows: selenium (5 mg/kg of DM) 100, 66, 70, 56, 16, 20, 40 and 20 (Table 3b). However, no clinical cases of selenium toxics or deficiency has been reported in all the eight regions studied.

Percentages of grass samples found to be above the normal recommended values for cobalt (in parentheses) for livestock in Mount Kenya, Coast, Eldoret, Nakuru, Nyanza, Kiambu, Nairobi and Machakos, respectively, were as follows: cobalt (4.2 mg/kg of DM) 100, 53, 53, 11, 52, 0, 40, and 24 (Table 3b). Percentages of grass samples observed to be below the normal recommended values for cobalt (in parentheses) for livestock in Mount Kenya, Coast, Eldoret, Nakuru, Nyanza, Kiambu, Nairobi and Machakos, respectively, were as follows: cobalt (4.2 mg/kg of DM) 0, 23, 16, 44, 23, 45, 30 and 40 (Table 3 b).

4.2 Regional and national levels of trace elements in Kenyan pastures.

The present study has indicated that copper had a national mean ± SD of 4.2 ±1.6 mg/kg of dry matter and a 95% confidence interval of 4.2, 4.2(Table 4).

Table 4: Mean (mg/kg DM) ± SD national levels of zinc, copper, selenium and cobalt in pasture grasses.

Element	**Zinc**	**Copper**	**Selenium**	**Cobalt**
Mean ±SD	44.1 ±20.5	4.2 ± 1.6	*	4.4 ± 6.3
95% CI	41.7 ± 46.6	4.2, 4.2	*	4.2, 4.7

(*)= Below detection limit.
N = 171

Mean (mg/kg of DM) ± SD copper levels for the eight regions studied were as follows: Coast (4.2 ± 2.3), Kiambu (4.1 ± 1.4), Machakos (4.5 ±1.8), Mount Kenya (4.6 ± 1.3), Nairobi (3.4 ± 1.2), Nakuru (3.3 ± 1.1), Homabay (4.4 ± 2.3) and Eldoret (4.3 ±1.6) (Table 5). Data generated from the present study found copper deficiency in all the eight regions studied. It is interesting to note that the lowest copper levels were observed in the region with the highest values of zinc, due to interactions between zinc and copper in the soil.. The region with the highest copper level was Mount Kenya, with a mean ± SD of 4.6 ± 1.3 mg/kg of dry matter and a 95% confidence interval of 3.9, 5.2 (Table 5), whereas Nakuru had the lowest mean copper level with a mean ± SD of 3.3 ± 1.1mg/kg of dry matter and a 95% confidence interval of 3.0, 3.6 (Table 5).

Table 5: Mean (mg/kg DM) ± SD regional levels of zinc, copper, selenium and cobalt in pasture grasses.

	Zinc	**Copper**	**Selenium**	**Cobalt**
Coast	38 ± 17	4.2 ± 1.2	*	8.3 ± 8
95% CI	19.9, 55.3	4.1, 4.3	*	4.5 ± 12
Kiambu	54 ± 19.7	4.5 ± 1.4	5.9 ± 7	0.13 ± 3
95% CI	16, 92	4.4, 5	1.4, 11	0.1, 3
Machakos	33 ± 10	4.5 ± 1.8	4.4 ± 5.2	1.5 ± 3.3
95% CI	25, 42	4.3, 4.8	2.1, 6.6	0.7, 2.4
Mt.Kenya	45 ± 26	4.6 ± 1.3	*	16 ± 6
95% CI	30, 42	3.9, 5.2	*	4, 27
Nairobi	33 ± 14	3.4 ± 1.2	*	3.7 ± 4.7
95% CI	16, 51	3.3, 3.6	*	1.7, 5.6
Nakuru	61 ± 13	3.3 ± 1.1	*	0.7 ± 3.2
95% CI	26, 96	3, 3.6	*	0.3, 5
Homabay	47 ± 23	4.4 ± 2.3	6.1 ± 6.2	4.4 ± 5.4
95% CI	14, 79	4, 4.7	3.7, 8.5	2.6, 6.2
Eldoret	53 ± 24	4.3 ± 1.6	*	5.5 ± 6.9
95% CI	15, 91	4.1, 4.4	*	2.4, 8.6

(*)= Below detection limit.
n = 171

Mean (mg kg of DM) ± SD zinc levels for the eight regions studied were as follows: Coast (38 ± 16.5), Kiambu (54 ± 19.7), Machakos (33 ± 10.4), Mount Kenya (45 ± 26), Nairobi (33 ± 14), Nakuru (61 ± 12.7), Homabay (47 ± 22.6) and Eldoret (53.2 ± 24) (Table 5). Data generated from the present study has found zinc deficiency in sixty three percent of all the eight regions studied. The regions with mean (mg/kg of DM) ± SD zinc levels suggestive of a deficiency were Coast (37.6 ± 16.5), Machakos (33.3 ± 10.4), Mount Kenya (44.9 ± 25.9),

Nairobi (33.4 ± 14) and Homabay (46.5 ± 22.6) (Table 5). Up to thirty seven percent of all the regions studied were found to contain zinc at levels that were found to meet nutrient requirements of ruminant livestock. These regions had mean (mg/kg of DM) ± SD zinc values of 53.5 ± 19.7, 60.8 ± 12.7 and 53.2 ± 24.0 for Kiambu, Nakuru and Eldoret, respectively (Table 5). It is worthy noting that the mean (mg/kg of DM) zinc value was observed to be highest in Nakuru (60.8 ± 12.7), which was observed to have the lowest mean (mg/kg of DM) copper value of 3.3 ± 1.1 (Table 5). Zinc had a national mean ± SD of 44.1 ±20.5 mg/kg of dry matter and a 95% confidence interval of 41.7, 46.6 (Table 4). The region with the highest mean zinc level was Nakuru, with a mean ± SD of 60.8 ± 12.7 mg/kg of dry matter and a 95% confidence interval of 25.7, 95.8 (Table 5), while Machakos had the lowest mean zinc level, with a mean ± SD of 33.3 ±0.4 mg/kg of dry matter and a 95% confidence interval of 24.4, 42.1 (Table 5).

The mean (mg/kg of DM) ± SD selenium levels generated from the present study in the eight regions studied were as follows: Coast (< 0), Kiambu (5.9 ± 6.8), Machakos (4.4 ± 5.2), Mount Kenya (< 0), Nairobi (< 0), Nakuru (< 0), Homabay (6.1 ± 6.2) and Eldoret (< 0) (Table 5). Data generated from the present study found selenium deficiency in five regions out of the eight regions studied and were observed to have mean (mg/kg of DM) ± SD values of less than zero (< 0). These regions were Coast, Mount Kenya, Nairobi, Nakuru and Eldoret (Table 5). Data from the present study found slightly elevated mean (mg/kg of DM) ± SD selenium levels in three out of the eight regions studied. These regions were Kiambu (5.9 ± 6.8), Machakos (4.4 ± 5.2) and Homabay (6.1 ± 6.2) (Table 5). Selenium had a national mean ± SD of -165± 295 mg/kg of dry matter and a 95% confidence interval of -674,343 (Table 4). The region with the highest mean selenium

level was Kiambu, with a mean± SD of 6 ± 7 mg/kg of dry matter and a 95% confidence interval of 1.4, 10.5 (Table 5). Mean (mg/kg of DM) ± SD cobalt levels observed from the present study for the eight regions were Coast (8.3 ± 7.6), Kiambu (0.13 ± 3), Machakos (1.5 ± 3.3), Mount Kenya (16 ± 6), Nairobi (3.7 ± 4.7), Nakuru (0.7 ± 3.2), Homabay (4.4 ± 5.3) and Eldoret (5.5 ± 6.9) (Table 5). Mean (mg/kg of DM) ± SD national cobalt levels observed in the present study found elevated levels of cobalt in Mount Kenya (15.5 ± 5.5) and Coast (8.3 ± 7.6) regions. Both Homabay and Eldoret were observed to have mean (mg/kg of DM) ± SD cobalt values of 4.4 ± 5.4 and 5.5 ± 6.9 respectively (Table 5). These values (in table 5) were observed to be slightly above the normal recommended levels for ruminant production. Data generated from the present study found cobalt deficiency in four out of the eight regions studied. These regions were Kiambu (0.1 ± 2.9), Machakos (1.5 ± 3.3), Nairobi (3.6 ± 4.7) and Nakuru (0.7 ± 3.2) (Table 5). However, clinical evidence of cobalt deficiency has not been reported of late. This could be due to supplementation. Cobalt had a national mean ± SD of 4.4 ±6.3 mg/kg of dry matter and a 95%confidence interval of 4.2, 4.7 (Table 4). The region with the highest mean cobalt level was Mount Kenya, with a mean ± SD of 15.5 ± 5.5 mg/kg of dry matter and a 95% confidence interval of 3.8, 27.3 (Table 5). Kiambu had the lowest mean cobalt level, with mean ± SD of 0.1±2.9mg/kg of dry matter and 95% confidence interval of 0.7, 0.9 (Table 5).

4.3: Levels of trace elements in individual grass types in Kenya

The mean (mg/kg of DM) ± SD zinc values for the grass pastures collected in the present study were as follows: Napier (39.8 ± 17), red oats (48 ± 25), star (45 ± 21), rhodes (39 ± 18.0), Bothriocloa (37 ± 16.7), Kikuyu (55 ± 22.0),

sudan (38 ± SD), hyperrhenia rufa (51 ± 18) and common setaria (47 ± 20.7) (Table 6).

Table 6: Mean (mg/kg DM) ± SD of zinc, copper, selenium and cobalt in different pasture grasses.

Grass	Zinc	Copper	Selenium	Cobalt
Napier	39.8 ± 17	5.4 ± 2	*	5.1 ± 6.6
95% CI	23, 57	5.2, 6	*	2.4, 8
Red oats	48 ± 25	3.3 ± 0.9	*	4.4 ± 7.2
95% CI	25, 30	3.2, 3.4	*	0.4, 8.5
Star	45 ± 21	5 ± 1.2	*	3.3 ± 6
95% CI	19, 71	4.4, 5	*	1.5, 5.1
Rhodes	39 ± 18	4 ± 1.2	*	4.4 ± 6
95% CI	18, 60	3.4, 3.6	*	2.1, 6.7
Bothriocloa	37 ± 17	3 ± 0.7	*	2.8 ± 5.4
95% CI	0.6, 74	2.4, 2.6	*	2.2, 6
Kikuyu	55 ± 22	4.9 ± 1.3	*	6.3 ± 7.8
95% CI	11, 98	4.7, 5	*	0.97, 12
Sudan	38.4 ± SD	2.3	1.3	*
C. setaria	47 ± 21	5.4 ± 1.2	*	4.8 ± 4.9
95% CI	40, 25	5, 5.7	*	0.57, 9
H. rufa	51 ± 18	1.8 ± 0.99	0.00 ± 7.1	8.2 ± 0.9
95% CI	42, 20	1.2, 1.3	0.01, 8.2	7.3, 9

(*) = Below detection limit.
n = 171

Data generated found zinc (mg/kg of DM) ± SD to be present in concentrations that meet nutrient requirements for ruminant livestock in Kikuyu grass (54.6 ± 22.0) and hyperrhenia rufa (50.6 ± 18) (Table 6). Kikuyu grass had the highest mean zinc level, with a mean ± SD of 54.6 ± 22 mg/kg of dry matter and a 95% confidence interval of 11.4, 97.8 (Table 6), whereas bothriocloa grass had the lowest mean zinc level, with a mean ±SD of 37.1± 16.2 mg/kg of dry matter and a 95% confidence interval of 0.6, 73.7 (Table 6).

