PERFORMANCE OF GRAZING CROSSBRED CATTLE SUPPLEMENTED WITH MINERALS:

CALCIUM, PHOSPHORUS AND ZINC

BY

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ABSTRACT

A study was conducted from 1997 to 1999 in Iringa region, Tanzania in order to evaluate the mineral status and performance of grazing crossbred dairy cattle as affected by season and nature of mineral supplementation. Initially concentration of calcium, (Ca), phosphorus (P) and zinc (Zn) in soil, grass pasture and blood plasma were measured. Seasonal effect was apparent ($P< 0.05$) in soil and pasture mineral concentration. Soil calcium (4.46 – 5.67 me /100g), was adequate during the dry and the rainy season. Soil phosphorus ($P< 0.05$) was low during the rainy season and high in the dry season (11.9 – 14.8 vs 28.1 – 31.8 ppm) whereas zinc (0.81 – 1.02 ppm) was deficient in both seasons. Pasture Ca (0.33 – 0.39%) and P (0.25 – 0.34%), were adequate whereas Zn (21.2 – 25.2 ppm) was inadequate for the sampled periods. Blood plasma Ca (<2.20 mmol/l), inorganic phosphate (< 1.28 mmol/l) and Zn (<12.2 μmol/l) were marginal to normal values in the sampled dairy cattle. Other minerals in the soil and grass pasture were also analysed. Sodium (Na) (0.31 – 0.37 me /100g), potassium (K) (0.36 – 0.42 me /100g), iron (Fe) (76.4 – 101 ppm) and copper (Cu) (1.38 –2.24 ppm) were adequate during the dry and the rainy seasons. However, magnesium (Mg) (0.99 – 1.57 me /100g) was deficient in both seasons. Pasture K (2.05 – 2.67%), Na (0.21- 0.23%) and Fe (154 – 190 ppm) were adequate whereas Mg (0.15 –0.17%) and Cu (2.53 – 4.57 ppm) were inadequate in the sampled periods. In an attempt to quantify the requirement for supplementation of Ca, P and Zn forty eight dairy cows in their first, second and third parity were allocated to eight groups, comprising six cows based on breed, parity, stage of lactation and milk yield.

Group 1 was control group receiving no mineral supplement; group 2 (Ca) received 10 g of Ca in the form of calcium carbonate ($CaCO_3$); group 3 (P), received 8 g of P in the form of sodium monophosphate ($Na_2HPO_4.2H_2O$) and group 4 (Zn) received 400 mg of Zn in the form of zinc oxide (ZnO). The rest of the groups received a combination of Ca and P or Zn or both. Animals were drenched with the minerals daily as of May 1997 to March 1999. Performance was evaluated in terms of cow's health status, milk yield and reproductive performance and by measurement of blood parameters and metabolites which affect health, energy and protein balance before supplementation started and thereafter at every two month intervals. Significant differences ($P< 0.05$) were observed between groups in terms of performance. Low plasma Ca, Pi and Zn were more prevalent in the dry season ($P < 0.05$) especially in the month of July and during calving period in the month of March. None of the Ca, P and Zn formulations were able to rectify the low plasma Ca observed during these periods, however, Ca supplementation in the form of $CaCO_3$, improved tissue P and Zn status. Dry season supplementation with concentrates, hay and fresh forage improved plasma Ca, Pi and Zn concentrations. Cows supplemented with Ca in the form of $CaCO_3$ only were superior ($P< 0.05$) in terms of body condition, less cases of mastitis and anaplasmosis, short calving interval (< 365 days) and high milk yield with high fat and protein content compared to the other groups. General health status, liveweight gain, body condition score were affected negatively with P supplementation in the form of $Na_2HPO_4.2H_2O$ alone. Supplementation with Ca/Zn in dairy cows maintained copper balance, however, the other formulations resulted in reduction in copper balance. Supplementation of P/Zn maintained

stable levels of blood selenium concentration during both the dry and rainy season. Calcium and Zn supplementation reduced the blood selenium concentration in the rainy season. Since incidences of low plasma Ca, Pi and Zn occurred during the dry season, supplementation with concentrate, hay and fresh forage during the dry season should be encouraged to the farmer and other farmers with similar problems for better metabolism of Ca, P and Zn. However caution should be taken with type and the amount of Ca, P and Zn fed to the animals to avoid interactions between these elements other minerals like Cu and Se. Since P requirements for tropical grazing cattle is not known with certainty, further research on P requirements in grazing crossbred cows is recommended. Magnesium and Cu were inadequate in forage, supplementation of these minerals in the diet was recommended. The effects of Ca, P and Zn supplementation on Cu and Se balance need more research inorder to quantify the effects and understand the mineral interaction mechanisms involved.

DECLARATION

I, ELLIOT CHIKULA JAILOS HENDERSON PHIRI, do hereby declare to the Senate of the Sokoine University of Agriculture that this thesis is my own original work and has not been submitted for a degree award in any other University.

Signature:.....

Date: 08. 11. 2001

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DEDICATION

This thesis is dedicated to my parents, my wife Nathalia, my daughters Tilaiciana and Happyness and my sons William, Stephen and David. " I know that, whatever God doeth, it shall be for ever: nothing can be put to it nor any thing taken from it: and God doeth it that men should fear before him. That which hath already been is now; and that which is to be hath already been; and God requireth that which is past" Ecclesiastes 3: 14 - 15.

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LIST OF ABBREVIATIONS

A	April
AA	Amino Acids
AAS	Atomic Absorption Spectroscopy
ABP	Arterial blood pressure
ADP	Adenosine diphosphate
AFRC	Agricultural and Food Research Council
ALP	Alkaline phosphatase
Al	Aluminium
AOAC	Artificial Official analytical Chemistry
AMP	Adenosine monophosphate
ARC	Agriculture Research Council (Australia)
ASAS	Name of the farm
ASAT	Aspartate aminotransferase
ATP	Adenosine Triphosphate
AU	August
BCS	Body Condition Score
BW	Body weight
Ca	Calcium
Cd	Cadmium
CEC	Cation Exchange Capacity
CMT	Calfornia Mastitis Test
Co	Cobalt

Cp	Ceruloplasmin
CT	Calcitonin
Cu	Copper
D	December
DCP	Diagnostic Products Corporation
DEB	Dietary electrolyte balance
DP	Dorsal Palmer
DM	Dry Matter
DNA	Deoxynucleotide Acid
DTPA	Diethylene triamine penta acetic acid
DWBC	Differential White Blood Counts
EC	Eosinophil Counts
EDTA	Ethylene diamine tetra acetic acid
EED	Early Embryonic Death
ESOD	Erythrocytic copper – zinc superoxide dismutase
F	February
Fe	Iron
Glu	Glucose
GR	Glutathione Reductase
GSH.Px	Selenium dependent glutathion peroxidase
GSSG	Oxidised glutathione
Hb	Haemoglobin
HMWK	High Molecular Weight Kininogen
IAEA	International Atomic Energy Agency

ICP	Inductively Coupled Emission Spectroscopy
J	June
JA	January
JU	July
K	Potassium
LAD	Lactation Days
LC	Lymphocyte count
M	May
MA	March
MC	Monocyte Count
MDH	Malate Dehydrogenase
MF	Milk Fat
Mg	Magnesium
MMY	Monthly Milk Yield
Mn	Manganese
MP	Milk Protein
N	November
NAA	Neutron Activation
NAS	National Academy of Science
NC	Neutrophil Count
NRC	National Research Council USA
O	October
P	Phosphorus
Pb	Lead

PCO	Interval between parturition and next conception
PCV	Packed Cell Volume
PD	Plantar Dorsal
Pi	Inorganic phosphate
Plu	Plasma urea
PRO	Resumption of oestrous activity
PTH	Parathyroid Hormone
RBC	Red Blood Cell
RBPT	Rose Bengal Plate Test
S	September
SAT	Serum Agglutination Test
SCA	Standing Committee in Agriculture (Australia)
Se	Selenium
SIM	Serum Immunoglobulin
SUA	Sokoine University of Agriculture
TCORN	Technical Committee on Ruminant Nutrition
TML	Total Milk Yield per Lactation
TP	Total Protein
TSP	Triple Super Phosphate
TWBC	Total White Blood Cell Count
VDR	Vitamin D Receptors
WBC	White Blood Cell
ZA	First cross Zebu x Aryshire
ZAA	Second cross Zebu x Aryshire

ZF	First cross Zebu x Friesian
ZFF	Second cross Zebu x Friesian
Zn	Zinc

CHAPTER ONE

INTRODUCTION

1.1. BACKGROUND

A healthy animal reflects proper nutrition whereas an unhealthy animal reflects malnutrition. The latter increases the risk for infection resulting in lower production and higher mortality rates (McDowell, 1992; Hogan *et al.*, 1996). Minerals make up only a relatively small amount of the diet of the animals, nevertheless they are vital to the health of the animal (Church, 1991; McDonald *et al.*, 1995).

Forage provides an important source of minerals for grazing ruminants (McDowell, 1992). In some instances, forages may provide adequate quantities of all essential minerals required by ruminants, however, in other situations, forages are deficient in one or more minerals (Underwood, 1981). Severe mineral deficiencies in ruminants still occur to some degree, but marginal mineral deficiencies are probably much more widespread. Marginal mineral imbalances or deficiencies may result in no clinical signs but only small decreases in metabolic functions, with the overall impact on growth, reproduction, or health in ruminants being substantial (Spears, 1994). Marginal deficiencies are frequently exacerbated by interactions between mineral elements and or confounded by variations in protein or energy supply (Lee *et al.*, 1999). McDowell (1987) suggested that tissue mineral concentrations or their functional forms must be maintained within narrow limits if growth, health and productivity of the animal are to be maintained.

In Tanzania deficiencies of calcium (Ca), magnesium (Mg), phosphorus (P), copper (Cu), selenium (Se), cobalt (Co) and zinc (Zn) in soil, pasture and animal tissue of

grazing ruminants have been reported (Rodgers, 1975; Mwakatundu, 1977; Maro *et al.*, 1980; Sendalo, 1986; Phiri, 1995). Currently published mineral requirements for cattle are based on data for animals fed above maintenance levels in feeding pens (NRC 1989; SCA, 1990; TCORN 1991) and may not be relevant to *Bos inducus* cross bred cattle grazing in tropical pastures. For instance, both the pen studies of Little, (1983) and field studies of Coates and Ternouth (1992) in the tropics have recorded satisfactory growth of cross bred cattle fed lower levels of P than what is recommended (0.28 – 0.48% per kg diet on dry matter basis).

To establish appropriate supplement regime, one needs sufficient chemical analyses and biological data to determine which minerals are required and in what quantities. Knowledge of soil, pasture and feed composition, mineral concentrations and metabolism in tissues and can facilitate the identification of biochemical criteria to diagnose deficiencies or imbalances and develop strategies for their prevention. The present study was geared at obtaining such information with more emphases on animal performance, enzymes and tissue analysis in relation to Ca, P and Zn supplementation in grazing crossbred cows.

1.2 JUSTIFICATION

Assessment of mineral status of grazing ruminants has been considered an important strategy to increase animal productivity in many countries, especially in tropical and sub tropical areas where mineral deficiencies or imbalances are commonly found (Cuesta *et al.*, 1993). In these countries studies have been carried

out to investigate the relationship between mineral supplementation and production for different classes of livestock under various type of production system (NRC, 1984; SCA, 1990; AFRC, 1991; McDowell, 1992). However, these studies in Tanzania are limited. Thus, there is lack of comprehensive knowledge on minerals and their effects on grazing ruminants, which need to be addressed.

1.3 OBJECTIVES OF THE RESEARCH

1.3.1 General objective

To investigate the effects of mineral supplementation (Ca, P and Zn) on the performance of grazing crossbred cattle in the Southern Highlands of Tanzania, mainly in Iringa Region.

1.3.2 Specific objectives

(a) To investigate Ca, P and Zn concentrations in soil, forage, feed concentrates and animal tissues in Iringa Region.

(b) To investigate the effect of Ca, P and Zn supplementation on the health and immune status of grazing crossbred dairy cows in Iringa Region.

(c) To determine the effects of Ca, P and Zn supplementation on milk production and reproduction performance of grazing crossbred dairy cows in Iringa Region.

(d) To determine the effects of Ca, P and Zn supplementation on copper and selenium balance in grazing crossbred dairy cows in Iringa Region. Copper and Selenium were suspected to be low in a previous study (Phiri, 1995).

CHAPTER TWO

LITERATURE REVIEW

2.1 CALCIUM

2.1.1 Chemical properties and distribution of calcium in soil and plants

Calcium in soil

Calcium is an alkaline, soft, silvery white earth metal with an atomic weight of 40.08 and atomic number of 20. Its occurrence in the earth's crust is 3.64% and is the fifth element in order of abundance (McDowell, 1992). Calcium is found naturally in compounds chiefly as limestone (calcium carbonate), calcium fluoride and calcium sulphate, (Bondi, 1987). In Tanzania available soil Ca range between 2.20 – 13.9 me/ 100g (Mwakatundu, 1977; Sinlapåå, 1982; Mtengeti, 1984; Sendalo, 1986; Muhikambele, 1990; Phiri, 1995). Mwakatundu, (1977) observed that humid conditions and acidity in soil are associated with lesser amount of available Ca. Under humid condition and acidity, Ca is very much depleted from such soils by leaching (Landon, 1991)

Calcium in plants

Variable amounts of Ca are present in almost all foodstuffs but generally low in grains and abundant in most forage (Underwood and Suttle, 1999). Its content in natural feeds varies widely depending on the species of the plant and plant part analysed. In general the leaf contains twice as much Ca as the stem (McDowell, 1992). Pasture Ca concentrations are increased by applying nitrogenous fertilisers and decrease with advancing maturity of the grass (Underwood and Suttle, 1999). Grains such as barley, maize, sorghum, oats and wheat are very low in Ca (0.02-

0.10%) (NRC, 1980). Calcium contents in grains range from 0.05 – 0.20% (Phiri, 1995) whereas oilseeds Ca levels averaged 0.3% (Thomke and Macha, 1986). The non legume roughage such as grass hay and mature range pastures are intermediate in Ca content (0.31 - 0.36%) where as legume forage such as alfalfa and clover hay are high in Ca content (1.2 -1.7% Ca), (NRC, 1980). The average Ca contents in pasture grasses examined in Tanzania range from 0.26% - 0.78% (Mwakatundu, 1977, Mtengeti, 1984; Sendalo, 1986; Muhikambele, 1990).