Mean (mg/kg of DM) ± SD national copper levels for the grass pastures collected in the present study were as follows: Napier (5.4±1.9), red oats(3.3 ± 0.9), star (5.0 ±1.2), rhodes (4 ± 1.2), bothriocloa (3 ± 0.7), Kikuyu (4.9 ± 1.3), sudan (2.3 ± SD), common setaria (5.4 ± 1.2) and hyperrhenia rufa (1.8 ± 0.9) (Table 6). Data generated from the present study has suggested copper deficiency in all the grass pastures collected. Grass pastures collected in the present study were found to contain copper in concentrations that were below the nutrient requirements of ruminant livestock; however no clinical cases of copper deficiency has been reported of late. This could be due to trace element supplementation. Common setaria had the highest mean copper level, with a mean ± SD of 5.4 ±1.2 mg/kg of dry matter and a 95% confidence interval of 5.1, 5.7 (Table 6), whereas brown hood grass had the lowest mean copper level, with a mean ± SD of 1.8 ± 0.9 mg/kg of dry matter and a 95% confidence interval of -6417, 6008 (Table 6).

Mean (mg/kg of DM) ± SD national selenium levels for the grass pastures collected in the present study were: Napier (< 0), red oats (< 0), star (< 0), Rhodes (< 0), bothriocloa (< 0), kikuyu (< 0), sudan (1.3 ± SD), common setaria (< 0) and hyperrhenia rufa (< 0) (Table 6). Mean (mg/kg of DM) ± SD national data generated from the present study found selenium deficiency in all grass pastures collected with the exception of sudan grass (1.3 ± SD).

Mean (mg/kg of DM) ± SD national cobalt levels observed in the present study for grass pastures analysed were: Napier (5.1 ± 6.6), red oats (4.4 ± 7.2), star (3.3 ± 6), Rhodes (4.4 ± 6), bothriocloa (2.8 ± 5.4), kikuyu (6.3 ± 7.8), sudan (< 0), common setaria (4.8 ± 4.9) and hyperrhenia rufa (8.2 ± 0.9) (Table 6). Observations from the present study has found mean (mg/kg of DM) ± SD national cobalt levels to be slightly elevated in kikuyu grass (6.3 ± 7.8) and hyperrhenia rufa (8.2

± 0.9) (Table 6), and to be present in concentrations that meet nutrient requirements for ruminant livestock in napier (5.1 ± 6.6), red oats (4.4 ± 7.2), rhodes (4.4 ± 5.9) and common setaria (4.8 ± 4.8) (Table 6).

4.4: National proportion of samples below, above and within the normal ranges in Kenya

Out of the 171 samples analyzed, 7 samples (4%) were below the normal recommended range for copper, with a mean ± SD copper level of 1.5 ± 0.3 mg/kg of dry matter (Table 7), whereas no sample was above the normal recommended range (Table 7). Out of the 171 samples analysed, two samples (1%) were below the normal recommended range, with a mean ± SD zinc level of 14 ± 0.9 mg/kg of dry matter, whereas 45 samples (26%) were above the normal recommended range, with a mean ± SD of 71 ±12.9 mg/kg of dry matter (Table 7). Out of the 171 samples analysed, 74 samples (44%) were below the normal recommended range with a mean ± SD selenium level of -387.0± 333.9 mg/kg of dry matter whereas no sample was above the normal recommended range (Table 7).

Table 7: Mean (mg/kg DM) ± SD national number of pasture samples below, above and within the normal range.

Element	***Below***	***Within***	***Above***
Zinc	*2 (14 ± 0.99)*	*124 (35 ± 12.4)*	*45 (71 ± 13)*
Copper	*7 (1.5 ± 0.3)*	*164 (4.3 ± 1.6)*	*0 (0 ± 0)*
Selenium	*74 (80 ± 15)*	*97 (7.4 ± 5.3)*	*0 (0 ± 0)*
Cobalt	*55 (60 ± 10)*	*46 (2.4 ± 1.8)*	*70 (10.5 ± 4.8)*

(*) = Below detection limit
n = 171.

Out of the 171 samples analysed, 55 samples (33%) were below the normal

recommended range, with a mean ± SD cobalt level of 1.7 ±1.5 mg/kg of dry matter whereas 70 samples (41%) were above the normal recommended range, with a mean ± SD of 10.5 ± 4.8 mg/kg of dry matter (Table 7).

CHAPTER FIVE

5. DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1: Copper

The study showed that 96% of all analyzed samples from all the regions were within the normal recommended range but below the nutrient requirements for ruminant livestock as recommended by ARC (1980). Copper had a national mean (mg/kg DM) ± SD of 4.2 ± 1.6 mg/kg of dry matter, the recommended nutritional value is 10 mg/kg DM (ARC 1980). Mean (mg/kg of DM) ± SD national data generated from the present study has found copper deficiency in all the eight regions studied. Copper levels were found to be below the nutrient requirements for ruminant livestock in all the regions surveyed. This finding is in agreement with the very early reports of French (1955) and Howard (1963), who found copper levels to lie between 4 mg/kg of dry matter and 12.2 mg/kg of dry matter. Howard (1963) reported copper concentrations in Kenyan pastures to lie between 4.0 and 12.2 ppm. Deficiency normally shows itself at levels lower than 5.0 ppm, and this has been shown in about 35% of pastures analyzed in Kenya (Howard, 1963).

Deficiency of copper has been reported along the rift valley and in parts of central province of Kenya (Howard, 1963). This is thought to be due to copper deficiency in the soil (Pinkerton *et al*., 1965). Soils in which the underlying rock is ash and pumice may be expected to be deficient in copper (Nyandat and Ochieng, 1976). Mwakatundu (1977) also found sub clinical copper deficiency widespread in Kenya as a result of copper deficiency either in the soil or pasture or bovine plasma and is more pronounced in dry periods (Nyandat and Ochieng, 1976). The functioning of dietary copper can be inhibited by excess molybdenum (Mo)

resulting in a conditioned copper deficiency. Animals consuming forages with molybdenum concentrations above 15 to 20 ppm showed copper deficiency symptoms even though the copper levels in the forage were higher than 5 ppm (Hodgson *et al*., 1962). Conditioned copper deficiency does not seem to be a problem in Kenya because molybdenum was found deficient in several areas surveyed (Mwakatundu, 1977).

The findings in the present study is also in agreement with reports from Latin American researchers who found 47% of forage samples to be deficient in copper (McDowell *et al.*, 1984). In other studies, McDowell *et al.*, (1984) also found most tropical grasses to be borderline to deficient in copper and many other essential micro minerals. Haro (1986) in another study involving three regions of Coahuila, Mexico, found copper to be the most likely micro mineral to be deficient. The present findings are also consistent with data from the States of Montana and Texas of the United States of America which indicated copper and zinc to be deficient in many of the forages for cattle feeding (Ward and Spears, 1999).

The finding in the present study is also in agreement with reports by Rodgers and Murphy (2000), who reported that Irish grasses had multiple deficiencies of trace elements copper, selenium, iodine and cobalt (file://A:/Chemical Composition of Irish Forages- Grass, Silage and Hay.htm). In the United States, the most severe copper deficiencies tend to be along the East and West Coast, Upper Midwest and Florida. Up to 50% of the samples analyzed have been found to be copper deficient (file://A:/Factors affecting the Trace mineral Composition of Feedstuffs.htm).

In Australia and New Zealand, deficiency syndromes of grazing sheep are well recognized for copper (file://A:/VEIN Sheep Health and Production: Chapter 11. Trace elements and vitamins: def.htm).

The apparent deficient copper levels observed in the study agree with previous observations that as many grasses mature, the micro mineral content declines due to a natural dilution process and translocation of minerals to the roots (Underwood, 1981; Minson, 1990). The low copper levels observed in the present study suggest that Kenyan grass pastures would be a poor source of this micro mineral (Long *et al.*, 1970; Jumba *et al*., 1995b), but are a relatively rich source of iron as reported by Mwakatundu (1977) and Youssef (1988). The present study has indicated that all grasses from the regions surveyed are deficient of copper and therefore cannot meet the nutrient requirements for ruminant livestock.

5.2: Zinc

Zinc had a national mean (mg/kg DM) ± SD of 44.1 ± 20.5, the recommended nutritional value is 50 mg/kg DM (ARC 1980). This value was found to lie within the normal recommended range (15 to 60 mg/kg DM) but below the nutrient requirements for ruminant livestock as recommended by ARC (1980), and thus found to be deficient. Up to 72% of the forage samples analyzed were below the normal nutrient requirements for ruminant livestock. The low zinc levels observed in the present study are in accordance with reports of decreased zinc values reported in tropical pasture grasses grown in Kenya (Mwakatundu, 1977). The observations in the present study are also in agreement with previous reports that, as many grasses mature, the zinc content declines due to a natural dilution process and translocation of minerals to the roots (Underwood 1981; Minson 1990). The low zinc levels observed in the eight regions surveyed suggested that grass pastures

would be a poor source of this micro mineral (Long *et al.*, 1970; Jumba *et al.*, 1995b), but a relatively rich source of iron as reported by Mwakatundu (1977) and Youssef (1988). This could be due to the inverse relationship between zinc and iron. The present study has shown that the levels of zinc in the grasses surveyed were similar and generally below those desired for optimum ruminant production This is in agreement with other reports (McDowell *et al.*, 1984), that most tropical forages have been found to be borderline to deficient in many essential elements.

The present observations are consistent with reports from Montana on grass samples collected over two years which found zinc to be the most deficient element (Greene *et al.*, 1998). In a Montana, study (Grings *et al.*, 1996), reported that the micro mineral that have been found to be most likely deficient in forages of the Northern Great Plains is zinc. The present findings also agree with reports by Corah and Dargatz (1996) who reported that 97.5% of forages analyzed were deficient to marginal in zinc. Herd (1997), published average traces mineral values for native grasses analyzed in the Texas A and M forage testing laboratory which were deficient to marginal in zinc. Haro (1986), reported zinc as the most likely micro mineral to be deficient in three regions of Coahuila, Mexico, with 75% of forage samples analyzed being deficient. This observation is comparable to the 72% of analyzed samples found to be zinc deficient in the present study. The slight difference in percentage deficiencies could be attributed to the soil type and mineral concentrations in the two regions.