2.1.2 Metabolism of calcium in animals

Tissue distribution

Calcium is the most abundant mineral element in the animal's body, comprising 1-2% of total body composition (Jonsson, 1999). About 99% of the animal body Ca is stored in bones and teeth. In the bones Ca occur in a molar ratio of 2:1 with P, primarily as hydroxyapatite crystals (Bondi, 1987). The remaining 1% is distributed in soft tissues and body fluids (Ballantine and Herbein, 1991).

Blood Ca concentration is usually reported as total Ca. It can be partitioned into three distinct fractions, 45 - 50% is ionised Ca and it is the biological active or free form, 45% is bound to plasma proteins, (80% to albumin and 20% to globulin) which is an important reservoir of Ca. The remaining 5% is bound to non-ionised inorganic mineral elements (Ballantine and Herben 1991). The normal blood concentration of calcium range between 9 and 11.5 mg / dl and is controlled by strong regulatory mechanisms (Littledike and Goff 1987; Shappel *et al.*, 1987). The

mechanisms involve hormones (calcitonin, parathyroid hormone and vitamin D 1,25 dihydroxycholecalciferol (1,25 $(OH)_2D_3$) (Goff *et al.*, 1991)

Absorption

The small intestine is the major site for Ca absorption, but occasionally the element is absorbed in small amounts in the rumen (Braithwaite, 1976; Underwood and Suttle, 1999). The absorption of Ca in ruminants ranges from 24% to 55% with an average of 45% (Bondi, 1987). Efficiency of Ca absorption in the small intestine of the dairy cow has been shown to rise in response to reduction in dietary Ca intake, pregnancy and onset of lactation (Braithwaite *et al.*, 1969, 1970; Braithwaite, 1974). The amount of Ca absorbed is directly related to milk production (Bondi, 1987). In early lactation when the demand for Ca is greatest, the increase in absorption falls short of requirement, hence the deficit being met by increased bone resorption under the activation of parathormone (Brairthwaite *et al.*, 1969, Goff, *et al.*, 1991).

Calcium absorption involves two processes, a passive diffusion between the intestinal epithelial cells (paracellular transport) and active transport (transcellular absorption) (Bronner, 1987). Experimental studies have suggested that if animals are fed a high Ca diet, more than 50% of the Ca absorbed will be by the paracellular route (Goff, *et al.*, 1991). When dietary Ca is low or when the demand is very high efficient absorption occur by active transport of Ca across intestinal epithelial cells. This process requires the active metabolite of vitamin D 1,25 dihydroxycholecalciferol (1,25 $(OH)_2D_3$) which induce Ca binding proteins (Goff, *et al.*, 1991) production hence increase the absorption rate. The absorption of Ca is

regulated to a large extent by the animal's requirements and is inversely related to intake. The mechanisms for absorption of dietary Ca are controlled primarily by the hormones 1,25 $(OH)_2D_3$, calcitonin and parathyroid hormone (PTH) (Goff *et al.*, 1991). Fall in plasma Ca concentration resulting from an increase in demand leads in turn to an increase in PTH (Fig. 1). This then, besides stimulating bone resorption, it stimulates the increased synthesis of (1,25 $(OH)_2D_3$) by the kidney which act on the gut to increase the production of Ca binding proteins which accelerates Ca absorption actively. In a reverse manner, an increase in plasma Ca concentration causes suppression of PTH release, reduction in 1,25 $(OH)_2D_3$ production and reduced Ca absorption.

Parathyroid hormone and 1,25 $(OH)_2D_3$ are hormones which normally respond to low plasma Ca (< 9 mg / dl) by increasing the entry of Ca into the blood from bone stores and dietary sources. Cows may develop parturient hypocalcaemia as a result of failure of one or both of these hormones to maintain adequate blood Ca at the onset of lactation (Horst and Reinhardt, 1983; Goff *et al.*, 1991).

Calcitonin (CT) lowers blood Ca. It acts on the bone to inhibit osteoclastic resorption and decreases renal tubular reabsorption of Ca and Mg. Secretion of CT is primarily stimulated by hypercalcaemia or hypermagnesemia (Littledike and Goff 1987; Shappel *et al.*, 1987). However, the degree of hypermagnesemia that causes CT release is generally seen only under experimental conditions (Littledike and Goff 1987). The main function of CT in Ca metabolism is to prevent hypercalcaemia after ingestion of a meal. When a high-Ca meal is ingested,

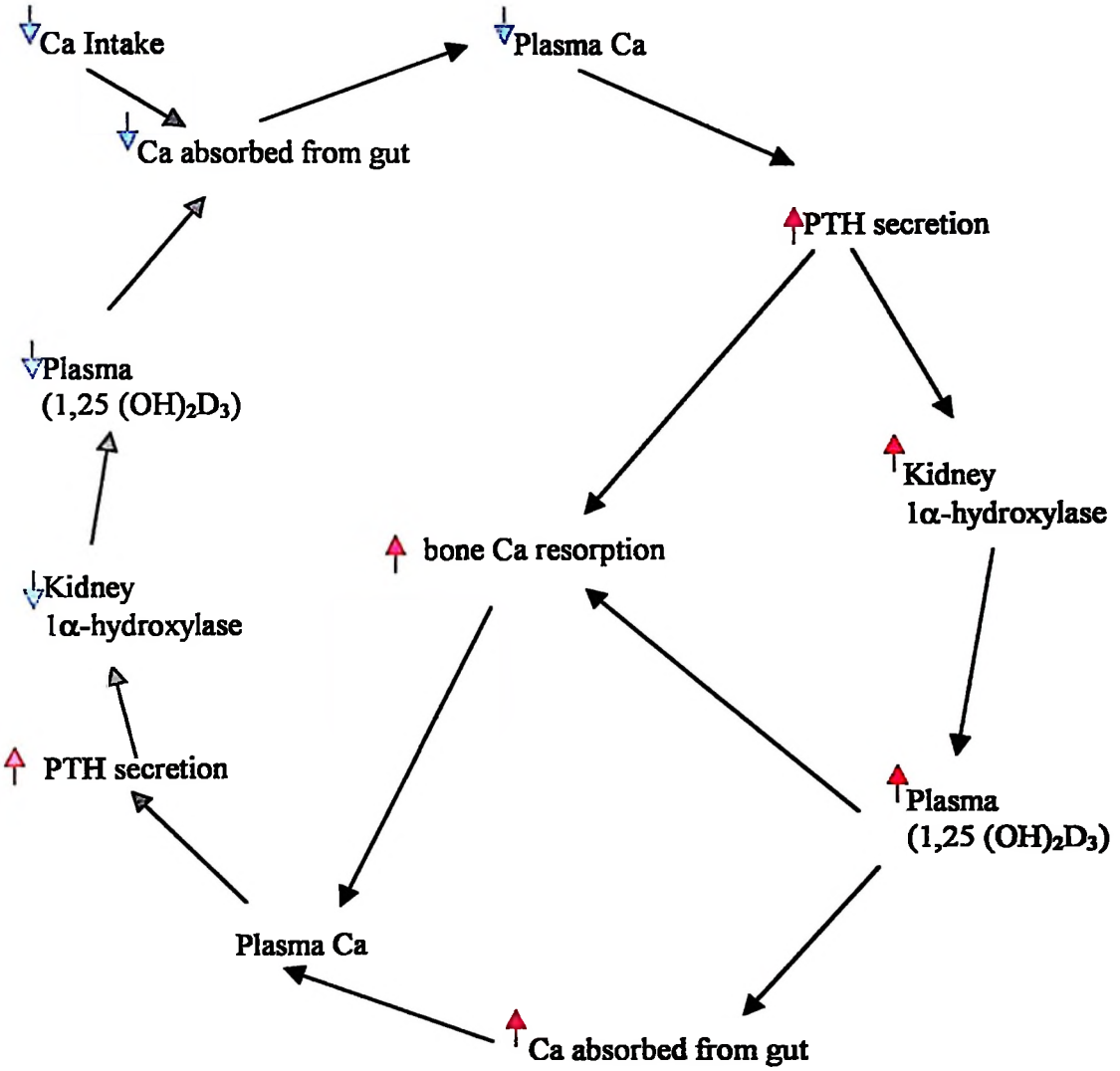


Figure 1. Mechanism of adaptation to alternations in dietary calcium, blue ↓ line indicates a decrease while red line ↑ indicate an increase. (After Horst (1986).

calcitonin secretion is stimulated, often before any hypercalcaemia develops. This effect is mediated by intestinal hormones such as gastrin, enteroglucagon and cholecystokinin (Care *et al.*, 1970; Cooper *et al.*, 1972).

The cellular mechanism of action of the Ca regulating hormone 1, 25 $(OH)_2D_3$ within intestine, kidney and bone is similar to that of the other steroid hormones. Tissue responsiveness to 1,25 $(OH)_2D_3$ is dependent on the circulating concentrations of the hormone in the extracellular *milieu* and the presence of vitamin D receptors (VDR) in the target tissue (Chen *et al.*, 1986; Feldman *et al.*, 1970).

Many factors have been identified that might reduce Ca absorption. An excess of P or Mg interferes with absorption of Ca by binding to the Ca binding protein, making the protein unavailable for Ca (Horst, 1986). There is a long held view that, a particular ratio of Ca to P in the diet is an important factor for the proper utilisation of the two elements (NRC, 1980). Several studies (Dowe *et al.*, 1957; Wise *et al.*, 1963; Smith *et al.*, 1966; Ricketts *et al.*, 1970; ARC, 1980; Miller, 1983c) have shown that dietary Ca to P ratios of between 1:1 and 7:1 in ruminants results in nearly equal performance provided the animal's P intake meets its requirement. Growing ruminants can tolerate a wide range of Ca: P ratio even larger as 7:1 (McDowell, 1992).

In monogastric animals absorption is impaired by presence of oxalic acid, which precipitates Ca in faeces. However in ruminants Ca in the presence of oxalates is

available, since oxalic acid is fully oxidised in the rumen by microbial enzymes to carbon dioxide and water, (Bondi, 1987). Nucleic acids, which are formed in the rumen and pass largely unchanged into the duodenum, are potent Ca binding substances at below pH 5 (McDowell, 1992). Furthermore it has been shown that dietary fibre can bind Ca and that the binding increases with increasing pH, which is related to the uronic acid concentration of the fibre (Underwood and Suttle, 1999). The importance of this binding in ruminant is yet unknown but unidentified Ca binding substances have been demonstrated in the calf ileum (Underwood, 1981). Dietary protein has been reported to have no effect on Ca absorption in dairy cattle (Visek, *et al* 1953) but Ca retention is decreased in pregnant sheep given low protein diets (Underwood, 1977). It was suggested that an inadequate protein intake might result in impaired formation of bone matrix and a consequent decrease in accretion of Ca in bone (Underwood, 1981).

Storage

Most of Ca in the body is stored in bones and teeth where it occurs as structural component of the tissues. Unlike the relatively inert mineral of tooth enamel, bone undergoes constant remodelling and turn over. If blood Ca concentration starts to decrease (< 9 mg/ dl), Ca is quickly mobilised from the bone under the effect of PTH to bring the blood level back to normal (McDowell, 1992).

Excretion

The three major routes of Ca excretion have been identified as faeces, urine and sweat, (Jonsson, 1999). Faecal output includes both the unabsorbed fraction and an

endogenous fraction, largely arising from secretions of the saliva and intestinal mucosa.

Urinary loss is minimal, owing to efficient reabsorption by the kidneys. About half of the plasma Ca, mainly ionised Ca, is filtered in the kidney, however more than 99% of this is reabsorbed under normal conditions (McDowell, 1992). The apparent Ca absorbability (feed Ca minus faecal Ca) generally approximates 50% although the percentage tends to decline as intake increases (Church, 1991).

Loss of Ca in sweat is of only minor significance in most species, but in man, horses and other species in which sweating is prominent, large amounts of Ca can be lost by this route (Bondi, 1987).

2.1.3 Physiological functions and manifestation of calcium deficiency

Approximately 99% of the total Ca in the body function as a structural component of bones and teeth, the remaining 1% is involved in such vital functions as blood clotting, membrane permeability, neuromuscular excitability, secretion of certain hormones and enzyme activation (McDowell, 1992).

Structure of bone and teeth

Bone is a metabolically active tissue with continuous turnover and remodelling both in growing and mature animals. The physiological control of bone metabolism is related to both endocrine and nutritional factors. The body maintains a relatively

constant Ca concentration in plasma (9 and 11.5 mg / dl) (Littledike and Goff 1987).

Dietary deficiency of Ca and or lack of vitamin D may impair absorption and utilisation of Ca, resulting in abnormalities of bones and teeth. The basic defect is a reduction or failure in the mineralization process of bone while the synthesis of the matrix continues (Miller, 1979). The bone of affected animals have a characteristically low ash content, they are soft and unable to maintain their normal shape (Underwood and Suttle, 1999).

Bone abnormalities caused by mineral disorders may occur at any age but more common in young animals. Rickets is the term used to denote the defective calcification of bones in young animals of all species. It is characterised by malformed bones, enlarged joints, lameness, fractures and stiffness of gait (McDowell, 1992). Osteomalacia is a term used in the mature animals and may be caused by the excessive mobilisation of minerals from bones. Pathologically both conditions are characterised by increased osteoclastic cell activity and osteoid (protein) formation (Benzie *et al.*, 1959). Osteoporosis is another failure of bone metabolism in mature animals caused by low Ca intake. In osteoporosis the mineral content of bones is normal in contrast to osteomalacia but absolute mass of bone is reduced. A deficiency of Ca is not the only cause of this disease. High intake of proteins associated with low Ca intake has been proposed to play a role in the pathogenesis (Underwood and Suttle, 1999). High protein results in considerable loss in total body Ca, hence bone resorption exceeds its formation and the synthesis of bone matrix (Miller, 1979).

Blood clotting

Calcium is essential for normal blood clotting. Calcium ions (Ca^{2+}) must be present for prothrombin to form thrombin (Fig. 2). Thrombin reacts with fibrinogen to form the blood clot, fibrin (Melvin, 1984).

Neuromuscular excitability

Calcium controls the excitability of the nerve and the muscle. Calcium influx is important in the regulation of cell function. While the onset of muscle contraction is mediated by a release of Ca^{2+} ions from the sarcoplasmic reticulum, the maintanance of contraction requires the presence of extracellular calcium. Calcium ions enter excitable tissues via two pathways (Martin, 1987). There is an entry which, coincides with the onset of the action potential which probably takes place via the Na^{+} channels. The second entry is through the channel activated by depolarization of the plasma membrane and is known as the voltage dependent channel. Reduced Ca^{2+} concentration produces increased excitability of pre- and postganglionic nerve fibres. Higher than normal Ca^{2+} concentration has the opposite effect on nerves and muscles, causing them to be hypoexcitable (Martin, 1987). Extremely low levels of serum Ca can result in tetany and convulsions. Excessive Ca depresses cardiac activity and leads to respiratory and cardiac failure. All these are followed by death, unless the cause is determined and treatment given (McDowell, 1992) promptly.

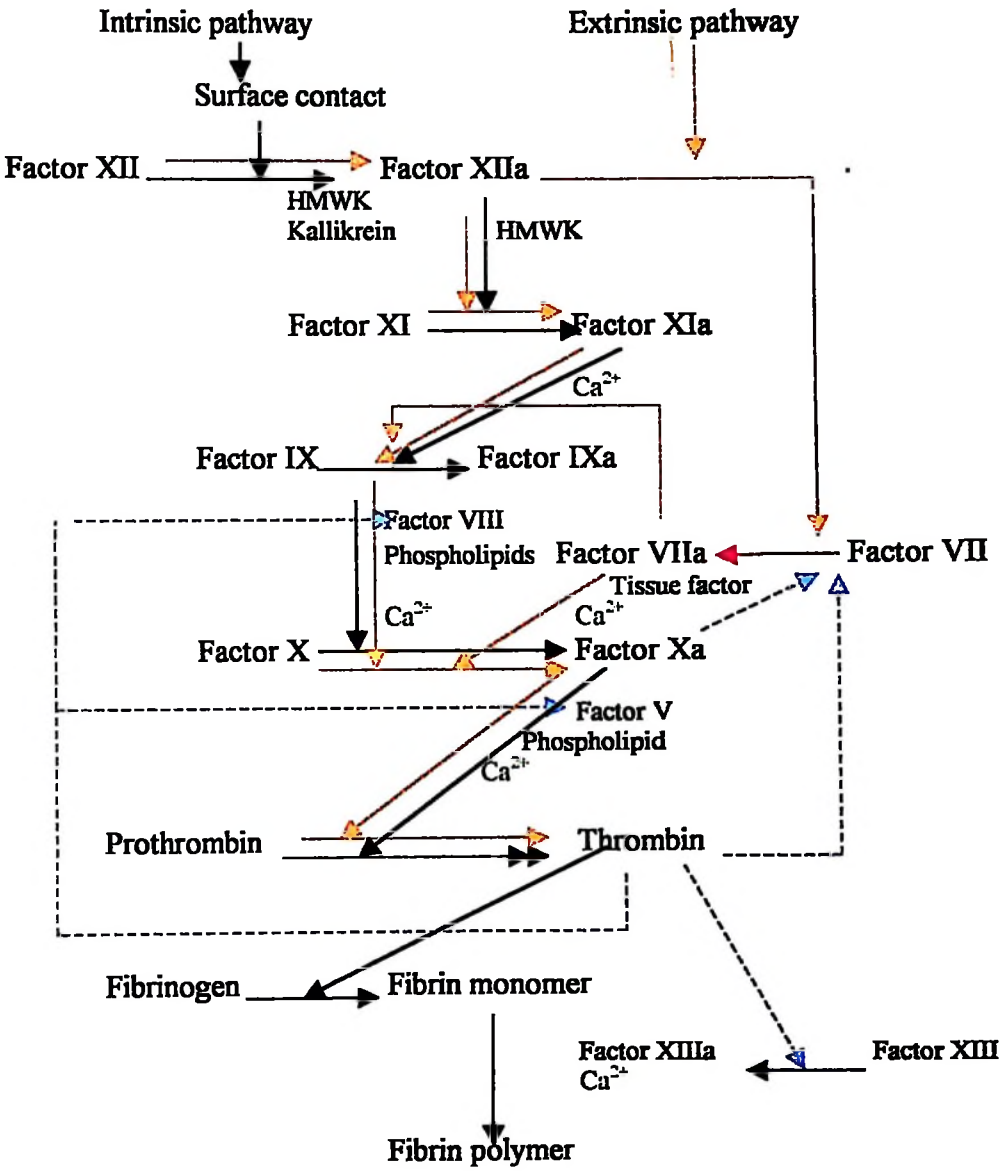


Figure 2 Simplified sketch illustrating involvement of calcium ions in the two pathways of blood clotting, the intrinsic (dark line) and extrinsic (red line). The blue broken lines illustrate the autocatalytic action of thrombin and factor Xa on both the intrinsic and extrinsic systems. HMWK, high molecular weight kininogen.(Modified from Melvin, (1984).

Milk secretion

Calcium is important for milk production as milk contains high amount of Ca. There is approximately 1 g of Ca and 0.8 g of P in every 1 kg of milk (NRC, 1989). The increased demand for Ca at the initiation of lactation may result in parturient hypocalcaemia in lactating animals. Parturient hypocalcaemia usually occurs within 72 hours after parturition and is manifested by circulatory collapse, generalised paresis, depression of consciousness and often hypothermia (McDowell, 1992).

At parturition, lactation creates a sudden high demand for Ca. Since colostrum contains about 2.3 g Ca/l, a cow producing 10 litres of milk on the day of calving would lose 23 g of Ca. This is about two or three times as much Ca present in the extracellular fluid (Littledike and Golf, 1987). For the cow to adapt to the Ca demands of lactation, dietary Ca absorption must be stimulated. Decreases in Ca concentrations stimulate PTH secretion which in turn favours the synthesis of 1, 25 $(OH)_2D_3$ and increased intestinal absorption of Ca to avoid development of hypocalcaemia (Goff *et al.*, 1995).

Tissue with low numbers of vitamin D receptors (VDR) like the kidney responds poorly to 1,25 $(OH)_2D_3$ when compared with tissue having higher numbers of VDR like the small intestine (Chen *et al.*, 1986). A defect in the response of the intestine to 1, 25 $(OH)_2D_3$ has been proposed as a possible mitigating factor in milk fever (Horst and Reinhardt, 1983). Intestinal VDR concentration has been shown to be lower immediately after calving (Golf *et al.*, 1995). Furthermore, metabolic alkalosis caused by prepartum diets high in cations, especially K and Na reduce

bone and renal responsiveness to PTH (Goff *et al.*, 1991; Goff and Horst, 1997).

Reproduction

Calcium is important in reproduction. Hypocalcaemia has been associated with poor reproductive performance (Scharp, 1980; Mckay (1994). Studies have shown that a cow requiring only 1 service per pregnancy had average serum Ca concentrations of 2.27 mmol Ca /l, compared with 2.17 mmol Ca /l in cows requiring more services (Scharp, 1980). Hypocalcaemia has been proposed as a cause of delayed postpartum ovulation in dairy cows (Ward *et al.*, 1971; Scharp, 1980) and delayed post partum uterine involution in cows (Al-Eknar and Noakes, 1989) and sheep (Robalo and Noakes, 1984). Cattle fed on Ca deficient diets showed delayed cervical and uterine involution (Morrow, 1980; Risco *et al.*, 1984). In such cases normal uterine involution occurred following the addition of Ca and Vitamin D in the diet. Furthermore, decreased blood Ca concentration had been associated with increased incidences of dystocia and retained placenta in cows (Morrow, 1980).

There are several mechanisms suggested by which hypocalcaemia might impair reproductive performance. These include poor uterine contractility, reduced blood flow to the hypothalamus and ovary thereby causing interference with normal endocrine signal transmission on a cellular level (Jonsson, 1999).

Impaired uterine contractility

There is evidence to suggest that hypocalcaemia might be associated with impaired uterine contractility. More rapid uterine involution (8 days earlier) was reported in

dairy cows fed Ca and vitamin D supplemented rations (Ward *et al.*, 1971). In another study, cows which had milk fever had thicker uterine horns during the first 32 days postpartum and had an average of 30.8 days to first ovulation compared to control cows with adequate Ca (2.40 mmol/l) which had 20 days to first ovulation (Risco *et al.*, 1994). In the same study, cows with low plasma Ca concentrations (2.17 mmol/l) were observed to have uterine prolapse and retained placenta.

Delayed uterine involution was associated with hypocalcaemia in a study by Al-Eknar and Noakes (1989), where parturient cows had reduced frequency and amplitude of uterine contractions when infused with Na_2EDTA. Robalo and Noakes (1984) using parturient ewes obtained similar findings.

Effect of subclinical hypocalcaemia on energy balance

Hypocalcaemia may induce negative energy balance as a result of impaired ruminal function. Daniel (1983) observed a linear relationship between plasma Ca concentration and rumen contraction frequency and amplitude as well as impaired abomasal contractility in Na_2EDTA induced hypocalcaemic cows. Huber *et al.* (1981) reported similar findings with respect to ruminal function in sheep. They noted that ruminal dysfunction was observed before any other clinical signs of hypocalcaemia. Complete ruminal paralysis was observed by Desmecht *et al.* (1996) when plasma Ca concentration fell by 25%. Reduced ruminal contractility due to hypocalcaemia reduces the absorption of nutrients including Ca.

Plasma glucose and Ca concentration have been reported to have a direct

relationship (Jonsson, 1999) to each other. Cows that suffer from parturient hypocalcaemia were reported to suffer from ketosis (Bendixen *et al.*, 1987) and cows with ketosis were found to have lower plasma Ca concentrations than normal cows (Eldon *et al.*, 1988). Relationship between Ca and insulin release has been reported by Wollheim and Sharpe, (1981). Furthermore, Littledike *et al.*, (1970) observed lower insulin concentration in cows with parturient hypocalcaemia. When Ca concentration in plasma falls below critical level (2.20 mmol/l) hyperglycaemia follows. However, whether this is due to change in insulin secretion or insulin binding is not clear (Jonsson, 1999).

Effects of hypocalcaemia on cardiovascular functions

It has been proposed that the effects of hypocalcaemia on reproduction may be due to alterations in the circulatory system (Kerr, 1992). Hypocalcaemia causes a substantial depression in the cardiac output (Daniel and Moodie, 1978; Barzanji and Daniel, 1987; Desmecht *et al.*, 1996). Daniel and Moodie (1978), demonstrated that when plasma Ca concentrations was reduced by 51% of normal levels, there was a reduction in cardiac output by 48% and a reduction in systemic arterial blood pressure (ABP). In sheep, blood flow to the ovaries declined by 16% when plasma ionised Ca concentration was reduced by 50% (Jonsson and Daniel, 1997). Desmecht *et al.* (1996) also demonstrated that reducing plasma ionised Ca concentration by 50% in calves resulted in a systemic reduction in ABP by 16%. The authors suggested that the decline in ABP was due to reduced vascular smooth muscle and myocardial contractility, thereby reducing the hydrostatic pressure and therefore blood flow through the blood vessels.

2.1.4 Assessment of calcium balance in animals

A number of response criteria have been used to evaluate Ca status in livestock and human beings including growth rate, feed intake and feed efficiency, serum/plasma Ca concentrations, bone alkaline phosphatase levels as well as dimensional, compositional and mechanical criteria for bones.

Plasma/serum calcium concentration

Total plasma Ca concentration may give an overall indication of Ca status, but ionised Ca concentration gives a better estimate of the amount available for uptake and utilisation by tissues (Ballantine and Herbein, 1991). Calcium is precisely regulated in blood plasma. In adult cattle the normal total plasma Ca range is 2.43 – 3.10 mmol/l (Kaneko, 1989). Several methods are available for measurements of plasma or serum Ca i.e. use of colorimeter, spectrophotometer and atomic absorption spectrophotometer (Gitelman, 1967; Kessler and Wolfman, 1964).