Florida researchers have reported zinc deficiencies in four regions of the State (file://A:/Zinc for Animals.htm). This observation is comparable to the findings in the present study, where zinc has been found to be deficient in six regions of

the country. Corah and Dargatz (1996) reported that only 2.5% of 352 forage samples analyzed contained adequate zinc. Similar deficiencies have been observed in forage samples that Vigortone (1990) (file://A:/The importance of Trace Mineral Nutrition.htm) has collected over the last seven years. In these samples, only 8.3% provided adequate zinc and is in accordance with the present findings.

Other investigators (Rodgers and Murphy, 2000) reported zinc to be very deficient in hay prepared from Irish grass. This report is in also in accordance with the present finding even though pastures sampled in the present study were in their early maturity stage. Spears (1994), had reported zinc deficiency in forage samples analyzed at the Pennsylvania state forage laboratory between 1969 and 1973. This report is in agreement with the findings in the present study.

5.3: Selenium

Selenium had a national mean (mg/kg DM) ± SD of below detection limit (< =0), the recommended value is 0.1 mg/kg DM (ARC 1980), and thus found to be present in sufficiently low concentrations to be suggestive of a deficiency in six regions out of the eight regions surveyed. Selenium was found to be slightly above the upper critical value in two regions (Homabay and Kiambu) and that was thought to be due to the mineral concentration in the soil. The marginal to deficient levels observed in the present study agree with previous reports that as many grasses mature, the selenium content declines due to a natural dilution process and mineral translocation to the roots (Underwood 1981; Minson 1990).

The low selenium levels observed in the present study is in agreement with the reports of Mwakatundu (1977) and Youssef (1988), that Kenyan pastures are a poor source of this micro mineral. The present study indicated that selenium levels in the nine grass pastures surveyed were similar and generally present in

marginal to deficient levels. These findings are in accordance with reports of Burdin and Howard (1963), Howard (1969), and Mwakatundu (1977), that surveys carried out in the country have revealed micro mineral deficiencies in Kenyan pastures; with possible consequences on animal growth and reproduction (Todd, 1954; Howard *et al.*, 1962).

In North West Poland, selenium deficiency has been found to be a serious problem affecting the effectiveness of animal production (file://A:/EJPAU 2002.htm) while selenium content of all forages from North Germany has been found to be below the recommended values (file://A:/EJPAU 2002.htm). These reports are in agreement with the present findings.

The present observations are also consistent with reports from states East and North East of Missouri (Illinois, Michigan, Ohio and Indiana), that feed grains and forages are generally deficient in selenium (file://A:/G2081.Mineral supplements for Beef Cattle.MU Extension.htm).

In Australia and New Zealand, deficiency syndromes of grazing sheep are well recognized for selenium (file://A:/VEIN Sheep Health and Production.htm). This is in agreement with the findings in the present study. Rodgers and Murphy (2000), reported multiple deficiencies of trace elements (cobalt, selenium, and copper) in Irish grass (file://A:/Chemical Composition of Irish Grass.htm). This report is in agreement with the findings in the present study.

Many investigators have also reported selenium deficiencies be present throughout the United States and in many areas of the world (file://A:/Selenium For Animals.htm). The present observations are in contrast to reports from Mexico that selenium was found to be above high critical values and suggestive of a toxicity (file://A:/Evaluation of the trace mineral status of ruminants in North East

Mexico.htm). This difference in levels could be was due to the differences in soil type and mineral concentrations. The present findings are in agreement with reports by McDowell *et al*., (1984), that most tropical forages have been found to be borderline to deficient in many essential elements.

5.4: Cobalt

Cobalt had a national mean (mg/kg of DM) ± SD of 4.4 ± 6.3, the recommended nutritional value is 0.1 to 4.2 mg/kg DM (ARC 1980). The mean (mg/kg of DM) ± SD national data generated from the present study observed cobalt to be borderline to deficient to the ruminant livestock in 75% of all the regions surveyed. Two of the eight regions surveyed in the present study were found to have slightly elevated cobalt levels which could have been due to the type and mineral concentration of the soil. However, clinical cases due to cobalt deficiency in these regions have not been reported of late. The low cobalt levels in the present study agree with reports of Hodgson *et al.,* (1962), who found it rare for grasses to contain cobalt in concentrations that meet the demands of grazing animals. This could lead to chronic starvation or wasting which is often indistinguishable from energy and protein malnutrition as reported (French 1952; Howard 1963; McDowell *et al.,* 1984).

In Kenya, cobalt deficiency is known as “Nakuruitis" (French, 1952; Howard, 1963) or as "Narurasha" among Maasai herdsmen (Hudson, 1944; French, 1952), “Pine” in Great Britain, “Bush sickness” in New Zealand and “Grand traverse disease” in the United States (Brander, 1982). Data generated from the present study found cobalt to be deficient in 75% of all the samples analysed. However, 25% of all the analysed samples were observed to have slightly elevated cobalt levels. This could have been due to the early stage of maturity of the forage

samples analyzed. This observation agrees with previous reports that, as many grasses mature, the mineral content declines due to a natural dilution process and translocation of minerals to the roots (Underwood 1981; Minson 1990). The low cobalt levels observed in the present study suggest that Kenyan grasses would be a poor source of this micro mineral (Long *et al.,* 1970; Jumba *et al.,* 1995b), but are a relatively rich source of iron as reported by Mwakatundu (1977) and Youssef (1988). The observations in this study indicated that cobalt levels were generally below those desired for optimum ruminant production and thus deficient.

The observations in the present study are also in agreement with reports by Hudson (1944), who found cobalt to be deficient along the Rift Valley and being seasonal in character, and Mwakatundu (1977), who confirmed cobalt deficiency in the Egerton, Kabete, and Molo areas of Kenya. The present findings, which suggests cobalt to be deficient in 75% of all the grass samples analysed, also agree with reports from Latin America that observed 43% of forage samples analysed from three regions of Coahuila, Mexico, to be deficient in cobalt (McDowell *et al.,*1977). The present findings are also in accordance with reports by McDowell *et al.,* (1984), who found tropical grasses to be borderline to deficient in many essential elements. Rodgers and Murphy (2000), have also found cobalt to be deficient in Irish grass.

Cobalt has also been found to be deficient in New Zealand and Australia (file://A:/VEIN Sheep Health and Production.htm). This observation is in agreement with observations from the present study. Most tall fescue grass collected from Missouri have also been found to be marginal or deficient in cobalt (file://A:/G2081. Mineral supplements for beef cattle. MU Extension.htm). This

observation is in agreement with the present findings even though in the present study, forage samples were in their early stages of maturity.

The present findings also agree with observations from North-West Poland, where cobalt deficiency has been found to be a serious problem affecting the effectiveness of animal production (file://A:/EJPAU 2002.htm). The findings of the present study are also in agreement with observations from New Zealand, Russia and South Africa, where cobalt has been found to be deficient (http: 11 www.publish.Csiro.au journal).

5.5. Conclusions

1. Findings from the present study has shown 68% of all the analysed pastures to be deficient in copper, 62% to be deficient in zinc, 61% to be deficient in cobalt and 42% of the 171 pasture samples analysed to be deficient in selenium.
2. The study has shown that the mean (mg/kg DM) ± SD national copper levels of 4.2 ± 1.6 was below the nutritional requirements (10 mg/kg DM) for proper livestock production.
3. This study confirms inverse relationship between copper and zinc availability in forages.
4. Data generated from the present study has shown cobalt levels to be below the nutritional requirements (4.2 mg/kg DM) for optimum livestock production in fifty percent (50%) of all the regions investigated.
5. Data generated from the present study has confirmed mean (mg/kg DM) ± SD cobalt levels in Nakuru region (Rift Valley province of Kenya) of 0.7 ± 3.2 to be below the nutritional requirements (4.2 mg/kg DM) for proper livestock production.

6. The present study has shown that the mean (mg/kg DM) ± SD national zinc levels of 44.1 ± 20.5 was below the nutritional requirements (50 mg/kg DM) for optimum livestock production.

7. Data generated from the present study has shown selenium levels to be below the nutritional requirements (0.3 mg/kg DM) for optimum livestock production in sixty three percent (63%) of all the regions investigated.

5.6. Recommendations

1. Data generated from the present study recommends copper, cobalt, zinc and selenium supplementation to ruminants in all the eight regions surveyed.

2. Further research is recommended in the areas studied to evaluate trace element status in biological tissues in ruminants and in the soil.

REFERENCES

Abate, A. (1985). Invitro estimation of the energy content of some feeds commonly fed to livestock in Kenya. *East African Agricultural and Forestry Journal* 45: 255-260.

Abate, A., Kayongo-Male, H. and C. N. Karue. (1981). Dry matter, protein, energy and fibre intake by dairy heifers grazing a Rhodes (*Chloris gayana*) pasture. *Animal feed science and technology* 6:15-26.

Abate, A.N. and Abate, A. (in press). Cassava as a source of energy in supplementary rations for weaner beef calves. *East African Agricultural and Forestry Journal.*

Abdurrahman, M. M., and R. L. Kincaid. (1995). Effect of selenium supplementation of cows on maternal transfer of selenium to fetal and newborn calves. *Journal of Dairy Science* 78:625-630.

Allen, J D. and J.M. Gawthorne. (1987). Involvement of the solid phase of rumen digesta in the interaction between copper, molybdenum, and sulfur in sheep. *British Journal of Nutrition* 58:265

Allen, J. G. (1978). The emergence of a lupinosis-associated myopathy in sheep in Western Australia. *Australian Veterinary Journal.* 54: p548.

Allen, J. G, Marcombe P. W, Masters H. G, Petterson D. S, and T. A. Robertson (1986). Acute zinc toxicity in sheep. *Australian Veterinary Journal.* 63: p 93.

Analytical Methods for Atomic Absorption Spectrophotometry, (1964) *Perkin-Elmer Corporation. Norwalk, Connecticut..*

Andrewartha, K.A; and I.W. Caple.(1980). Effects of changes in nutritional copper on erythrocyte super oxide dismutase activity in sheep. *Research in Veterinary Science.* 28:101-104.

ARC (1980). The nutrient requirements of ruminant livestock. *Farnham: Commonwealth agricultural bureaux.* Jaetzold R. and Schmidt H. (1983) Farm management handbook of Kenya, vol. 11a-c. Nairobi, Kenya: Ministry of Agriculture.