Serum/plasma and urine hydroxyproline

An animal's body skeleton is an important source of Ca for maintaining the concentration of Ca in plasma (Kronfeld, 1976). In the skeleton a continuous process of bone remodelling takes place characterised by a close coupling of resorption and formation of bones. During the process of resorption, not only do the bone minerals become available to the central pool but the organic bone matrix is also broken down (Underwood, 1981). Nearly 90% of the total body collagen is present in the bone matrix, with the degradation of collagen, the amino acid

hydroxyproline is freed and partly excreted in urine. Hydroxyproline is found almost exclusively in collagen, determination of its concentration in plasma may reflect the bone collagen turnover. Determination of hydroxyproline concentration can be done routinely more easily in the plasma than in urine. Hydroxyproline excreted through the urine account only for 10% of hydroxyproline freed by hydrolysis of collagen (Underwood, 1977).

Alkaline phosphatase

The enzyme alkaline phosphatase (ALP) is widely accepted as an indicator of bone formation as it is abundantly available in osteoblasts and chondroblasts (Kaplan, 1972; Kaneko, 1989). This enzyme is also present in many cells such as the epithelial cells of the kidney and intestine. When disease in these organs is excluded, a positive correlation can be found between serum ALP and Ca accretion rate (Boyd *et al.*, 1983).

Other methods

Other methods of investigating Ca balance include:

(a) Determining the bone mineral content either chemically or by dichromatic photon absorption, radiographic photometry and ultrasound techniques (Williams *et al.*, 1991b),

(b) Kinetic studies with radio - labelled Ca (Ramberg *et al.*, 1976),

(c) Histological and micro radiographic examination of bone biopsies (Rowland *et al.*, 1970; Black *et al.*, 1973; Yarrington *et al.*, 1977).

2.1.5 Calcium requirements and method of supplementation

The requirements for minerals, as for energy and other nutrients are estimated factorially and by the complementary method of feeding experiments (SCA, 1990). The factorial method does not make direct allowance for the requirements of the microbial population in the gut, particularly in the rumen, for interactions between nutrients during their utilisation, or for the effect of disease (including parasitism) and other disorders (SCA, 1990). The estimated requirements tend to be minimal values. Because of these reasons factorial estimates reference must be made from results of feeding experiments. In feeding trials the mineral being studied is given to the animals in two or more amounts and measurements are made of their performance e.g. growth, concentrations of the mineral in the body fluids and tissues and physiological states (McDowell, 1996).

Normal concentrations of minerals in feed or animal tissues, when stated, are those which show no evidence of deficiency or toxicity and impaired animal productivity at the given intake of energy and other nutrients (McDowell, 1992). Optimal productivity implies that the animal has fully achieved the level of production that can be sustained by the dietary energy supply. Unless stated otherwise, estimates are made of requirements (McDowell, 1992; Underwood and Suttle, 1999). Requirements are the minimum amounts of the mineral necessary for optimal productivity and will not necessarily maintain tissue concentrations as high as can be found in healthy animals.

Calcium requirements

The animal body skeleton provides an enormous reserve for both Ca and P, which can be drawn upon during a period of dietary deficiency (Bondi, 1987). This makes the estimation of the requirements of the adult, non pregnant, non lactating ruminant particularly difficult because diets deficient in Ca results in no obvious detrimental effect for a considerable period (Church, 1991). Requirements for Ca increase substantially with the onset of lactation as milk contains substantial amounts of calcium. The inability of a cow to adjust rapidly to this increased Ca demand as mentioned earlier can result in parturient hypocalcaemia. Dietary formulations for dairy animals should be formulated with the intent of meeting the cow's Ca requirements. Calcium needed for maintenance, growth, pregnancy and lactation in dairy cattle is 0.43 - 0.77% per kg diet expressed on dry matter basis (McDowell, 1992).

Source of calcium

The nutritional availability (true digestibility) of Ca may vary with different sources and therefore one may want to take this into account when formulating diets (NRC, 1989). Calcium from inorganic sources appears to be utilised more efficiently than that from plant origin (Bondi, 1987). Availability of Ca from different sources for cattle can be classified into two groups i.e. Ca from bone meal, mono or dicalcium phosphate being highly available and that from hay and forages being of lowest availability (Underwood and Suttle, 1999). Relative availability of Ca to cattle was about 20% lower in tropical grasses containing calcium oxalate than in grasses containing little calcium oxalate (Wiseman and Coles, 1990). Calcium in the soft

phosphate sources has been reported to have a low availability whereas the availability in gypsum (calcium sulphate,$CaSO_3.2H_2O$) is about 90% of that in calcium carbonate and in dolomite limestone (calcium magnesium carbonate, $CaMg(CO_3)$) the average availability is 66% (Church, 1991).

The most common sources of Ca supplementation in animal diets is ground limestone or calcium carbonate. Other common sources include oyster shell, calcium sulphate, calcium chloride, calcium phosphate and bone meal. These range in Ca content from 16 to 38% (McDowell, 1992).

Methods of Ca supplementation

Calcium deficiency can be prevented or overcome by direct treatment of the animals through supplementation of the diet or water supply or indirectly by appropriate fertiliser treatment of the soils on which the plants to be consumed are grown (Underwood, 1981). The choice of supplementation procedure depends on the management system. In most feedlot cattle and dairy cows, Ca supplements are incorporated into concentrate diets which generally ensures that animals are receiving the required quantities (McDowell, 1996).

Special Ca supplementation considerations need to be implemented for high producing dairy cows to prevent metabolic disorders such as parturient paresis. Parturient paresis can be prevented by feeding a prepartum cow low Ca and adequate P diet (McDowell, 1992). Treatment with high levels of vitamin D has been successful but toxicity problems sometimes have been encountered (Littledike

and Goff, 1982). Recently there has been considerable interest in altering dietary electrolyte balance (DEB) or fixed ions balance to prevent milk fever (Underwood and Suttle, 1999). Feeding late gestation cows anionic or acidogenic diets decreases incidence of parturient paresis, improves subsequent lactation performance and health (Gaynor *et al.*, 1989). High anion levels in acidic diets in cows is thought to induce metabolic acidosis that facilitates bone-Ca resorption (Beede *et al.*, 1992).

2.1.6 Calcium toxicosis and interaction with other minerals

Under normal condition dietary Ca is considered to be non toxic when single large doses are consumed by animals because it is under homeostatic regulation, i.e. the element is absorbed according to need and the excess is excreted (NRC, 1980). Excess of Ca may cause bone disorders and reduce feed consumption (McDowell, 1992). Feed intake was reduced by 3.2%, weight gain by 1.8% and feed efficiency by 1.59% when feedlot cattle received diets containing more than 1% Ca (Bondi, 1987). Maximum tolerance level of Ca for cattle is 2% Ca when adequate dietary P is provided (McDowell, 1992).

Dietary Ca, at a level of 4.4%, may cause significant depression in protein and energy digestion, whereas high incidence of bone and joint abnormalities (osteoporosis, vertebral ankylosis and degenerative osteoarthritis) have been reported in bulls fed three to five times recommended Ca levels (McDowell, 1992). Some research has shown that excess Ca can reduce digestibility of fat and other organic nutrients (Underwood, 1981). Animals grazing on pastures with excess Ca

in several parts of the world, develop calcinosis, a disease characterised by deposition of Ca salts in soft tissues (McDowell, 1992). In most cases the condition is caused by ingestion of plants which contain water soluble glycoside of 1,25-$(OH)_2D_3$ Ca like *Solanum malacoxylon, Cestrum diurnum and Trisetum flavescens* (Wasserman and Taylor, 1976). The digestive system of the animal release the sterol which promotes a massive increase in the absorption of the dietary Ca and phosphate such that adjustment of these by the normal physiological process become ineffective and soft tissue calcification results (McDowell *et al.*, 1983).

The addition of excess Ca to an otherwise adequate diet may result in a deficiency of other essential elements such as P, Mg, Fe, I, Cu, Zn and Mn (NRC, 1980; Shupe *et al* ., 1988). In every case it appears that the injurious effect of the Ca is attributable to an interaction with these elements rather than to a harmful effect of Ca itself (McDowell, 1992).

2.2 PHOSPHORUS

2.2.1 Phemical properties and distribution of phosphorus in soil and plants

Phosphorus in soil

Phosphorus has an atomic weight of 30.97 and its atomic number is 15. It forms about 0.12% of the earth's crust (Bondi, 1987). Phosphorus does not occur free in nature because it is very reactive (McDowell, 1992). Essentially all of the naturally occurring P compounds are phosphates and they occur as apatite, $Ca_{10}(PO_4)_6(F,$

$Cl,OH)_2$ or $3Ca_3(PO_4)_2.Ca(F,Cl,OH)_2$, (Bondi, 1987). The ultimate source of P is igneous rocks which can be divided into four principal types of large deposits i.e. igneous appatite, marine phosphorites, phosphatized rock and guano (McDowell, 1992). Pellet phosphorite and guano are the two major groups of sedimentary deposit important for production of feed phosphates. The high level of fluoride (F) in the natural rock phosphates limits them as P sources for animal nutrition (McDowell, 1992).

It has been reported that, in East Africa most of P comes from the organic matter and very little from the inorganic sources (Mwakatundu, 1977). In Tanzania available soil P range between 3.6 – 358 ppm (Mwakatundu, 1977; Mtengeti, 1984; Sendalo, 1986; Muhikambele, 1990; Phiri, 1995). Mwakatundu, (1977) observed that volcanic soils at Iwambi Mbeya and West Kilimanjaro had high available P levels in both the dry and wet seasons. The author further noted that high P was also related to high organic matter.

Phosphorus in plants

Phosphorus is present in all common foodstuffs. The phosphorus status of forage varies widely and is influenced primarily by the P status of the soil, the stage of maturity of the plant and climate (Underwood and Suttle, 1999). Temperate forages generally contain more P than tropical forage (0.35% vs 0.25% P) per kilogram dry matter (DM) (Minson, 1990). Distribution of P between leaf and stem is relatively uniform, but there is a marked reduction in whole plant P as the forage matures particularly during the dry season (McDowell, 1992). Cereals contain relatively

uniform and apparently adequate P concentrations (0.27 % - 0.43 %), vegetable protein sources contains 0.5 % - 1.2 % P (McDowell, 1992). Seeds by- products such as wheat bran and oil meals are especially rich in P and most of this (50 – 80 %) is present as phytate which is well utilized by ruminants (Underwood and Suttle, 1999). Feeds containing milk and bone are high in both P and Ca (McDowell, 1992).

In Tanzania the average P contents in pasture range from 0.06% - 0.67% (Mwakatundu, 1977, Mtengeti, 1884; Sendalo, 1986; Muhikambele, 1990). Phosphorus contents in grains range from 0.14 – 0.40% (Phiri, 1995) whereas oilseeds P levels averaged 0.20 – 1.5% (Thomke and Macha, 1986).

2.2.2 Phosphorus metabolism in ruminants

Tissue distribution

Phosphorus is the second most abundant mineral element found in the body of cattle (Ternouth, 1990). Approximately 80% of the P of the body is present in bones and teeth. The remainder is distributed in soft tissue and body fluids. Phosphorus is present as hydroxyapatite ($Ca_{10}(PO_4)_6$ $(OH)_2$) in bones. Hydroxyapatite has an "ideal" molar Ca:P ratio of 1.67:1.0. (Corbridge, 1985). Small amounts of Mg can substitute for Ca and carbonate or hypophosphate for phosphate or hydroxide to form an amorphous phase containing ($Ca_3(PO_4)_2CaCO_3$ $Mg_3(PO_4)_2$ and small amount of citrates, Na, K, Cl and F so that Ca:P ratio (w/w) in bones become 2.0:1.0 (Bondi, 1987; Ternouth, 1990). In the hydroxyapatite of teeth dentine and

enamel, small quantities of fluoride may substitute for the hydroxide to confer extra hardness. In bones, hydroxyapatite is present as rod shaped crystals intimately associated with the fibrous protein, collagen, which spatially anchor the crystals to form organic matrix of the bone (Ternouth, 1990).

In times of deficiency, up to 30% of P have been observed to be removed from the bones (Benzie *et al.*, 1959; Little, 1983; Sevilla, 1985). Cancerous (spongy) bones like the vertebrae and ribs will reduce their concentration of P and Ca per unit of bone most readily when deficiency occurs (Benzie *et al.*, 1959; Little, 1983). Bones of the limbs can also lose considerable amounts of mineral (Ternouth *et al.*, 1980) although this loss is in the form of a reduction of cortical volume rather than concentration (Little, 1984; Davis, 1985).

Absorption

In ruminants large amounts of inorganic phosphate enter the gastrointestinal tract by salivary secretion, and this endogenous secretion is well balanced by the net Pi absorption from the intestinal tract (Ternouth, 1990). Thus the overall absorption rate from the intestinal tract is substantially higher in ruminants compared with single stomach animals (Breves and Schroder, 1991). The major site of absorption of dietary P in cattle is the duodenum, however some amounts of P (of endogenous origin) are secreted into the lower part of the small intestine (jejunum and ileum) (Ternouth, 1990).

Although it is well known that, in ruminants the upper small intestine is the major

site for net Pi absorption, little is known about the epithelial transport processes involved and their hormonal regulation (Underwood and Suttle, 1999). This is in contrast to many single- stomached species where a calcitriol - stimulated secondary active Na^+ -Pi-co-transport system has been identified as the predominant mechanism for active Pi absorption (Brever and Schroder, 1991).

For sheep, a carrier mediated mechanism, has been assumed for mucosal Pi uptake from in vivo studies (Care *et al.*, 1980; Scott *et al.*, 1984). Schroder *et al.* (1995) found that 65% of active transported Pi in sheep jejunum was mediated by a Na-dependent active transport mechanism. The mechanism for the remaining Na^+-independent active Pi transport has not yet been identified. The same authors further found that dietary P depletion induced a significant stimulation of net Pi absorption in goat duodenum and jejunum. This increase was independent of dietary Ca supply and was not associated with increased plasma calcitriol concentrations. They concluded that substantial differences in hormonal regulation of Pi transport exist in small ruminants in comparison with single stomach species.