Arthur, R.J. (1997). Selenium biochemistry and function. In: Fischer, P. W. L; Abba, M. R; Cockell, K. A and Gibson, R. (ed) Proc. Ninth Intl. Symposium on trace elements in man and animals TEMA 9*). NRC Research Press, Ottawa, Canada, pp* 1-15.

Arthur, R.J; Beckett, G.J (1994). New metabolic roles for selenium *Proceedings of Nutrition society* 53: 615-624.

AOAC Official Methods of Analysis (1998); 1975; 58, 456 (1975); 36, 411 (1953); 58, 764 (1975); 36 (1975); 36 (1953); 68,499 (1975); 34, 710 (1953); 25, 520 (1942); 36, 397 (1953); 52, 627 (1969), 14th edn. Washington DC: Association of Official Analytical Chemists.

Australian Journal of Experimental Agriculture, 2003; 43: 47-52. (http: 11 www. Publish. Csiro. au journal)

Awadeh, F. T., M. M. Abdelrahman, R. L, R. L. Kincaid, and J. W. Finley. (1998). Effect of selenium supplements on the distribution of selenium among serum proteins in cattle. *Journal of Dairy Science.* 81: 1089-1094.

Babior, B. M. (1975) Cobamides as cofactors: adenosylcobamide-dependent reactions. In: Babior, D. M. (ed.) Cobalamin: Biochemistry and pathophysiology. *John Wiley and Sons, London,* pp 141-212.

Babu, II, Failla, M. C. (1990). Copper status and function of neutrophils are reversibly depressed in marginally and severely copper deficient rats. *Journal of nutrition* 120, 1100.

Blakely, B.R., and D.L. Hamilton. (1985). Ceruloplasmin as an indicator of copper status in cattle and sheep. *Canadian Journal of Medicine.* 49: 405-408.

B.P. Fabricand, R.R. Sawyer, S.G. Ungar, and S. Adler, Geochim. (1962) Cosmochim. *Acta* 26, 1023.

Brander, G. C. (1982). Veterinary applied pharmacology and therapeutics, pp.142. Ames, Iowa 50010. Fourth edition.

Branum, J.C., G.E. Carstens, E.H. McPhail, K.W. McBride, and A.B Johnson. (1998). Effects of prenatal dietary copper level on immune function of calves at birth and at 56 days of age. *Journal of Animal Science.* 76 (Suppl.1): 43 (Abstract).

Boyne, R. (1978). Changes in leucocyte cytochrome oxidase activity associated with deficiency of copper in laboratory and farm animals. *Research in Veterinary Science* 24: 134-138.

Boyne, R., Auther, J. R. (1986). Effects of molybdenum or iron induced copper deficiency on the viability and function of neutrophil from cattle. *Research in veterinary science* 41, 417.

Burdin, M.L. and Howard, D.A. (1963). Mineral survey of Kenya. *Kenya Veterinary Research Laboratories, Kabete, Kenya.*

Bush, J. A., Jensen, W. N., Athens, J. N., Ashenbuchem, H., Cartwright, G.E., Wintrobe, M. M. (1956). Studies on copper metabolism 19. The kinetics of irons 19. The kinetics of iron metabolism and erythrocyte lifespan in copper deficient swine. *Journal of experimental medicine* 103, 701-712.

Cardoso, E. C., Pereira, W. L. A., Aquiar, F. C. O., McDowell, L. R. (2001). Hypochromic anemia related to copper deficiency in buffaloes from Marajo Island. Para state. *Brazil International Journal of Animal Sciences* 76, 19-23.

Carvens, R. L., Vaden, P. (1994). Selected trace elements, vitamin and Various antibody titers in Mexico. *Agriculture practice* 15, 17-19.

Chanarin, I., Deacon, R., Perry, J. and Lumb, M. (1981). How vitamin B_{12} acts. *British journal of haematology* 47, 487-491.

Claypool, D.W., F.W. Adams, H.W Rendell, N.A. Hartman, Jr., and J.F Bone. (1975). Relationship between the level of copper in the blood plasma and liver of cattle. *Journal of Animal Science.* 65: 1702-1711.

Corah, L. R. and D. Dargatz. (1996). Forage analysis from cow/calf herds in 18 states. Beef cow/calf Health and Productivity (CHAPA). *USDA Animal and Plant Health Inspection Services, National Health Monitoring System.*

Engle, R.W., W.A. Hardison, R.F. Miller, N.O. Price, and J.T. Huber. (1964). Effect of copper intake on concentration in body tissue and on growth, reproduction and production in dairy cattle. *Jeanie. Science.* 23: 1160-1164.

Engle, T. E., C. F., C. F. Nockles, C. V. Kimberling, D. L Weaber, and A. B. Johnson. (1997). Zinc repletion with organic and inorganic forms of zinc and protein turnover in marginally zinc- deficient calves. *Journal of Animal Science.* 75: 3074- 3081.

Erskine, R.J., and P.C. Bardett. (1993). Serum concentrations of copper, iron and zinc during Escherichia coli-induced mastitis. *Journal of Dairy Science* 76:408-413.

Etzel, K.R., M.R. Swerdel, J.N. Swerdel and R.J. Cousins. (1982). Endotoxin induced changes in copper and zinc metabolism in the Syrian hamster. *Journal of Nutrition.* 112: 2363-2373.

Ferguson, E.G.W. Mitchell, G.B.B and Macpherson, A. (1989). Cobalt deficiency on the efficacy and safety of selenium and cobalt included in an anthelmintic for sheep, *Veterinary Records* 123:97-100.

(file://A:/Cobalt for Soil and Animal Health.htm).

File://A:/Copper for Animals.htm.

File://A:/EJPAU 2002. MALECKI J., MALINOWSKI E., SUPERA K., BALICK A-RAMISZ A.htm.

File://A:/Factors Affecting the Trace mineral composition of feedstuffs.htm.

File://A:/G 2081 Mineral Supplements for Beef Cattle, MU Extension.htm.

(file://A:/The importance of Trace Mineral Nutrition.htm)

File://A:/VEIN Sheep Health and Production.htm.

File:// A:/Zinc for Animals.htm

Field, A. C., Suttle, N. F., Brebner, J. and Gunn, G. W. (1988). An assessment of the efficacy of selenium and cobalt included in an anthelmintic for sheep. *Veterinary Record* 123, 97- 100.

Fischer, P.W.L; L. Abba, M.R. Cockell, K.A and Gibson, R.,(ed) Proc. Ninth Intl. Symposium on trace elements in man and animals

(TEMA 9). *NRC Research Press, Ottawa, Canada*, PP 1-15.

French, C. E., Smith, C. B., Fortmann, H. R., Pennington, R. P., Taylor,G. A., Hinish, W. W., and Swift., R.W. (1957). Survey of ten nutrient elements in Pennsylvania forage crops. I. Red clover. *Bulletin No. 624, Pennsylvania Agricultural Experiment station*.

French, M.H. (1952). Mineral deficiencies in farm livestock. *East African Agricultural and Forestry Journal* 10: 4-8.

French, M.H. (1955). Earth eating and mineral needs of livestock. *East African Agricultural and Forestry Journal* 20: 168-175.

Gallop, J. M. and Smith, R. M. (1972). Folic acid metabolism in vitamin B12 deficient sheep: Effects of injected methionine on methotrexate transport and the activity of enzymes associated with folate metabolism in liver. *Biochemical journal* 142, 119-126.

Gawthorne, J. M. and Smith, R.M. (1974). Folic acid metabolism in vitamin B12 deficient sheep: Effects of injected methionine on methotrexate transport and the activity of enzymes associated with folate metabolism in liver. *Biochemical journal* 142:119-126.

Gengelbach, G.P., and J.W Spears. (1998). Effects of dietary copper and molybdenum on copper status, cytokine production, and humoral immune response of calves. *Journal of Dairy Science.* 81: 3286-3292.

Gerloff, B. F. (1992). Effect of selenium supplementation on dairy cattle. *Journal of Animal Science.* 70: 3934-3940.

Gladstones, J. S. and Loneragen, J. F. (1967). Mineral elements in temperate crop and pasture plants. 1. Zinc. *Australian Journal of Agricultural Research* 18: 427-466.

Grace, N. D. (1972). Observations on plasma zinc levels in sheep grazing New Zealand pastures. *New Zealand Journal of Agricultural Research* 15, 284-288.

Greene, L. W., A. B. Johnson, J. A. Paterson and R. P. Ansotegui. (1998). Role of trace minerals in cow/calf cycle examined. *Feedstuffs* 70:34.

Grings, E. E., M. R. Haferkamp., R. K. Heitshmidt, and M. G. Karl. (1996). Mineral dynamics in forages of Northern Great Plains. *Journal of range management* 49: 234.

Grover, J. and Dougall, H.W. (1961). Milk production from pastures. *Journal of Agricultural Science (Cambridge)* 56: 201-204.

Halpine C. G, Hanrahen, P and McDonald. J. W. (1987). Selenium fertilizer for the control of selenium deficiency in grazing sheep in temperate pastures: Their production, use and management-edited by J. L. Wheeler, C. J. Pearson, and G. E. Robards, *Australian wool cooperation-CSIRO, Melbourne*, p 386.

Haro, I. M. (1986). Determination of mineral deficiencies and or toxicities in three regions in Coahuila, Mexico. *Master of Science Thesis. Agrarian University "Antonio Narro", Sactillo. Coahuila. México.*

Hart, E. B., Steenbock, H., Waddel, J. and Elvenhjem, C. A. (1928). Iron in nutrition.7. Copper as a supplement to iron for hemoglobin building in the rat. *Journal of Biological Chemistry* 77, 797-812.

Healy, W.B. (1974). Ingested soil as a source of elements to grazing animals. In W.C Hoekstra, J.W suttee, H.E gentler and W.Mertz. (eds). Trace element metabolism in animals-2 Baltimore. *University Park Press*.

Herd, D.B. (1997). Mineral supplementation of beef cows in Texas. *Texas Agricultural Extension Services Publication.* B-6056.

Hidiroglou, M., and C.J. Williams. (1982). Trace element status of fetuses from ewes fed a copper deficient ration. *American Journal of Veterinary Research.* 43: 310-313.

Hill, C.H; Starchei, B. and Kim, C. (1967). Role of copper in the formation of elastin. *Fed Proceedings* 26:129.

Hodgson, J.F., Leach, R.M. and Allway, W.H. (1962). Micronutrients in plants and soils in relation to animal nutrition. *Journal of Agriculture, Food and Chemistry* 10: 171-174.

Hoekstra, W.G. (1974). Biochemical role of selenium. In Hoekstra; J.W Suttie; H.E Gonthei; and W Mertz, eds. Trace element metabolism in animals-2 Baltimore, *University Park Press*.