A number of factors can affect the absorption of P. It is known that high levels of Ca in the diet may reduce the absorption of P when its levels are low. (Theiler and Green 1932; Preston and Pfander 1964; Young *et al.*, 1966; Field *et al.*, 1985; Sevilla, 1985). This reduction is due to either precipitation of P in non-absorbable forms within the intestine as the pH rises or to the homeostatic mechanisms concentrating on regulating plasma Ca (Schneider *et al.*, 1985).

Large intakes of iron, aluminium and magnesium salts interfere with the absorption of P by forming an insoluble phosphate (Bondi, 1987) . Phosphorus present in phytates is poorly available to simple stomached animals, but is available in ruminants, since phytates are hydrolysed by microbial phytases occurring in the rumen to inositol and phosphoric acid (Underwood and Suttle, 1999). Parasitism may also reduce P absorption (Wilson and Field 1983).

Storage

Bones serve as a store for P, which can be mobilised when the provision of this mineral is not sufficient to meet the body requirements. Thus the mineral metabolism of bone involve not only the accretion of P during growth, but also continuous exchange between the bones and blood (McDowell, 1992).

Excretion

Secretion of P into the intestinal lumen (endogenous faecal P) occurs, but this loss does not represent as high a proportion of the daily loss as for Ca. Most of the Pi excretion occurs through the kidneys, which appears to be the main regulator of blood Pi concentration. Work with sheep has shown that when the level of plasma Pi is above 1.90 mmol P/l the reabsorptive capacity of the kidney tubules is exceeded and significant amount of P is lost in the urine. When intestinal absorption of P is low, urinary P falls to a low level with reabsorption by the kidney tubules approaching 99% (Field *et al.*, 1983; Scott *et al.*, 1984; Davis 1985; Sevilla, 1985). Cattle fed concentrate diets, excrete more P in the urine; this is largely due to the low rates of salivary secretion and the diets being rich in P (Ternouth, 1990).

2.2.3 Physiological functions and manifestation of phosphorus deficiency

Phosphorus is involved in a great variety of vital functions probably more than any other mineral (Corbridge, 1985). In grazing livestock, P deficiency has been described as the most prevalent deficiency mineral throughout the world (McDowell, 1992). Hypophosphatemia may be directly related to low levels of P in soils and forages, wider Ca:P in soils and forage or high levels of plasma Mo which may cause increased urinary and faecal excretion of P (Underwood and Suttle, 1999).

Carbohydrate, protein, nucleic and fat metabolism

Phosphorus is involved in feed metabolism and utilisation of fat, carbohydrate, protein and other nutrients in the body (Bondi, 1987). As a major constituent of cell walls, phospholipids are involved in maintaining the structure and integrity of all cells in the body. Furthermore, phospholipid formation allows fatty acids to be transported throughout the body. As part of DNA in cell nuclei, P is involved in the determination of the genetic characteristics of the animal and in the expression of those characteristics through the P in RNA. Phosphorus is involved in many enzymatic reactions involving energy metabolism in cells e.g. in ATP, ADP, cyclic AMP and creatine phosphate (Wang *et al.*, 1985). Phosphorus is important in formation of protein, nucleoproteins and phosphoproteins (Petri *et al.*, 1989; Ternouth and Sevilla, 1990b).

Buffer system

Phosphorus is an essential component of the buffer systems in the blood and other body fluids including those of the rumen (Ternouth, 1990). Salivary inorganic P has two important functions, to act as a buffer against large depressions in pH resulting from the production of organic acids and to provide adequate P for the rumen reticular microbes. The rumen and reticulum have relatively high concentrations of P (200 – 600 mg /l) (Evans and Davis, 1966; Witt and Owens, 1983). Salivary glands of cattle have the ability to concentrate plasma inorganic P three to eight fold depending on salivary flow rate and plasma P concentration (Clark, 1953; Ternouth *et al.*, 1985).

Microbial nutrition

Phosphorus is essential for proper functioning of the rumen microorganisms especially those that digest plant cellulose (Bryant *et al.*, 1959; McDowell, 1985). Thus the gastro intestinal microbes have a requirement for P which is distinct from the requirements of cattle. Microbial activities are reduced when inorganic P levels are less than 50 – 80 mg /l in the rumen (Komisarzuk *et al.*, 1987). These critical levels of P for adequate microbial fermentation apply to both ruminal and caecocolic microbes (Milton and Ternouth 1984).

Food intake and utilisation

Phosphorus is involved in the control of appetite and in the efficiency of feed utilisation (Underwood, 1981). The primary effect of feeding low P diets is to reduce food intake and digestibility; this reduction may be as much as 50% in sheep

(Call *et al.*, 1986; Read *et al.*, 1986b). Four possible feedback mechanisms to the satiety centre have been suggested to be involved in reduced feed intake i.e. (i) low ruminal P results in reduction in fibre digestion by limiting microbial activity; (ii) low ruminal P reduces microbial protein synthesis thus reducing the intestinal absorption of amino acids (AA) so the animal becomes AA deficient; (iii) low P in metabolically active tissues (for example, muscle and liver) results in reduced P for intermediary metabolism; (iv) low P in metabolically active tissues results in reduced RNA synthesis affecting the metabolic activities of cells (McDowell, 1992).

The reduction in intake do not necessarily occur immediately and appear to be dependent upon the previous history of the animal. Animals that do not have a high net requirement may be fed a moderately P deficient diet and not suffer from a reduced food intake for a considerable period of time (weeks, even months) (Milton and Ternouth, 1985). Animals fed high Ca diets (> 4 g/kg DM), which inhibit the reabsorption of Ca and P from bone, appear to reduce their food intake much more rapidly (Field *et al.*, 1975; Boxebeld *et al.*, 1983, Milton and Ternouth, 1985).

One of the consequences of deprived appetite is craving for and consumption of abnormal materials such as soil, wood, flesh, and bones. This behaviour may predispose the animals to ingest the botulism micro organism *Clostridium botulinum*. (Underwood, 1981). Significant quantities of soil can be consumed by cattle, especially during periods of forage shortage and after heavy rain (soil splash) (Healy, 1968; McDowell, 1992).

Body weight gain

Phosphorus supplementation experiments have shown benefits in liveweight gain (Preston and Pfinder 1964; Whinks *et al.*, 1977; Little *et al.*, 1978). Heifers receiving higher dietary P of 0.20% versus 0.12% had higher gains (257 kg versus 205 kg) per year and produced calves with greater birth weights (26.9 kg versus 22.7 kg), respectively (McDowell, 1992). Since liveweight gain is closely related to dry matter intake (Minson and McDonald, 1987), these results are strongly suggestive of an effect of P supplementation on the increase in dry matter intake.

Bone demineralisation

Lameness and stiffness of gait, enlarged and painful joints, bending, deformation or fractures of the pelvis and long bones, arching of the back, facial enlargements, malformed teeth and jaws are characteristics of P deficiency (Underwood, 1981). The basic defect is a failure or reduction in the mineralization process so that the bones contain insufficient minerals to develop or maintain normal shape and strength and therefore to sustain mechanical functions. Stiffness in the joints and reluctance to stand exacerbate the P deficiency as they reduce the ability of the animal to look for food (Ternouth, 1990). Rickets and osteomalacia as in Ca deficiency are manifestations of long standing dietary P deficiency in young and adult animals, respectively (Jones and Hunt, 1983).

Reproduction

Poor reproduction has been a regular feature of flocks and herds confined to P deficient grazing pasture (Underwood and Suttle, 1999). The subnormal fertility is

associated with wide range of conditions including low ovarian activity, low conception rates, retained placenta, abortions and long calving intervals (Read *et al.*, 1986a; Call *et al.*, 1986). However there is no evidence to determine whether these are primary effects of the P deficiency per se or secondary effects of a reduction in feed intake (Ternouth, 1990).

Milk production

Phosphorus is needed for normal milk secretion. A lactating animal responds to a dietary deficiency of P by reducing its milk yield without affecting the concentration of the minerals in the milk produced (Muschen *et al.*, 1988). Even in extreme deficiency the composition of the milk remains within normal limits (McDowell, 1992). The depression of milk yield may be a secondary consequence of loss of appetite or reduced synthesis of rumen microbial protein (Underwood and Suttle, 1999). It has been reported that cows on a P deficient diet will increase milk production when fed a P supplement (Kincaid *et al.*, 1984) but if too much P is given, milk production may be depressed (Carstairs *et al.*, 1980).

2.2.4 Assessment of phosphorus balance in animals

Blood serum or Plasma inorganic phosphate (Pi)

Strong positive relationship has been found between plasma Pi and dietary P intake levels in both cattle and sheep (Tomas *et al.*, 1967; Little 1968; Belonje 1978; Gartner *et al.*, 1980; Ternouth *et al.*, 1980; Read *et al.*, 1986d). Serum P is a good indicator of P status of ruminants only if stress factors, time of sampling and blood

preparation (hemolysis, temperature and serum separation time) can be strictly controlled (McDowell, 1992).

Protein and P deficiencies commonly occur concurrently in the dry season under tropical conditions. Under these conditions food intake is reduced, cattle lose weight and bone resorption results in elevation of plasma Pi (Ternouth, 1990). Plasma levels of 1.80 – 2.90 mmol Pi /l are considered normal while, 1.25 – 1.75 mmol Pi /l are considered marginal and below 1.25 mmol Pi /l is considered low (Betteridge *et al.*, 1989; Ternouth, 1990; Underwood and Suttle 1999). Older beef cattle on pasture of low nutritive value can have mean serum Pi values around 1.0 mmol / l and do not benefit from P supplementation (Engels, 1981; Wadsworth *et al.*, 1990).

Plasma (or) serum alkaline phosphatase (ALP)

Plasma or serum alkaline phosphatase activity is a potential criterion, which is used as a supportive measure in determining P adequacy (Boyd, *et al.*, 1983). Alkaline phosphatase has been reported to increase in P deficiency (Kaplan, 1972). Plasma ALP had been reported to be a sensitive index for P at day 14 post treatment with P (Kaplan, 1972). This rapidity, in combination with relatively easy sample acquisition and chemical analyses lends ALP to routine assay. However, plasma ALP should be carefully scrutinized because; (a) plasma ALP is derived from both skeletal and nonskeletal sources (Kaplan, 1972) and (b) that being a blood constituent, it is subjected to minute fluctuations thus can lead to serious error.

Additional parameter

As in calcium status evaluation, it has been suggested that, bone condition is more sensitive to P status than are other parameters (McDowell., 1992). Williams *et al.* (1991b) analysed P concentration in blood, milk, faeces, bone, saliva, rumen liquor, various tissues and hair of growing cattle fed adequate (0.20%) or deficient (0.12%) dietary P. Of the parameters studied, rib bone P concentration best reflected dietary P intake. Williams *et al.*, (1991a) demonstrated that chemical, physical and mechanical properties of bone could be used to evaluate the P status of cattle. Breaking load and breaking strength were significantly higher for the adequate (0.20% P) versus low-P (0.12% P) diets, 1348 versus 1179 kg and 202.5 versus 189.2 MPa (unit of measurement) for stress respectively.

Bone characteristics are good indicators as they respond to a wider range of dietary Ca-P levels than are growth rate and feed efficiency or blood components, hence are more recommended (McDowell, 1992).

2.2.5 Phosphorus requirements and method of supplementation

Researchers have identified P as a major limiting nutrient for cattle in the tropical and subtropical environments (Whinks, 1977; Read *et al.*, 1986a,b,c; Betteridge *et al.*, 1989). However, the supplementary P requirements of grazing cattle are not known with certainty. Consequently, recommendations for P supplementation are based largely on speculation because of the two difficult conditions, which exist. i.e. large amount of recycling P from digesta to blood and to saliva in ruminant animals

as well as the dependency of P requirements on the levels of protein and digestible energy of the pasture available for grazing animals (McDowell, 1992; De Waal *et al.*, 1996). Many producers may over supply cattle with supplementary P while others may under supply. Both scenarios may have an impact on animal production and financial returns (Read *et al.*, 1986a). The cost of P supplementation must be justified by an increase in animal production or in value of the animals (McDowell, 1992).

The phosphorus (P) requirements for ruminants have traditionally been estimated using liveweight gain response curves and factorial technique (ARC 1980; NRC, 1989; Gueguen *et al.*, 1989). Live weight response curves pose difficulties associated with the complex interaction of P with other dietary nutrients and the animal's ability to withstand short and medium term P deficiency with little or no detrimental effect on production. Factorial estimates vary according to the values assigned to each factor and their differences are reflected in published recommended requirements (e.g. ARC 1980; NRC 1984; Geay and Micol 1989; SCA, 1990).

Phosphorus requirements

Phosphorus requirement is highly dependent on the level of productivity and the physiological status of the animal (NRC, 1984, 1989). High yielding milking cows requires much more dietary P than do low yielding cows. Young, pregnant animals that are lactating and still growing have high P needs (McDowell, 1992). The amount of P absorbed by the animal depends on the source of the P, the amount of

intake, the Ca to P ratio, intestinal pH, the age of the animal and dietary levels of Fe, Al, Mn, K and fat (NAS, 1988). The normal range recommended in dairy cattle is 0.28 to 0.48% in Units per kg diet, based on a 100% dry matter (McDowell, 1992).

Source of phosphorus

Different feeds vary widely in their content of P. Animal by-products such as fish meal, meat meal and bone meal are comparatively rich sources of P (Underwood, 1981). All the cereal grains and their by-products, oilseeds and oilcakes are rich in P (McDowell, 1992). Forages particularly legumes have higher levels of Ca than P. The phosphorus content of forage is low during the vegetative period. Thus the content of P in green and preserved forages depends on the vegetative stage of the plants when harvested (Underwood, 1981).

In practice, rations deficient in P should be supplemented with the mineral. A number of sources of P are available for supplementation including calcium phosphates (dicalcium phosphate, monocalcium phosphate, defluorinated rock phosphate, bone meal, guano- origin phosphates), ammonium phosphates (monoammonium phosphate, diammonium phosphate, ammonium polyphosphate), sodium phosphates (monosodium phosphate, disodium phosphate, sodium tripolyphosphate) and phosphoric acid (Cohen, 1975; McDowell, 1992). Dicalcium phosphate accounts for 70% of the Western European source of P (McDowell, 1996). In general the orthophosphate are well absorbed and utilised by all species of animals. High temperature lead to an increase of the pyrophosphate and metaphosphate radicals which are of lower availability (Bondi, 1987).

Fertiliser phosphates are sometimes used to provide P to livestock. Super juices are sources of P from P fertilizers that are safe (low F) and economical to use (McDowell, 1992). The super juice are supernatant made by mixing the fertilisers with water and allowing the slurry to settle for 12 to 24 hours (McDowell, 1992). The mixing allows most of the toxic F to precipitate as CaF_2 (McDowell, 1992). Triple superphosphate (TSP) containing 21% P and 2% F is quite commonly used where feed grade phosphates are difficult to obtain or prohibitively expensive. Triple superphosphate is quite commonly used as a P supplement in northern Australia, because it is cheap and available. It is also used in developing countries in which fertiliser phosphates are used to provide part of the P requirement (McDowell, 1992).

Method of phosphorus supplementation

As for calcium supplementation, P deficiency can be prevented or overcome by direct or indirect treatment of the affected animals. On sparse P deficiency in grazing animals the direct supplementation method is preferred (McDowell, 1996). High transport and application cost and poor herbage productivity prevents the use of the indirect method using phosphate fertilisers. In more climatically favoured and intensively farmed areas, phosphate applications designed primarily to increase pasture yields also increase P concentrations of forage (Underwood, 1981). In Australian studies, superphosphate applications at the rate of 125 kg/ha doubled pasture yields and increased forage P by 50% (Underwood, 1981). Phosphorus fertilisation to raise forage P in very acidic soils is not effective because soil P will be slightly available for plant uptake (McDowell, 1985). Superphosphate fertiliser

does not only increase herbage P but it also improves palatability and digestibility of the forage (McDowell, 1992). Unless there are definite forage yield increases that can be utilised effectively by grazing herbivores, use of mineral containing fertilisers is economically prohibitive (McDowell, 1996).

Individual dosing or drenching with P supplements ensures the right dosage for each type of animal and therefore getting maximum economic value. However, it is tedious, costly in labour and requires frequent handling of the animals. This procedure has therefore limited applicability unless it can be linked to other practices such as dipping of cattle once weekly for ticks control (McDowell, 1992).

Dissolving soluble phosphates in water is applicable only where the access of animals to water is controlled (McDowell, 1996). Studies with disodium phosphate in heifers indicated that by dissolving the compound in water at a rate of 1 g/l litre increased performance of the animals (McDowell, 1992).

Increased incidences of mineral deficiencies during the wet season are less related to forage concentration than to the greatly increased requirements for the P in grazing animals (McDowell, 1992). During the wet season, livestock gain weight rapidly since energy and protein supplies are adequate and thus the mineral requirements are high, while in the dry season inadequate protein and energy result in animals losing weight which lowers mineral requirements (McDowell, 1992).

2.2.6 Phosphorus toxicity and interactions with other minerals

Phosphorus is not considered to be toxic when single large doses are administered or consumed by animals, although mild diarrhoea may occur. However, prolonged consumption of high P diets may cause severe hypocalcaemia because of disorders associated with Ca absorption and metabolism (Cunha, 1973).

In cattle, the maximum P tolerance level is 1.0% when expressed as a percentage of requirements. The amounts of P that can be safely tolerated appear to be quite dependent on other factors including amount of other nutrients fed, especially Ca and Mg (McDowell, 1992). High P intake predispose animals to urinary calculi in sheep this is more likely to happen when the diet also provides excess magnesium leading to the formation of magnesium phosphates, an integral part in the formation of phosphatic calculi (Suttle and Field, 1970).

2.3 ZINC

2.3.1 Chemical properties and distribution of zinc in soil and plants

Zinc in soil

Zinc is a bluish white, relatively soft metal with atomic number of 30, atomic weight of 65.37 and is found in ores principally as the sulphide (ZnS) (McDowell, 1992). It is often found associated with sulphides of other metals especially lead (Pb), copper (Cu), cadmium (Cd) and iron (Fe), (Bondi, 1987).

Zinc occurs in the earth's crust to the extent of about 70-130 ppm and ranks the twenty fourth in abundance (Bondi, 1987). Normal soil Zn contents range from 10 to 300 ppm with an average of 50 ppm (NRC, 1980). Soils near highways can be contaminated with Zn from tires (containing Zn oxide) and emissions from motor oil to which Zn dithiophosphite has been added (McDowell, 1992).

Zinc concentration in plants

Zinc values (dry weight) for forage range between 17 and 60 ppm (McDowell, 1992). Differences between plant species contribute little to reported variation in forage Zn, however the state of maturity is important (Minson, 1990). It has been reported that Zn concentrations may be reduced by 50 % with advancing maturity (Minson, 1990). Leguminous plants invariably carry higher Zn levels than grasses grown and sampled under the same condition (Underwood, 1981). Heavy dressing with lime and to a lesser extent with superphosphate can greatly reduce pasture Zn contents (Underwood, 1977). The zinc content of cereal grains and other seeds varies little among plant species but can vary greatly in accordance with soil Zn status (Underwood and Suttle, 1999). The zinc concentrations of wheat, oats, barley and millet lie between 30 – 40 ppm, with slighty lower values in maize grain and in all cereals grown on soil low in Zn (Underwood, 1977). Like manganese (Mn), Zn is highly concentrated in the outer layer of the grain (Underwood, 1977). Protein sources such as soybean, sesame seed, cottonseed and groundnuts meals are higher in Zn than cereal grains (50-70 ppm) (Underwood and Suttle, 1999). Animal protein sources such as meat and bone meal have Zn contents between 90 to 100 ppm (McDowell, 1992). Drinking water has a standard of 5 ppm, with industrial

pollution greatly increasing Zn in both water and plant sources (NRC, 1980).

2.3.2 Zinc metabolism in ruminants

Tissue distribution

Zinc is fairly evenly distributed throughout the animal tissues and normal total Zn content is approximately 2.2 g (McDowell, 1992). Higher concentrations of Zn are present in bones, liver, kidney, skin, wool, hair and wool, eye tissues and male sex organs (Underwood and Suttle, 1999). Zinc is an integral constituent of various enzymes and tissue distribution of Zn is associated with tissue distribution of enzyme systems to which it is related (Bondi, 1987). For example, when bone Zn is high, ALP of bone is high. The high concentration of Zn in the pancreas probably is related to its association with the hormone insulin, which is secreted by the pancreas (Bondi, 1987). Plasma Zn is bound loosely to albumin (2/3) and more firmly to globulin (1/3) while most of the Zn in RBC's is present as a component of carbonic anhydrase (McDowell, 1992).

Absorption

The main site for Zn absorption is the small intestine. However, all segments of the gut have the capacity to absorb Zn depending on the species of the animal (Swinkles *et al.*, 1994). In cattle high absorption occurs in the duodenum and ileum segments but small amounts are absorbed also in the large intestine (McDowell, 1992).

The process of Zn absorption can be physiologically divided into two separate events. First the uptake of Zn from the lumen into the cell and secondly Zn

transport from the cell into the circulatory system (Swinkels *et al.*, 1994). Uptake or cellular entry of Zn appears to occur by means of active transport and facilitated diffusion (Davies, 1980; Menard and Cousin, 1983; Blakeborough and Salter, 1987; Bronner, 1987; Oestreicher and Cousins, 1989). A small portion of Zn uptake and transport occur through simple diffusion (Cousin, 1985). The active uptake of Zn may involve binding of Zn by low molecular weight ligands, which are present within the intestinal lumen. The Zn ligand complex either enters the cell intact or donates Zn to a membrane bound receptor (Swinkels *et al.*, 1994).

A number of factors are known to interfere with Zn absorption. Metal binding protein, intestinal metallothionein, may limit Zn absorption at high intake (Hempe and Cousin, 1992; Cousin, 1985). Absorption of Zn is impaired by non starch polysaccharide in rats (Rubio *et al.*, 1994) and by elements such as Cu and Cd, which increase the mucosal binding of Zn by metallotheionein (Bremmer, 1993). The interaction between Cu and Zn may result from competition for binding with metallothionein. Metallothionein has a high affinity for both Cu and Zn (Cousin, 1985) but thermodynamically binding with Cu is preferred to binding with Zn (Williams, 1984). High selenium may inhibit Zn absorption from diets by forming insoluble complexes with Zn, this is particularly important in seleniferous regions (House and Welch, 1989).

Furthermore Zn absorption is affected by other dietary factors such as phytate or fibres in mono gastric animals (Davis and Reid 1979; Simmons *et al.*, 1990). A decrease in plasma Zn level was observed by Sturniolo *et al.* (1991) after selective

inhibition of gastric acid secretion in man. Their explanation was that a more alkaline environment in the stomach induces the formation of insoluble Zn compound that cannot be absorbed further down the gut.

Tissue storage

Following absorption, Zn in plasma is distributed between two fractions. Two thirds of plasma Zn is loosely bound to albumin and the remainder is tightly bound to alpha 2 macroglobulin. Zinc complexed with albumin is readily removed from the blood by the tissues, however, the mechanism of tissue uptake has not been characterised (McDowell, 1992). The highly saturated tissue i.e. muscle usually translocates Zn to unsaturated tissues like liver, pancreas and kidney (McDowell, 1992).

About 30 to 40% of the Zn entering the hepatic venous supply is extracted by the liver, from which it is subsequently released into the blood. Circulating Zn is incorporated at differing rates into various extrahepatic tissues, which have different rates of Zn turnover (Hambidge *et al.*, 1986). Zinc uptake by the central nervous system and bones is relatively slow and this remains firmly bound for long periods. Zinc in bone and hair is also unavailable to tissues. The most rapid accumulation and turnover of retained Zn occur in the pancreas, liver, kidney and spleen (McDowell, 1992).

Intracellular Zn is largely found in the cytosol (60-80%), with about 10 to 20% in the crude nuclear fraction and smaller amounts in the microsomal and mitochondrial

fractions (Underwood and Suttle, 1999). In the cytosol Zn is mostly bound to proteins in the cell membrane. Under normal dietary conditions only small amounts of Zn are bound to metallotheioneins (McDowell, 1992).

Readily available stores of Zn are quite small and this can be reflected by decreases in the plasma Zn values to the deficiency range within 24 hours after changing to a diet with very low Zn concentration (Underwood, 1977). Metallotheionein and superoxide dismutase have been suggested to be the major storage forms of Zn in the liver and is mobilised during metabolic need (Underwood and Suttle, 1999).

Excretion

Zinc is excreted largely in faeces. In animals on adequate Zn intake, faecal Zn includes both unabsorbed and endogenously secreted Zn (Stake *et al.*, 1975). The pancreas is the main route of excretion of endogenous Zn (McDowell, 1992). Except in abnormal conditions such as nephrosis or hypertension, urinary losses of Zn are very low (Stake *et al.*, 1975).

In animals that sweat freely, Zn loss by this route can be extensive in hot environment. Prasad *et al.* (1963) showed that humans may lose 5 mg Zn/day on diet adequate in Zn whereas Zn deficient individuals lose less than half the amount in the same environment, illustrating the homeostatic control of body Zn stores.

2.3.3 Physiological functions and manifestation of zinc deficiency

Zinc is critical for the metabolism of many nutrients including proteins, nucleic acids and carbohydrates (Spears, 1994). Zinc activates a number of enzymes and over 200 enzymes from various sources have been shown to be Zn metalloenzymes (Hambidge *et al.*, 1986). Severe Zn deficiency is rare, but it has been observed in grazing ruminants (McDowell, 1992). The extent to which marginal or subclinical Zn deficiency exists is unknown (Spears, 1991). Zinc functions and manifestation of deficiency are as follows:

Enzymes

Zinc, as a constituent of RNA and DNA polymerases, is involved in protein biosynthesis. Carbonic anhydrase is another Zn containing enzyme which plays an important role in maintaining the desired acid - base equilibrium of the body and in calcification of bone (Miller and Miller, 1962).

Since Zn containing enzymes are involved in primary processes of protein, nucleic, lipid, carbohydrate metabolisms and cell division, Zn deficiency results in subnormal growth, depressed food consumption, poor food conversion, impaired reproductive performance and abnormalities of the skin and its outgrowths (hair, wool and hoofs) (Bondi 1987; Spears, 1994; Hynd, 2000).

Hormones

Zinc has many biologically significant interactions with hormones (Kaneko, 1989).

It plays a role in the production, storage and secretion of individual hormones as well as in the effectiveness of receptor site and end organ responsiveness. Among the most notable effect of Zn deficiency on hormone production and secretion are those related to testosterone, insulin and adrenal corticosteroids (Kaneko, 1989).