Howard, D.A. (1963). Notes on Animal diseases. XXIII. Mineral deficiency diseases. *East African Agricultural and Forestry Journal* 28:191-194.

Howard, D.A. (1969). Notes on animal diseases. Mimeographed report. *Veterinary Research Laboratories, Kabete, Kenya.*

Howard, D.A., Burdin, M.L. and Lampkin, G.H. (1962). Variation in mineral and crude protein content of pastures at Muguga in the Kenya highlands. *Journal of Agricultural Science (Cambridge)* 59:251-256.

Howell, J.McC (1983). Trace elements in Animal Production and Veterinary Practice. *Occasional Publication no.7. British Society of Animal production*, pp, 107-117.

Humphires, W. R., Phillipo, M., Yound, B. W., Bremmer, I. (1983). The influence of dietary iron and molybdenum on copper metabolism in calves. http://www.publish.csiro.au journals (Australian Journal of Experimental Agriculture 2003:43:47-52).

Hudson, J.R. (1944). Notes on animal diseases. XXIII. Deficiency diseases. *East African Agricultural and Forestry Journal.* 10:51-55.

Jones, L. M. (1977). Veterinary pharmacology and therapeutics. Ames, Iowa 50010. Fourth edition.

Jones, D. G., Suttle, N. F. (1981). Some effects of copper deficiency on leucocytes function in sheep and cattle. *Research in veterinary science* 31, 151-156.

Judson, G. J. (1991). Long acting selenium treatments for sheep. *Australian Veterinary Journal.* 68: p 263.

Judson, G. J., Brown, T. H, Gray, D, Dewey, D.W, Edwards, J. B, and M. C. Farlane. J. D. (1982). Oxidized copper wire particles for copper therapy in sheep. *Australian Journal of Agricultural Research.* 30 pp 695.

Jumba, I. O., Suttle, N. F., Hunter, E. A. and Wandiga, S. O. (1995a). Effects of soil origin and mineral composition and herbage species on the mineral composition of forages in the Mount Elgon region of Kenya. 1. Calcium, phosphorous, magnesium and sulphur. *Tropical grasslands* 29, 40-6.

Jumba, I. O., Suttle, N. F., Hunter, E. A. and Wandiga, S. O. (1995b). Effects of soil origin and mineral composition and herbage species on the mineral composition of forages in the Mount Elgon region of Kenya. 2. Trace elements. *Tropical grasslands* 29, 47-52.

Kariuki, J. N., G. K. Gitau, C. K. Gachuiri, S. Tamminga, K. R. G. Irungu and Muia J. M. (1999). Effects of maturity on the mineral content of napier grass (*Pennisetum purperium*) *Tropical Science*. 39**,** 56-61.

Kellog, D.W., J.M. Rakes, and D.W. Gliedt. (1989). Effect of zinc methionine supplementation on performance and selected blood parameters of lactating dairy cows. *Nutritional Rep. International* 40: 1049-1057.

Kennedy, D. G., F. P. M. O. Harte, W. J. Blanchflower, and D. A. Rice. (1991). Sequential changes in propionate metabolism during the development of cobalt/vitamin B_{12} deficiency in sheep. *Biological Trace element Research.* 28: 233-241.

Kennedy, D. G., P. B. Young, S. Kennedy, J. M. Scott, A. M. Molloy, D. G. Weir, and J. Price. (1995). Cobalt-vitamin B_{12} deficiency and the activity of

methylmalonyl CoA mutase and methionine synthase in cattle. *International Journal of Vitamin Nutritional Research.* 65:241-247.

Kennedy, D. G., S. Kennedy, W. J. Blanchflower, J. M. Scott, D. G. Weir, A. M. Molloy, and P. B. Young. (1994). Cobalt-vitamin B_{12} deficiency causes accumulation of odd-numbered, branched chain fatty acids in the tissues of sheep. *British Journal of Nutrition.* 71: 67-76.

Kincaid, R.L. (1980). Toxicity of ammonium molybdate added to drinking water of calves. *Journal of Dairy Science.* 63: 608-610.

Kincaid, R.L. (1995). The biological basis for selenium requirements of animals. *Professional Animal Science.* 11: 26-29.

Kincaid, R. L., and A. S. Hodgson. (1989). Relationship of selenium concentrations in blood of calves to blood selenium of the dam and supplemental selenium. *Journal of Dairy Science.* 72: 259-263.

Kincaid, R.L., C.C.Hay, and R.I Krieger. (1986). Relationship of serum and plasma copper and ceruloplasmin concentrations of cattle and the effects of whole blood sample storage. *American Journal of Veterinary Research*. 47: 1157-1159.

Kincaid R.L., and C. L White. (1988). The defects of ammonium tetrathiomolybdate intake on tissue copper, and molybdenum in pregnant ewes and lambs. *Journal of Animal Science* 66: 3252-3258.

Kincaid, R. L., and J. D. Conrath. (1979). Effects of dietary zinc upon tissue zinc and percent unsaturated plasma- zinc binding capacity. *Journal of Dairy Science* 62: 572-576.

Kincaid, R. L., and M. J. Rock. (1999). Selenium intakes during late gestation on immunoglobulin and thyroid hormones in sheep *Faseb Journal.* 13(4): A249.

Kincaid, R.L., W.J Miller, P.R. Fowler, R.P. Gentry, D.L. Hampton, and M.W. Neathery. (1976). Effect of high dietary zinc metabolism and intracellular distribution in cows and calves. *Journal of Dairy Science.* 59: 1580-1584.

Knowles, S. O., N. D. Grace, K. Wurms, and J. Lee. (1999). Significance of amount and form of dietary selenium on blood, milk, and casein selenium concentrations in grazing cows. *Journal of Dairy Science.* 82: 429-437.

Lambourne, D. A., and Mason. R. W. (1969). Mortality in lambs following overdosing with sodium selenite. *Australian Veterinary Journal.* 45: 208.

Langlands, J.P., J.E Bowels., G.E. Donald, and A .J. Smith. (1984). Deposition of copper, manganese, selenium, and zinc in merino sheep. *Australian Journal of Agricultural Research.* 35: 701-707.

Lee, H.J. and Marston, H. R. (1969). Australian Journal of Agricultural Research, 20, 905.

Lee, J., B. P. Treloar, and N. D. Grace. (1994). Metallotheinein and trace element metabolism in sheep tissues in response to high and sustained zinc dosages. II. Expression of metallotheinein m-RNA. *Australian Journal of Agricultural Research.* 45: 321-332.

Levander, O. A. (1986). Selenium. In: W. Mertz (Ed.) Trace elements in Human and Animal Nutrition, Vol. 2. pp. 209-279. *Academic Press, New York.*

Lewis, G and Anderson, P.H. (1983). Trace elements in animal production and veterinary practice. *Occasional publication no.7, British society for animal production,* pp. 11-16.

Lilley, J., S.R., W.T Cuckly, and D.A Christenen. (1985). Review of copper deficiency and metabolism in ruminants. *Canadian Journal of Animal Science.* 69:819-845.

Lines and Marston. (1935). The effect of the ingestion of minute quantities of cobalt by sheep affected with "coast disease": A preliminary note. *Journal of the council for scientific and industrial research, Australia* 8, 117-119.

Lloyd and Chandrasekaran. (1980). Review of copper deficiency and metabolism in ruminants. *Australian Journal of Experimental Agriculture,* 43: 47-52. (http: 11 www. publish. Csiro. au journal)

Long M. I. E., Thornton D. D., Ndyanabo W. K. and Marshalls. B. (1970). The mineral status of dairy farms in Buganda and Busoga bordering lake Victoria, Uganda. 11. Nitrogen and mineral content of pastures. *Tropical Agriculture (Trinidad)* 47, 327-50.

Maas, J., D. Galey, J. R. Peauroi, J. T. Case, E. S. Littlefield, C. C. Gay, L. D. Koller, R. O. Crisman, D. W. Weber, D. W. Warner, and M. L. Tracy. (1992). The correlation between serum selenium and blood selenium in cattle. *Journal of Veterinary Diagnostic Investigations.* 4:48-52.

MacPherson, A. (1994). Selenium-vitamin E and biological oxidation. In: Garnsworthy, P. C. and Cole, D. J. (eds) Recent advances in nutrition. *Nottingham University Press, Nottingham,* pp. 3-30.

MacPherson, A; Grey, D; Mitchell, G.B.B and Taylor, C.N. (1987). *Ostertagia* infection and neutrophil function in cobalt deficient and cobalt supplemented cattle. *British Veterinary Journal.* 143: 348-355.

Mason, J. (1982). The putative role of thiomolybdates in the pathogenesis of Mo-induced hypocupraemia and molybdenosis: Some recent developments. *Irish Veterinary Journal*.. 36:164-168.

Mason, J., Williams, S., Harrington, R. and Sheahan, B. (1983). Some preliminary studies on the metabolism of 99 mo-labelled compounds in deer. *Irish Veterinary Journal* 38, 171-175.

Marston, H. R. (1935). Problems associated with "coast disease" in South Australia. *Journal of the council for scientific and industrial research, Australia* 8, 111-116.

Marston, H. R. and Lee, H. J. (1948). Selenium-vitamin E and biological oxidation. *Journal of Agricultural Science*, Cambridge 38, 229.

Masters, D. G. and Fels, H. E. (1985). Zinc supplements and reproduction in grazing ewes. *Biological trace element research* 7, 89-93.

Maynard, L. A., Loosli, J. K, Hintz, H. F, and Wayner, R. G. (1979). Animal Nutrition. 7th edition. *McGraw-Hill Book Co. New York.*

McC. Howell, J., and A.N. Davidson. (1959). The copper content and cytochrome oxidase activity of tissues from normal and swayback lambs. *Biochemistry Journal.* 72: 365-368.

Mc Donald, I.W. (1975). Selenium responsive unthriftness of young Merino sheep in central Victoria. *Australian Veterinary Journal.* 51: pp. 433.

Mc Donald, I.W. (1993). *Ostertagia* infection and neutrophil function in cobalt deficient and cobalt supplemented cattle *Australian Journal of agricultural research.* 44, 347.

McDowell, L.R. (1985). Nutrition of grazing ruminants in warm climate. *Academic Press Incorporation., New York.*

McDowell, L.R. (1992). Minerals in Animal and Human Nutrition. *Academic Press, New York.*

McDowell, L. R., B. J. Gordon, R. C. Merkel, V. Fadok, N. S. Wilkinson and G. A. Kunkle. (1991). Mineral status comparison in goats of Florida with emphasis on zinc deficiency. *Small Ruminants Research.* 5: 327-335.

McDowell L.R, Conrad J.H, and Ellis. G. L. (1984). Mineral deficiencies and imbalances and their diagnosis-In: *Symposium on Herbivore nutrition in subtropics and tropics. Academic press, Sydney*, 5 pp 316-324.