Spermatogenesis and the development of the primary and secondary sex organs in the male and all phases of the reproductive process in the female can be adversely affected by Zn deficiency (Hidiroglou, 1979; Underwood 1981). However, the extent to which Zn deficiency affects reproductive outcome in domestic animals is not well characterised (Kaneko, 1989). It has been suggested that abnormal nucleic acid and protein synthesis, alterations in the differential rates of cellular growth necessary for normal morphogenesis, impairment of tubulin polymerization, chromosomal defects and excessive cell membrane lipid peroxidation may be involved in pathogenesis (Kaneko, 1989).

Impaired glucose metabolism with Zn deficiency has been reported in humans, rats and cattle (Kaneko, 1989). The impairment in glucose metabolism is reported to be secondary to reduced insulin release, increased insulin degradation via glutathione insulin transhydrogenase and an increase in peripheral insulin resistance. In addition to its putative effects on insulin metabolism, Zn deficiency is associated with reduction in growth hormone production and output (Kaneko, 1989). This defect may be secondary to the Zn deficiency induced by reduction in food intake rather than due to direct role in growth hormone synthesis and release (McDowell, 1992).

Growth Rate

Growth retardation is universally observed in Zn deficiency, perhaps because of impairment of nucleic acid biosynthesis (Droke *et al.*, 1993). Zinc deficiency also results in impaired amino acid utilisation or protein synthesis (Kaneko, 1989). Loss of appetite is one of the first signs of Zn deficiency, with poor growth being the only obvious sign (Droke *et al.*, 1993).

Relationship to Vitamin A

Zinc is also required in maintenance of normal concentration of vitamin A in plasma. Deficiency in Zn results in decreased synthesis of the retinol binding protein (RBP) (McDowell, 1992). The precise mechanism involved is not clear but it has been speculated that thymidine kinase and DNA dependent RNA polymerase depend on Zn for their activity (Underwood, 1981). Activity of alcohol dehydrogenase is depressed in the liver of Zn deficient lambs and could be related to the night blindness observed in some lambs (Arora *et al.*, 1973; Chhabra and Arora, 1993). Alcohol dehydrogenase is necessary for the interconversion of retinol to retinal, a process essential for normal vision (McDowell, 1992).

Immune response

Zinc is required for maintaining the integrity of the immune system through energy production, protein synthesis, stabilisation of membrane against bacterial endotoxins, antioxidant enzyme production and antibody production (Nackels, 1994). A low percentage of lymphocytes in the blood have been observed in animals consuming diets deficient in zinc (Droke *et al.*, 1993). Supplementing zinc

to dairy cows during lactation resulted in fewer infections of mammary glands (Spain *et al.*, 1993). Diversity of effects on immunocompetence as a result of Zn deficiency is related to thymic hormone production and activity, lymphocyte functions, neutrophil function and lymphokines production (Hambidge *et al.*, 1986; Fraker *et al.*, 1986; Keen and Gershwin, 1990). Deficiency causes rapid atrophy of the thymus with the predominant influence on various T cell functions (Chester and Arthur, 1988). Virtually, complete loss of lymphoid tissues including thymus, tonsils and lymph nodes has been reported in patients suffering from acrodermatitis enteropathica, a hereditary disease characterised by impaired utilisation of Zn (Chester and Arthur, 1988).

Protection of tissue from oxidative damage

Several investigators (Otelza, *et al.*, 1995; Har-el and Chevion, 1991) suggest that Zn deficiency can contribute to tissue oxidative damage. Zinc is a component of the well known antioxidant enzyme Superoxide dismutase (SOD), but may also be involved in a number of other oxidant defence systems (Miller *et al.*, 1996). Through its association with metallothionein, Zn may have a potential protection of sulfhydryl groups against oxidation and production of oxygen radicals by transition metals. Zinc has also the ability to compete with Fe (maybe Cu also) for membrane binding sites, thus reducing the potential for site specific damage of hydroxyl radicals (.OH) formation (Miller *et al.*, 1996).

Skin and wound healing

Zinc plays an important role in the prevention of diseases by stabilising epithelial

cells (Moynaham, 1981). The skin, particularly rich in Zn, in severe Zn deficiency, show parakeratosis, scaling and cracking of the paws with deep fissures as well as loss of hair and dermatitis (Miller, 1979). Damage of epidermal tissues and open lesion of the skin may render the animals more susceptible to infections (Bondi, 1987).

Wound healing is impaired in Zn deficient animals. Zinc is preferentially concentrated in healing tissue, skin and muscle wounds as well as bone fractures (Miller, *et al.*, 1965). This suggests a heightened metabolic demand for Zn in tissue synthesis during the healing process. The decrease in collagen synthesis observed in Zn deficiency explains the slower wound healing in the absence of sufficient Zn (Miller *et al.*, 1969).

Zinc deficiency can result in prolonged clotting time, which, in rats has been shown to be related to defective platelet function (Emery and O'Dell, 1990). Zinc deficiency in rats decreases thrombin stimulated platelet aggregation by lowering protein kinase C activity secondary to impaired calcium uptake (Emery and O'Dell, 1990).

Additional functions

Additional functions of Zn include prostaglandin and lipid metabolism; metabolites of prostaglandin are affected by Zn deficiency whereas glucose incorporation into fatty acids is greatly reduced (Underwood and Suttle, 1999). Zinc is also necessary for microbial growth in the rumen (McDowell, 1992).

2.3.4 Assessment of zinc balance in animals

Various attempts have been made to develop a sensitive, specific and reliable measure for Zn status but as yet there is no single, sensitive and specific method available (Golden, 1989). Laboratory diagnosis of severe Zn deficiency is relatively simple but marginal Zn deficiency is extremely difficult to confirm due to lack of suitable methods. At present, the best indication of Zn deficiency is the biochemical and clinical response made to Zn supplements (Fairweather-Tait *et al.*, 1988). Biochemical functional tests measure changes in the activities of certain enzymes or blood components dependent on Zn. Zinc is a constituent of over 200 metalloenzymes which vary in their responses to Zn deficiency depending on the tissue examined, their Zn affinity and rate of turnover of the enzyme (Gibson, 1994).

Plasma zinc

Plasma or serum Zn is most frequently used as an index, however it has several limitations (Gibson, 1994). The level of Zn in plasma or serum does not always reflect body status because of other conditions unrelated to Zn nutrition. It can only be used when serum or plasma sample are not haemolysed or contaminated and condition such as stress and infections are absent which reduces the plasma level (King, 1987). Erthyrocytes have high Zn content and in cases of Zn deficiency red blood cell fragility is increased (Bettger *et al.*, 1978). Parasitaemia confounds the interpretation of serum Zn concentrations, during infection, values are low because Zn is redistributed from the plasma to other tissues (Filteau and Tomkins, 1994).

Other important limiting factors which must be controlled when collecting blood samples for plasma Zn analysis include time interval between blood collection and separation of plasma, contamination of the blood sample from evacuated tubes with rubber stoppers and non acid washed glassware (Aggett, 1991; Wallock *et al.*, 1993).

Low plasma or serum Zn levels indicate deficiency or a redistribution of Zn, but normal levels do not necessarily preclude deficiency. For instance, in cases of chronic but mild Zn deficiency states, plasma concentrations are often normal (Gibson *et al.*, 1989; Ruz *et al.*, 1991), making diagnosis difficult. With a very low dietary Zn for ruminants there is an immediate sharp decline in plasma Zn (often within 24 to 36 hours), with reductions in feed intake and cessation of growth within less than 1 week (McDowell, 1992). Normal plasma Zn concentration range between 12.0 to 18.5 μmol /l in cattle (Underwood and Suttle, 1999), concentration between 6 to 9 μmol Zn /l are characterised with severe Zn lesions, whereas concentration between 9 - 12 μmol Zn /l are characterised with mild signs of Zn deficiency (McDowell., 1992).

Plasma or serum alkaline phosphatase

Activity of plasma or serum alkaline phosphatase has been widely used to assess Zn status (Gibson, 1994). It is reduced in severe Zn deficiency (Rothbaum *et al* 1982) but not in mild Zn deficiency (Gibson, *et al.*, 1989; Ruz *et al.*, 1991; Cavan *et al.*, 1993b). The specificity of alkaline phosphatase as an index for Zn status is also poor. Its activity is influenced by many factors other than Zn such as low diet, type

of protein, magnesium or manganese deficiency, season and increased bone turnover (Gibson *et al.*, 1989).

Measurement of alkaline phosphatase activity in neutrophils (Ruz *et al.*, 1991), lymphocytes (Schiliro *et al.*, 1987) and red blood cell membranes (Ruz *et al* 1992; Cavan *et al.*, 1993b) has also been investigated as indices of Zn status. To date there is no universally accepted Zn dependent enzyme, which can be used to assess mild Zn deficiency (Underwood and Suttle, 1999).

Leukocyte zinc

The Zinc contents in leukocytes or specific cellular types of leukocytes (e.g. neutrophil) have been used as an index for tissue Zn status (Jones *et al.*, 1981). They reflect soft tissue Zn and have a shorter half-life than erythrocytes and hence should detect changes in Zn status over a shorter time period (Jones *et al.*, 1981; Prasad and Cossack, 1982; Thompson, 1991; Ruz *et al.*, 1992). Relatively large volumes of blood are required and isolation of the leukocytes and specific cellular types as well as subsequent analysis is lengthy and technically difficult limiting their use in many countries (Gibson, 1994).

Radioimmunoassay

Levels of the Zn binding protein metallothionein have been investigated in serum, urine and erythrocytes as indices of Zn status in rats (Golden, 1989). Metallothionien is an important metal binding protein that occurs in varying amounts in wide range of tissues (Bremner, 1993). Levels may fall in Zn deficiency

as a result of impaired synthesis. The specificity of the method is poor and Fe deficiency, diurnal rhythm and acute infection may affect Zn concentration. Metallothionein is said to be much less responsive to stress and infection in erythrocytes than in plasma (Grider *et al.*, 1990) and hence may provide a useful index of Zn status once suitable radioimmunoassays are available for other animals.

Serum thymulin and plasma somatomedin-C

Serum thymulin has also been assessed as a potential index for Zn status. Thymulin is a Zn metallo peptide that controls cell mediated immune function (Prasad *et al.*, 1988), its activity falls in mild Zn deficiency. Plasma somatomedin C, a peptide of low molecular weight, which is regulated by growth hormone, nutrition and insulin, is increased in response to increases in Zn concentration in plasma and tibia of rats. Nevertheless, more work is required to establish the sensitivity, specificity and validity of these methods (Gibson, 1994).

Other methods

Hair Zn concentration depend not only on the delivery of Zn to the root of hair but also on the rate of hair growth. Zinc deficiency itself may impair the growth of hair and in such condition hair Zn concentration may be normal or elevated (Bradfield and Hambidge, 1980; Mederois *et al.*, 1987). Platelet aggregation has been shown to be impaired when plasma Zn levels are low following Zn deprivation but is restored to normal within 19 hours of supplementation (Gordon *et al.*, 1982). Hence, platelet and Zn hair are not good indicators for Zn status in the animal (Fairweather-Tait, 1988).

2.3.5 Zinc requirements and method of supplementation

Zinc requirements

Zinc requirements for farm animals vary with species, breed, age and productive functions of the animal. Furthermore, composition of the diet, particularly the amounts and proportions of factors that affect Zn absorption and utilisation may be important (Underwood, 1981). Lack of knowledge on the physiological availability of Zn to ruminants from feeds and forages grown in varying conditions, makes estimation of Zn requirements difficult (McDowell, 1992). Not only are Zn requirements of ruminants fed forage diets poorly defined, but also little is known regarding factors that affect Zn availability in forages (Wiseman and Cole 1990).

Estimates of requirements based on semi purified diets under controlled experimental conditions tend to be lower than is suggested from field studies (Underwood 1977). Zinc requirements for optimum growth and fertility of beef cattle is 30 ppm of the dry diet. For dairy animals the requirement is 40 ppm Zn (McDowell 1992).

Source of zinc

Zinc must be present in the diets of all animals and must be supplied almost continuously, because animals have only small amounts of readily available stored body Zn (McDowell, 1992). Dietary Zn source is important in determining whether an inorganic supplement is required (Underwood, 1981). Animal protein sources such as meat meal or fishmeal are generally richer in Zn and of higher biological

availability than plant protein supplements. It is suggested that all plant diets should be supplemented with an inorganic Zn salt (McDowell, 1992). Zinc intake may be increased when animals have access to forage or pasture that provides forage potentially rich in Zn and also allows Zn to be obtained through consumption of soil or plants with soil contamination (Underwood and Suttle, 1999).

Zinc as sulphate, carbonate, oxide and in several natural ores has been shown to be available as supplements when provided in suitable physical form for mixing (Ammerman *et al.*, 1995). The two predominant sources used by the animal feed industry are zinc oxide (72% Zn) and sulphate (36% Zn) (McDowell, 1996). Studies with dairy cows have shown that an organic Zn compound, Zn methionine, is a promising nutritional feed additive (Kellogg, 1990). Dairy cows receiving Zn methionine produced more milk with lower somatic cell counts and had higher hoof quality than cows receiving Zn oxide and methionine separately (Kellogg, 1990).

Method of supplementation

Zinc supplementation is easily and cheaply provided by fortifying complete diets as mineral concentrates, mineralised salt, salt blocks, molasses blocks and sometimes added in water (McDowell, 1992). Premixes of Zn and other trace minerals may be added to feeds at the farm (McDowell, 1996). Zinc provided as intraruminal pellets, oral drenching and as injections have been effective in preventing or curing Zn deficiency. Although very effective, oral drenching is costly in time and labour unless animals are routinely caught and restrained for other purposes (McDowell, 1992).