McDowell, L.R, Conrad J.H, Thomas J.E, Hariss L.E, and Fick. K.R. (1977). In Forage in ruminant nutrition. *Academic press, Sydney*, pp. 326-334.

Minson, D.J. (1990). Copper: In: Forage in ruminant nutrition. *Academic Press, Sydney*, pp 316-324.

Mills, C.F. (1987). Biochemical and Physiological indicators of mineral status in animals: Copper, cobalt, and zinc. *Journal of Animal Science*. 65: 1702- 1711.

Mills, C. C. and Dalgarno, A. C. (1972). Copper and zinc status of ewes and lambs receiving increased dietary concentrations of Cadmium. *Nature, UK* 239, 171-173.

Mills, C. C., Dalgarno, A. C., G. Wenhen. (1976). Biochemical and pathological changes in tissues of Friesian cattle during experimental induction of copper deficiency. *British journal of nutrition* 35, 309-311.

Mitchell, R. L. (1982). Cobalt and nickel in soils and plants. *Soil Science* 60, 63.

Mitchell, R.L. (1954). The trace element content of plants. *Research. UK* 10:357.

Moxon, A. L. (1937). Alkali disease or selenium poisoning. Bulletin No. 311, *South Dakota Agricultural Experiment Station*.

Muth, O.H; and Allaway, W.H. (1963). The relationship of white muscle disease to the naturally occurring selenium. *Journal of American Veterinary Medical Association.* 142: 1379.

Mwakatundu, A.G.K. (1977). A study of the nutritive value of East African pastures for ruminant livestock with special reference to mineral nutrition in grazing dairy cattle. *PhD thesis, University of Nairobi, Kenya.*

National Research Council. (1984). Nutrient requirements of domestic animals.

Neathery, M. W., W. J. Miller, D. M. Blackmon, R. P. Gentry, and J. B Jones. (1973a.). Absorption and tissue zinc content in lactating dairy cows as affected by low dietary zinc. *Journal of Animal Science* 37: 848-852.

Neathery, M. W., W.J. Miller, D. M. Blackmon, F. M. Pate and R. P. Gentry. (1973b). Effects of long term zinc deficiency on feed utilization, reproductive characteristics, and hair growth in the sexually mature male goat. *Journal of Dairy Science 56*: 98:-105.

No.4. Nutrient requirements of beef cattle. 6th edition. *National Academy of Sciences-National Research Council, Washington DC.*

Nockles, C.F. (1994). Micronutrients and the immune response. In: Montana Nutrition Conference Proceedings. *Montana State University*, p 3.1.

NRC (National Research Council). (1996). Nutrient requirements of beef cattle (7th Ed.) *National Academy Press. Washington, DC.*

Nyandat, N.N. and Ochieng, P.N. (1976). Copper content and availability in soils: A survey of arable and range areas of Kenya. *East African Agricultural and Forestry Journal* 42: 1-7.

Ott, E.A., W.H. Smith, R.B. Harrington, and W.M. Beeson. (1966a). Zinc toxicity in ruminants.111. Physiological changes in tissues and alterations in rumen metabolism in lambs. *Journal of Animal Science* 25: 424-431.

Ott, E. A., W. H. Smith, R. B. Harrington, H. E. Parker, and W. M. Beeson. (1966b). Zinc toxicity in ruminants. IV. Physiological changes in tissues of beef cattle. *Journal of Animal Science.* 25:432- 438.

Pagot, J. (1992). Animal production in the tropics. *Macmillan, BTA* pp 270-276.

Paterson, J. E., and A. MacPherson. (1990). A comparison of serum vitamin B_{12} and serum methylmalonic acid as diagnostic measures of cobalt status in cattle. *Veterinary records.* 126:329-332.

Paynter, D.I. (1982). Differences between serum and plasma ceruloplasmin activities and copper concentrations: Investigations of possible contributing factors. *Australian Journal of Biological Science.* 35: 353-361.

Paynter, D. I, Anderson, J. W, and McDonald. (1979). Glutathione peroxidase and selenium in sheep 11. In: Montana Nutrition Conference Proceedings. *Montana State University*, p 3.1.

Pehrson, B., J. Hakkarainen, and J. Tyopponen. (1986). Nutritional muscular degeneration in young heifers. *Nord. Veterinary Medicine* 38: 26-30.

Perkin-Elmer, Atomic Absorption Newsletter vol. 3, No.11, Dec. (1964).

Perkin, E. (1982). The guide to techniques and applications of atomic spectroscopy.

Pinkerton, A., Garret, M.W. and Guthrie, E.J. (1965). A note on copper deficiency in the Njoro area, Kenya. *East African Agricultural and Forestry Journa*l 30: 257258.

Poston, J. M. (1980). Cobalamin-dependent formation of leucine and β -leucine by rat and human tissue. *Journal of biological chemistry* 255, 10067-10072.

Poston, J. M. and Stadman, T. C. (1975). Cobamides as cofactors: Methylcobamides and the synthesis of methionine, methane and acetate. In: Babior, B. M (ed.)

cobalamin: Biochemistry and pathophysiology. *John Wiley and Sons, New York*, pp. 111-140.

Prasad, B. P. (1998). Metallotheinein and trace element metabolism in sheep tissues in response to high and sustained zinc dosages. II. Expression of metallotheinein m-RNA. *Australian Journal of Agricultural Research.* 45:321-332.

Price, J., S. Ueno, and S. G. Wood. (1993). Recent developments in the assay of plasma vitamin B12 in cattle. In: M. Anke, D, Meissner, and C. F. Mills (Ed) Proc. *Eighth International Symposium on Trace Elements in man and animals*. pp 317-318 Verlag media Touristik, Gersdoff, Germany.

Price, N. O. and Hardison, W. A. (1963). Minor element content of forage plants from the central Piedmont region of Virginia. *Bulletin No. 165, Virginia Agricultural Experiment station.*

Quirk, M. F., and B. W. Norton. (1988). Detection of cobalt deficiency in lactating heifers and their calves. *Journal of Agricultural Science.* 110:465-470.

Radostits, O. M., Gay, C. C., Hinchcliff, K. W. (2000). In: Veterinary medicine, 9th edition. *W. B. Saunders Company Limited pp. 990.*

Rice, D. A., M. Mc Loughlin, W. J. Blanchflower, C. H. McMurray, and E.A.Goodall. (1989). Sequential changes in plasma methylmalonic acid and vitamin B_{12} in sheep eating cobalt deficient grass. *Biol. Trace elements. Res*earch. 22:153-164.

Richards, D. A., McLoughlin, C. H., Blanchflower, W. J., Good all, E. A. and McMurray, C. H. (1981). Methylmalonic acid as an indicator of vitamin B_{12} deficiency in sheep. *Veterinary Records* 121, 472-473.

Rickes, E. L., Brink, N. G., Koniuszky, F. R., Wood, T. R. and Folkers, K.L. (1948). Vitamin B_{12}, a cobalt complex. Science 108, 134.

Rodgers and Murphy (2000) (file://A:/ Chemical composition of Irish forages-Grass, Silage and Hay.htm).

Rottruck, J.J; Pope, A.L; H.E. (1973). Selenium: Biochemical role as a component of glutathione peroxidase, USA, 179: 588-590.

Saba, L., Bismuth.,W. H., Nowakowiez, D. B., Stenzel, R., Ondersovic, M. (1999). Effects of mineral and herbal mixture administered to calves at other raising period on their haematologic indices. *Annales, Universetalis Marie Curie Skladowaska section* 17, 347-352.

Saenko, E. L., Yuaroplou, A. I., Harries, E. D. (1994). Biological functions of ceruloplasmin expressed through copper bindings. *Journal of experimental medicine* 7: 69-88.

Scholz, R. W., and L. J. Hutchison. (1979). Distribution of glutathione peroxidase activity and selenium in the blood of dairy cows. *American Journal of Veterinary Research.* 40: 245-248.

Schubert, J.R; Muth, O.H, Oldfield. (1961). The interactions between Copper, molybdenum and sulfur in ruminant nutrition. *Annals of Rev. Nutrition,* 11: 121-140.

Scott, J. M. (1992). Folate-vitamin B_{12} interrelationships in the central nervous system. *Proceedings of the nutrition society* 51, 219-224.

Selinus, O. (1988). Biogeochemical mapping of Sweden for geomedical and environmental research. *In: Thornton, 1. (ed.) Proceedings of the second international symposium on geochemistry and health. Science reviews, Norwood, UK*, pp. 13-20.

Shanker, A. H., and A. S. Prasad. (1998). Zinc and immune function: the biological basis of altered resistance to infection. *American Journal of clinical Nutrition.* 68 (Suppl.): 447S- 463S.

Sharma, M. C., Raju, S., Joshi, C., Kaur, H., Varshney, V. P. (2003). Studies on serum micro mineral, hormone and vitamin profile and its effect on production and therapeutic management of buffaloes in Haryana state of India. *Australian journal of animal sciences* 16 (4), 519-528.

Sheppard, A. D., L. Blom, and A. B. Grant. (1984). Levels of selenium in blood and tissues associated with some selenium deficiency diseases in New Zealand sheep. *New Zealand Veterinary Research.* 32: 91-95.

Singh, J. L., Sharma, M. C., Kumar, M., Rastogi, S. K., Gupta, G. C., Singh, S. P., Sharma, L. D., Gandhi, V. K., Kalicharan. (2002). Assessment of therapy in goitrous goat, through cardiac function tests. *Small ruminant research* 44, 119-124.

Small, D. H. and Carnegie, P. R. (1981). Myelopathy associated with vitamin B_{12} deficiency: New approaches to an old problem. *Trends in neurosciences 4, X-X1.*

Small, J.A. (1997). Serum mineral concentrations in relation to parturition in beef heifers and cows fed conserved forage. *Canadian Journal of Veterinary Science.* .77: 63-68.

Small, J.A., E. Charmley, A.V. Rodd, and A.H Fredeen. (1997). Serum mineral concentrations in relation to estrus and conception in beef heifers and cows fed conserved forage. *Canadian Journal of Veterinary Science.* 77: 55-62.

Smith, E. L. (1948). Presence of cobalt in the anti-pernicious anemia factor. *Nature, UK* 162, 144-145.

Smith, K. L., J. S. Hogan, and H. R. Conrad. (1988). Selenium in dairy cattle: Its role in disease resistance. *Veterinary Medicine* 83: 72-78.

Smith, R. M. (1997). Cobalt. In: B. L. O Dell and R. A. Sunde (Ed). Handbook of nutritionally essential mineral elements. Pp. 357-387. Marcel Dekker, New York.

Smith, S. E., Koch, B. A. and Turk, K. L. (1951). the response of cobalt deficient lambs to liver extract and vitamin B_{12}. *Journal of nutrition* 144, 455-464.

Spears, J.W. (1994). Minerals in forages. P.281. in George C. Fahey, Jr. (ed.) Forage quality, evaluation, and utilization. *American Society of Agronomy. Madison* WI. 53711.

Spencer, V.E., reading, R.E., and Thren, L.W. (1958). *Univ.Nevada Agricultural Experimental Station.* Bull 202.

Stake, P. E., W. J. Miller, R. P. Gentry, and M. W. Neathery. (1975). Zinc metabolic adaptations in calves fed a high but non-toxic zinc level for varying time periods. *Journal of Animal Science.* 40: 132- 137.

Stowe, H. D., and T. H. Herdt. (1992). Clinical assessment of selenium status of livestock. *Journal of Animal Science* 70: 3928-3933.

Suleiman, H. B., A. I. Abdelrahim, A. M. Zakia and A. M. Shomein. (1998). Zinc deficiency in sheep: Field cases. *Tropical Animal Health Production.* 20: 47-51.

Sutherland, R. J., Cordes, D. O., and Carthew, G. C. (1979). Ovine white liver disease: a hepatic dysfunction associated with vitamin B_{12} deficiency. *New Zealand Veterinary Journal* 27, 227-232.

Suttie, N.F. (1991); Schubert, J.E; Muth, O.H, Oldfield; J.E and Remmart, L.F. (1961). Experimental results with selenium in white muscle disease of lambs and calves. *Federation Proc*eedings. 20: 689-694.

Suttle, N. F. (1988). The role of comparative pathology in the study of copper and cobalt deficiencies in ruminants. *Journal of comparative pathology* 99, 241-258.

Suttle, N.F. (1991). The interactions between copper, molybdenum and sulfur in ruminant nutrition. *Annals of Nutrition.* 11:121.

Suttle, N.F., and A.C. Field. (1968). Effect of intake of copper, molybdenum and sulphate on copper metabolism in sheep.1. Clinical condition and distribution of copper in blood of the pregnant ewe. *Journal of Comparative Pathology. Comp.*. 78: 351, 362.

Suttle, N. F., Jones, D. G., Woolliams, J. A. (1987). Heinz body anaemia in lambs with deficiency of copper and selenium. *British journal of nutrition* 58: 539-548.

Thompson, R. H., C. H. McMurray, and W. J. Blanchflower. (1976). The levels of selenium and glutathione peroxidase activity in blood of sheep, cows and pigs. *Research in Veterinary Science.* 20: 229-231.

Torre, P. M., R.J. Harmon, R. W. Hemken, T. W. Clark, D. S. Trammel and B. A. Smith. (1996). Mild dietary copper insufficiency depresses blood neutrophil function in dairy cattle. *Journal of Nutrition and Immunology* 4:3- 24.

Todd, J.R. (1954). The phosphorous status of cattle in parts of central province, Kenya. *East African Agricultural and Forestry Journal* 20: 66-68.

Todd, W. R., Elvehjem, C. A. and Hart, E. B. (1934). Zinc in nutrition of the rat. *American Journal of Physiology* 107, 146-156.

Ullrey, O.E. (1974). The selenium deficiency problem in animal Agriculture. In W.G Hoekstra, J.W Suttie, H.E Ganther and W. Mertz (eds). Trace element metabolism in animals-*Z Baltimore, University Park Press.*

Underwood, E.J. (1971). Trace element in animal and human nutrition. 3rd ed, p.469. *New York: Academic Press.*

Underwood, E.J. (1981). The mineral nutrition of livestock. *Common Wealth Agriculture Bureaux, London.*

Underwood, E.J. (deceased), Suttle, N.F. (1999). Mineral nutrition of Livestock, 3rd ed. Pp.283-513, CAB. Pub.

Underwood, E. J. and Filmer, J. F. (1935). The determination of the biologically potent element (cobalt) in limonite. *Australian veterinary journal* 11, 84-92.

Waldner, C., J. Campbell, G. K. Jim, P. T. Gulchon, and C. Booker. (1998). Comparison of three methods of selenium assessment in cattle. *Canadian Veterinary Journal.* 39: 225-231.

Ward, J.D., and J.W. Spears. (1997). Long-term effects of consumption of low copper diets with or without supplemental molybdenum on copper status, performance and carcass characteristics of cattle. *Journal of Animal Science. .* 75: 3057-3065.

Ward, J.D., and J.W. Spears. (1999). The effects of low copper diets with or without supplemental molybdenum on specific immune responses of stressed cattle. *Journal of Animal Science.* 77:230-237.

Wegner, T. N., D. E. Ray, C. D. Lox and G. H. Stott. (1973). Effect of stress on serum zinc and plasma corticoids in dairy cattle. *Journal of Dairy Science.* 65: 748- 752.

Wellinghausen, N., and L. Rink. (1998). The significance of zinc for leucocyte biology. *Journal of Leucocyte Biology* 64: 571- 577.

Wikse, S. E., D. Herd, R. Field, and P. Holland. (1992). Diagnosis of copper deficiency in cattle. *Journal of American Veterinary Medical Association.* 200: 1625- 1629.

Whelan. B.R Barrow, N.J and Peter, D.W. (1994). Selenium fertilizers for pasture grazed by sheep. II. Wool and live weight response to selenium. *Australian Journal of Agricultural Research* 45: 875-886.

Wikse, S.E. (1992). The relationship of trace elements deficiencies to Infectious diseases of beef cattle. *Proceedings of the 1992 TAMU beef cattle short course.*

Williams, D. M., Kennedy, F. S., Green, B. G. (1983). Hepatic iron accumulation in copper deficient rats. *British journal of nutrition* 50, 653-660.

Woolliams, J.A., G. Weiner, C. Woolliams, and N.F Suttle. (1985). Retention of copper in the liver of sheep genetically selected for high and low concentrations of copper in plasma. *Animal Production* 41: 219-226.

Xin, Z., D.F. Waterman, Hemken, and R.J. Harmon. (1993). Copper status and requirement during the dry period and early lactation in multifarious Holstein cows. *Journal of Dairy Science* 76: 2711-2716.

Young, R. S. (1979). "*Cobalt in biology and biochemistry*". Academic Press, New York, pp. 50- 86.

Youssef F. G. (1988). Some factors affecting the mineral profiles of tropical grasses. Outlook on Agriculture 17, 104 -11.

APPENDICES

Appendix 1: Symbols for units and prefixes

Å	Angstrom.
^{o}C	Degree Celsius.
g	Gram.
h	Hour.
Ml	Milliliter.
s	Second (Time).
Mg/L	Milligram per litre.
%	Percentage.
nm	Nanometre.
µ	Micron.
µg	Microgram.
µg/ml	Microgram per millilitre.
µg/L	Microgram per litre.
Mg/kg	milligram per kilogram.
m	Metre.
mm	millimetre.
No.	Number.
L	litre.
G/l	Gram per litre
amp	Ampere.
(")	Inch.
K/ml	Potassium per millilitre.

ML/min	Millilitre per minute.
(%)[a]	Concentration based on 1g sample taken up in 5ml buffer.

Appendix 2: Formulae

Cu	Copper.
Se	Selenium.
Zn	Zinc.
Co	Cobalt.
1	One.
V/v	Volume by volume.
HNO_3(1+1)	One part nitric acid to one part of water.
HCL(1+1)	One part hydrochloric acid to one part of water.
HNO_3	Nitric acid.
$HCLO_4$	Perchloric acid.
La	Lanthanum solution.
N_2O	Nitrous oxide.
C_2H_2	Acetylene.
HCL	Hydrochloric acid.
H_2O	Water.
N	Normal.
K	Potassium.
Ca	Calcium.
Mg	Magnesium.
P	Phosphorous.
Na	Sodium.
Fe	Iron.
Mn	Manganese.
Al	Aluminium.

Zn	Zinc.
Ba	Barium.
Sr	Strontium.
B	Boron.
Mo	Molybdenum
GSSG	Oxidised glutathione.
GSG	Reduced glutathione.
S_1	First standard (least concentrated standard).
S_2	Second standard (concentration thrice the concentration of S_1)
S_3	Third standard (concentration is twice the concentration of S2).
S.D	Standard deviation

Appendix 3: Levels (mg/kg) of dry matter for various trace elements in pasture grasses

A = above normal recommended range, B = below normal recommended range, N = within the normal recommended range, (-) =trace value (< 0), Obs=observation number.

Obs No.	Sample No	Zn	Cu	Se	Co
1	MKK_1	89.8A	6.1N	-5.6B	15A
2	MKN_1	37.9 N	5.8N	-700B	13.8A
3	MKO_1	39.8N	3.6N	-722.5B	25A
4	MKS_1	33.1N	3.9 N	-736B	11.3 A
5	$MKSS_1$	23.8N	3.4N	-575B	12.5A
6	CTK_1	34.1 N	3.3 N	-725B	3.75 N
7	CTN_1	16.8N	4 N	-703.8 B	10 A
8	CTO_1	30.4N	3.5 N	-578.8 B	11.3A
9	CTS_1	21.1 N	5.4 N	-683.8 B	18.8 A
10	CTR_1	37.1N	1.5B	-677.5B	13.8 A
11	CTK_2	56.2 N	4.4 N	-715 B	6.3A
12	CTN_2	17N	5.4 N	-668.8B	22.5 A
13	CTO_2	43.9N	3.5N	-733.8 B	15 A
14	CTS_2	32.6N	3.9 N	-705 B	3.8 N
15	CTR_2	40.4N	3.9 N	707.5 B	13.1A
16	CTK_3	20.8 N	2.3 N	-576 B	13.8 A

17	CTN_3	35.1N	4.3 N	-737.5 B	10 A
18	CTO_3	68.9 A	5.5 N	-732.5 B	18.8A
19	CTS_3	52.3 N	5N	-655B	2.5 N
20	CTR_3	29 N	4.3 N	-742.5 B	15 A
21	CTK_4	41.4N	4.4 N	-631.5 B	16.3 A
22	CTN_4	29.9 N	3.8 N	-747.5 B	13.8 A
23	CTO_4	29.3 N	2 N	-752.5 B	10 A
24	CTS_4	60.3 A	2.9 N	-585 B	15 A
25	CTR_4	28.9 N	3N	-753.8 B	17.5 A
26	CTK_5	61.3 A	4 N	1.3 N	-2.5 B
27	CTN_5	20.2 N	5.5 N	7.5 N	-1.25B
28	CTO_5	27.2 N	2.9 N	1.3 N	2.5 N
29	CTS_5	26.7 N	4.8N	10 N	0 B
30	CTR_5	79.2 A	5.1 N	15 N	-1.25 B
31	CTK_6	52.6N	4.9N	3.8 N	2.5 N
32	CTN_6	16.4 N	5.6 N	8.8 N	-2.5 B
33	CTO_6	50.3 N	5.1 N	10 N	-1.25 B
34	CTS_6	22.9 N	6.4 N	12.5 N	0 B
35	CTR_6	46.2 N	4.5 N	1.3 N	1.25 N
36	UGN_1	50.4 N	4.9 N	-585 B	12.5 A
37	UGR_1	58.8 N	4.6 N	-487.5 B	-1.25 B
38	UGO_1	103.8 A	3.4 N	-730 B	-0.75 B

39	UGS_1	66.8 A	5.5 N	-490 B	-1.25 B
40	UGK_1	78.9 A	5.6 N	-610 B	22.5 A
41	UGN_2	48.8 N	2.3 N	-580 B	6.25 A
42	UGR_2	54.9 N	3.9 N	-487.5 B	8.75 A
43	UGO_2	122.4 A	2.1 N	-752.5 B	3.75 A
44	UGS_2	38.8 N	3.5 N	-485 B	10 A
45	UGK_2	76.4 A	7.6 N	-606.3 B	10 A
46	UGN_3	47.7N	2.5 N	-725.5 B	13.8 A
47	UGR_3	47.4N	3.5 N	-532.5 B	1.25 N
48	UGO_3	67.8 A	2.2 N	-642.5 B	2.5 N
49	UGS_3	59.3 N	3.6 N	-525 B	2.5 N
50	UGK_3	79.5 A	6.5 N	-622.5 B	21.3 A
51	UGN_4	50.4 N	4.9 N	-710 B	10 A
52	UGR_4	54.4 N	4.1 N	-492.5 B	-3.75 B
53	UGO_4	71 A	3.6 N	-512.5 B	3.75N
54	UGS_4	57.1 N	3.1 N	-527.5 B	-1.25 B
55	UGK_4	65A	3.8 N	-530 B	12.5 A
56	UGN_5	17.5N	4.8 N	3.8 N	5 A
57	UGR_5	19.3 N	2.4 N	12.5 N	8.8 A
58	UGO_5	36.6 N	4.5 N	13.8 N	-2.5 B
59	UGS_5	25.3 N	5.4 N	13.8 N	1.25 N
60	UGK_5	37.8 N	5.3 N	6.3 N	5 A

61	UGN_6	44.8N	8.8 N	7.5 N	-3.75 B
62	UGR_6	20.6 N	4N	8.8 N	1.25 N
63	UGO_6	31.7 N	3.3 N	0 B	12.5A
64	UGS_6	32.9 N	2.6 N	7.5 N	5 A
65	UGK_6	29.7 N	5.3 N	2.5 N	-1.25B
66	NKS_1	63 A	3.9 N	0 B	-1.25B
67	NKK_1	70.5 A	3.9 N	-395 B	2.5 N
68	$NKBI_1$	69.3 A	3.1 N	-532.5 B	0 B
69	$NKSS_1$	71.8 A	5.3 N	3.8 N	2.5 N
70	$NKSD_1$	38.4 N	2.3 N	1.3 N	-5B
71	NKN_1	66.8 A	2.6 N	-305 B	5 A
72	NKS_2	64.3 A	1.6 B	27.5 N	-2.5 B
73	$NKSS_2$	39.8N	4.3 N	11.3 N	3.75 N
74	NKN_2	63 A	2.8 N	-32.5 B	1.25 N
75	NYR_1	50.9 N	3 N	15 N	6.25 A
76	NYS_1	23.8 N	5.4 N	1.25 N	-2.5 B
77	$NYSS_1$	63.6 A	6.9 N	-1.25 B	7.5 A
78	$NYBI_1$	65.2A	2.6 N	12.5 N	11.3 A
79	NYN_1	62.4 A	8 N	8.75N	7.5 A
80	NYR_2	36.1 N	1.6 B	2.5 N	11.3 A
81	NYS_2	63.6 A	4.5 N	1. 25 N	8.8 A
82	$NYSS_2$	64.2 A	6.1 N	0 B	11.3 A

83	$NYBI_2$	25.1 N	0.8 B	13.8 N	0 B
84	NYN_2	58.1 N	8 N	22.5 N	3.8 N
85	NYR_3	19.8 N	2.3 N	2.5 N	0 B
86	NYS_3	62.6 A	6.1 N	7.5 N	6.3 A
87	$NYSS_3$	61.1 A	6.9 N	0 B	1.3 N
88	$NYBI_3$	23.1 N	3.1 N	7.5 N	-3.8 B
89	NYN_3	58.1 N	7.4 N	1. 25 N	11.3 A
90	NYR_4	11.9 N	1.3 N	-2.5 B	-3.8 B
91	NYS_4	106.9 A	5. 5 N	7.5 N	6.3 A
92	$NYSS_4$	63.1 A	5.9 N	12.5 N	3.8 A
93	$NYBI_4$	49.3 N	3.1 N	5.6 N	7.5 A
94	$NYHR_4$	37.9 N	1.1 N	5 N	8.8 A
95	NYR_5	12.9 N	2.6 N	3.75N	-3.8 B
96	NYS_5	70.3 A	4.6 N	0 B	2.5 N
97	$NYSS_5$	62.4 A	5.4 N	3.75 N	-2.5 B
98	$NYBI_5$	49.1 N	1.8 B	5 N	12.5 A
99	$NYHR_5$	63.3 A	2.5 N	-5 B	7.5 A
100	NYN_6	58.1 N	6.8 N	5 N	10 A
101	NYR_6	20.5 N	3.4 N	13.8 N	5 A
102	NYS_6	21.2 N	5.5 N	10 N	2.5 N
103	$NYSS_6$	18.7 N	6.3 N	5 N	1. 25 N
104	$NYBI_6$	14.7 B	1.6 B	2.5 N	-3.8 B

105	NYN_7	44.8 N	6.4 N	12.5 N	-1.3 B
106	KBN_1	68.7A	5.6 N	1.25 N	-5 B
107	KBO_1	49.2 N	2.8 N	0 B	0 B
108	KBK_1	74.9 A	3.8 N	10 N	3.8 N
109	KBS_1	55.9 N	3.8 N	5 N	3.8 N
110	KBR_1	60.2A	4 N	16.3 N	-3.8 B
111	KBN_2	46.2 N	6.5 N	-3.75B	0 B
112	KBO_2	74.1 A	4.1 N	5 N	-3.8 B
113	KBK_2	71.3 A	3 N	1.25 N	-2.5 B
114	KBS_2	63.1A	3.6 N	3.75 N	-2.5 B
115	KBR_2	70.2 A	4 N	5 N	1. 25 N
116	KBN_3	41.3	6N	7.5 N	2.5 M
117	KBO_3	46.7 N	3.3 N	10N	0 B
118	KBK_3	86.4 A	5.6 N	0 B	2.5 N
119	KBS_3	61.1 A	4 N	2.5 N	1.25 N
120	KBR_3	69.3 A	5.1 N	1.25N	1.25 N
121	KBN_4	27.8 N	7.4 N	10 N	1.25 N
122	KBO_4	30.2 N	4.3 N	11.3 N	3.75 N
123	KBK_4	32.3N	4.5 N	24.8N	-3.8 B
124	KBS_4	18.9 N	6.6 N	-2.5 B	1.25 N
125	KBR_4	21.8 N	2.5 N	10 N	2.5 N
126	NBO_1	32.1 N	2.7 N	0 B	0B

127	NBS_1	32 N	5.8 N	5 N	7.5 A
128	NBK_1	17.9 N	6.1 N	11.3 N	7.5N
129	$NBBI_1$	28.8 N	3.6 N	5 N	8.8 N
130	NBN_1	31.9 N	5.6 N	10N	12.5 A
131	NBR_1	42.4 N	2.4 N	-530 B	5 A
132	NBO_2	63.9 A	3.3 N	0 B	0 B
133	NBS_2	36.9 N	3.1 N	7.5 N	6.3 A
134	$NBBI_2$	22.3 N	1.8 B	-2.5 B	0 B
135	NBN_2	29.6 N	3.3 N	2.5 N	0 B
136	NBR_2	43.8 N	2.5 N	7.5 N	10A
137	NBO_3	40.7 N	3.1 N	-1.25 B	-2.5 B
138	NBS_3	65.1 A	3.9 N	-40 B	11.3 A
139	$NBBI_3$	53.4 N	2.3 N	1.25 N	5 A
140	NBN_3	23.4 N	3.6 N	5 N	2.5 N
141	NBR_3	37.9 N	3.8 N	-5 B	7.5 A
142	NBO_4	13.3 B	2N	1.25N	-1.25B
143	NBS_4	26.8 N	3.6 N	7.5 N	-3.8 N
144	NBN_4	16.6 N	4N	7.5 N	1.3 N
145	NBR_4	18.1N	2.4 N	7.5 N	1.3 N
146	NBK_4	31.8 N	4.4 N	5 N	0 B
147	$NBBI_4$	26.3 N	2.4 N	-1.25 B	1.3 N
148	MSN_1	28.2 N	4.5 N	7.5 N	-2.5 B

149	MSS_1	27.6 N	5.3 N	-2.5 B	0 B
150	MSR_1	38.1 N	3.9N	5 N	1.3 N
151	MSO_1	24.6 N	2.5 N	5 N	6.3 A
152	MSB_1	31.2 N	2.5 N	-5B	6.3 A
153	$MSSS_1$	22.9N	3.6 N	7.5 N	7.5A
154	MSN_2	21.4 N	4.4 N	20 N	0 B
155	MSS_2	22.7 N	5.4 N	5 N	-3.8 B
156	MSR_2	18.7 N	3.5 N	-2.5 N	5 A
157	MSO_2	32.9 N	2.3 N	6.25N	1.3 N
158	$MSBI_2$	27.7 N	2.6 N	5 N	1.3 N
159	$MSSS_2$	26.6 N	5.3 N	10N	0 B
160	MSN_3	40.3 N	9.3 N	7.5 N	2.5 N
161	MSK_3	46.4 N	7.3 N	0 B	6.3 A
162	MSS_3	50.3 N	6 N	3.75 N	-1.3 B
163	MSO_3	40.9 N	3 N	0 B	2.5 N
164	MSR_3	45.6 N	5.4 N	6.25 N	2.5 N
165	$MSBI_3$	27.0 N	2.6 N	-2.5 B	-2.5 B
166	MSN_4	53.3 N	7.4 N	2.5 N	-1.3 B
167	MSK_4	46.2 N	5.1 N	3.75N	-2.5 B
168	MSS_4	19.1 N	4.5 N	10 N	0 B
169	MSO_4	29.4 N	3.4 N	5 N	3.8 N

170	$MSBI_4$	44.6 N	3.3 N	6.25N	-1.3B
171	MSR_4	33.8 N	5.8 N	1.25 N	5 A

(-) Below detection limit.
n = 171.