Fertilizers can be used as source of Zn for grazing animals. Zinc fertilisers are successful in increasing forage and grain Zn concentrations (McDowell, 1992). Australian research on Zn deficient soils indicated that 5-7 kg of Zn sulphate per ha every 2 to 3 years maintained increased Zn concentrations in forage (Underwood, 1981).

2.3.6 Zinc toxicity and interactions with other minerals

Cattle can exhibit considerable tolerance to high intake of Zn; the extent of the tolerance depend partly on the nature of the diet especially its relative contents of Ca, Cu, Fe and Cd with which Zn interacts in the process of absorption and utilisation (Underwood, 1981). It has been reported that dairy cows which received 2000 ppm Zn during lactation period decreased milk yield and feed intake (McDowell 1992).

High dietary Zn (> 1000 ppm) can accentuate borderline deficiencies of other elements including Fe and Cu (McDowell, 1992). In sheep a high Zn intake (750 ppm) was superimposed on a marginal Cu intake in two groups of ewes; only 2 out of 10 lambs in one group and 1 out of 10 of the other survived beyond 24 hours after birth. No adverse effect was noted in lambs receiving 150 ppm Zn (ARC, 1980).

2.4 Calcium, phosphorus and zinc interactions

Element interactions can be additive, synergic or antagonistic and such information is crucial in the assessment of dietary requirements and element toxicity (Wiseman and Coles, 1990). A high level of Ca, P or Zn reduces the efficiency of absorption or utilisation of the other (McDowell, 1992). The addition of excess Ca to an otherwise adequate diet may result in a deficiency of P nd Zn (NRC, 1980). Excess Ca or P has been reported to influence Zn requirements in monogastric diets, however in normally fed ruminants, there is no direct evidence that Ca or P decreases Zn absorption (NRC, 1989). Elevated amounts of dietary Ca as ground limestone, reduced absorption of Zn in rats fed soy protein but did not had effect in lactating cows (McDowell, 1992).

Ruminants can tolerate a wide range of Ca:P particularly when their vitamin D status is high (Underwood, 1981). Franklin *et al.* (1948) as quoted by Underwood (1981) reported accelerated onset of signs of Ca deficiency in sheep when they were fed on very low Ca diets having a Ca:P ratio of 0.3:1. In another trial with sheep the high Ca:P ratio of 10:1 did not have an effect on P absorption with a moderately high P diet (0.26%) but severe bone disorders were observed when the diet contained only 0.08% P (Underwood, 1977). In another critical experiment with calves given three levels of dietary Ca (0.27, 0.81 and 2.43%) and three level of P (0.27, 0.34 and 0.68%), nine Ca:P ratio ranging from 0.4:1 to 14.3:1 were tested. Dietary ratios between 1:1 and 7:1 all gave satisfactory and similar results but with Ca:P ratios below 1:1 and over 7:1 growth and feed efficiency decreased

significantly (Underwood, 1981). A dietary Ca:P ratio between 1:1 and 2:1 is considered ideal since this is approximately the ratio of the two minerals in bones and blood (Underwood, 1981; NAS, 1988).

2.5 MINERAL NUTRITION IN RELATION TO LAMENESS

2.5.1 Background

Lameness is a departure from the normal stance or gait resulting from some structural or functional disorder of one or more limbs or the trunk. Lameness used to be considered an important clinical manifestation of a musculoskeletal disorder in modern dairy and beef production where intensive management is practised, however to-date lameness is a common problem in dairy cattle under semi intensive system in urban and peri urban areas (Mgasa, *et al.*, 1994). Studies in Europe have identified lameness as the third most costly health problem in dairy cows, after mastitis and disorders of reproduction (Whitaker *et al.*, 1983; Collick *et al.*, 1989). Welfare implication of lameness include reduced mobility and effects on physiology, behaviour and increased susceptibility to metabolic disorders as well as pain and discomfort, (Hassall *et al.*, 1993). All of these are detrimental to productivity and the economics of the livestock industry.

Approximately 90% of lameness in cattle is located in the foot and mainly localised in the claws and can have a traumatic, systemic or infectious origin (Politiek *et al.*, 1986). The claw quality is a product of nutrition, claw shape, characteristics of the

horn and anatomy of the inner structure of the claw (Greenough, *et al.*, 1981). Improvement of claw quality in short term can be achieved by management procedures, which would reduce claw problems in confinement. However genetic improvement can give a contribution in the long term (Politiek *et al.*, 1986)

Feeding and management are considered to be the most important factors associated with digital diseases throughout the world where high energy concentrate, low fibre diets are implicated to be the major predisposing factors (Greenough and Vermunt, 1991). Feeding of high energy rations and limited roughage from an early age causes animal to become heavy early and upset the balance of nutrients essential for proper skeletal development (Fraser *et al* 1986). This increases the stress on young bones and contributes to lameness. Lameness has also been associated with highly digestible protein intake, deficiency in Cu, vitamin D and Ca in cattle fed highly concentrate ration and chronic Se toxicities (McDowell, 1992).

Mineral toxicity or deficiencies are of minor importance in Europe as the cause of lameness (Politiek *et al.*, 1986). However, these may be important in tropical conditions (McDowell 1992). Sub acute and chronic selenosis has been reported to be the cause of lameness (Underwood 1981). These conditions are most frequently observed in grazing livestock that have consumed Se-accumulator plants or exposed to large doses of Se over a longer period (weeks or months). The clinical observations include abnormal hoof development, which may be more pronounced as the age of animal's increases (Underwood 1981).

2.5.2 Calcium, phosphorus and zinc nutrition in relation to lameness in cattle

The horny capsule, which forms the outer part of the claw, is largely responsible for protecting the foot from internal and external factors (Greenough, *et al.*, 1981). The ability of the claw horn to withstand the environment depends mainly on its physical properties in particular hardness, toughness and viscoelasticity. These in turn are determined by the structure and chemical composition of the keratin forming the horn (Kempson and Logue, 1993). Dietary P and Ca level has been shown to affect bone development and associated chemical and physical properties in different species (Williams *et al* 1991a). Baggort *et al.* (1988) found that high Ca, P and Zn were related to hardness of the claw keratin, whereas low levels of Cu, Zn and Mg were associated with lameness. Shupe *et al.* (1988) reported that cows fed low amount of dietary P (less than 7 g/ day) for 14 to 24 months showed signs of osteoporosis as well as traumatic fractures. Williams *et al.* (1991a) observed that marginal P levels (0.12% of DM) caused severe lameness in cattle, thus affecting the useful productive life of the animal.

2.6 SUPPORTING PARAMETERS IN ASSESSING CALCIUM, PHOSPHORUS AND ZINC BALANCE IN CATTLE

2.6.1 Soil, plant and feed analysis

Analyses to determine the available forms of soil minerals can provide clues to livestock mineral deficiency but more often they are unreliable and difficult to

interpret. Mineral correlations among soil, plant and animal tissue concentrations are highly variable among locations, (Suttle, 1986). Chemical analyses of foodstuffs are of very limited values as they give no information concerning the chemical nature or digestibility of the element in question, (Masters, 1984). Feeds and forage mineral analyses are preferable to soil analyses, while appropriate animal tissue and fluid analyses most accurately portray the total contribution of the total dietary environment (forage, soil, water) in meeting livestock mineral requirements (Paynter, 1987). Theoretically plant analysis is more correct than soil analysis as it measures just such fraction of a micronutrient, which has been available to a plant, thus absorbed by the plant.

Mineral elements are determined in soil, plants and feed by a wide range of chemical and physical procedures. The more recent developed procedures include atomic absorption spectroscopy (AAS), inductively coupled emission spectroscopy (ICP) and neutron activation (NAA) (Wiseman and Cole, 1990). The atomic absorption spectrophotometer has provided the most notable advances in mineral analyses. The sample is subject to a high energy thermal environment in order to produce excited state atoms. The ground state atom absorbs light energy of a specific wavelength as it enters the excited state. As the number of atoms in the light path increases, the number of atoms in the light path increases. By measuring the amount of light absorbed a quantitative determination of the amount of analyte can be made. The use of special light sources and careful selection of wavelength allow specific determination of individual elements (McDowell, 1992). The method can be used for more than 60 elements including Ca, P and Zn. The method offers

the advantage of excellent sensitivity, speed and convenience and is well suited for routine measurement of minerals in various matters (Wiseman and Cole, 1990).

Inductively coupled emission spectroscopy is a technique established 20 years ago. The technique use inductive coupled plasma generators as atomizer source for optical emission spectrometry. It offers several advantages over both flame emission and atomic absorption procedures. Multi element assays can readily be carried out. The determination of major minerals Ca, Mg, P, K, Na and trace elements Cu, Zn, Fe, Mn, Co, Mo in plants or animal tissues present no problems. Neutron activation is widely used in mineral analyses in human subject; its use in animal science is limited (Wiseman and Cole, 1990).

2.6.2 Clinical examination of animals

Assessment of mineral status solely based on clinical signs is fraught with error. Few of these clinical signs are specific for an individual element; e.g. unthriftness may be due to bacterial or viral infections, Cu imbalance, parasitic infestation or energy and protein imbalances (Underwood, 1977). Bone fragility has been associated with factors other than Ca or P deficiency including Cu deficiency, (Radostitis *et al.*, 1994). In many instances an investigation of the pathology associated with the clinical signs may be of considerable benefit in obtaining a specific diagnosis (Paynter, 1987). However, the assessment of Ca, P and Zn status in animals showing clinical signs, which have been associated with Ca, P or Zn deficiency, must be regarded as essential part of the deferential diagnosis of these

diseases. Treatment should not be recommended on the basis of clinical signs alone (Paynter, 1987).

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY AREA AND CLIMATE

3.1.1 Area

The work was carried out in Iringa Region on a private farm called ASAS, situated in a village called Nduli. The farm is located 10 - 12 km from Iringa Town along the highway to Dodoma. Iringa town is located at 7^0 48′ S latitude and 35^0 43′ E longitude. Asas Dairy Farm is a relative medium sized semi commercial farm. The farm had 368 heads of cattle including 100 lactating cows at the commencement of the study. The primary complaint from the farmer were reduced growth, delayed conception rates, long calving intervals, abortions, poor milk production and lameness which was suspected to be connected to mineral imbalances; hence a need for investigation so as to improve production and general health status of the animals. The present study was based on a master's degree research work on mineral nutrition at the farm (Phiri, 1995). In the study chronic selenosis, low P, Ca, Mg, Cu and Zn in the pasture were suspected to be contributing to poor animal performance in terms of health, reproduction and milk production.

3.1.2 Climate

The study was conducted from April 1997 to April 1999. The climate during the study period was characterised by two dry seasons (mid May 1997 to mid November 1997; mid May 1998 to mid December 1998) and two rainy seasons (Mid November 1997 to April 1998; Mid December 1998 to April 1999). The average monthly rainfall, temperature and relative humidity are presented in (Fig. 3, 4 and 5).

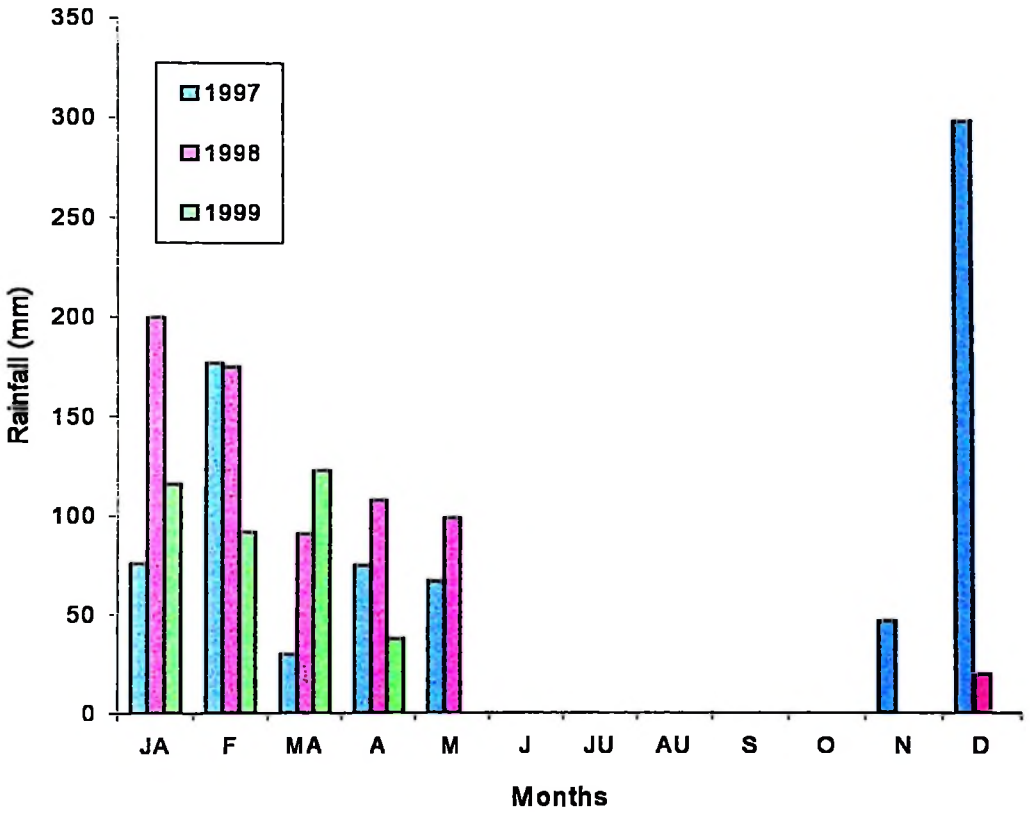


Figure 3 Rainfall (January 1997 to March 1999) for Nduli village, Iringa, Tanzania. (Source: Iringa Meteorology 1999).

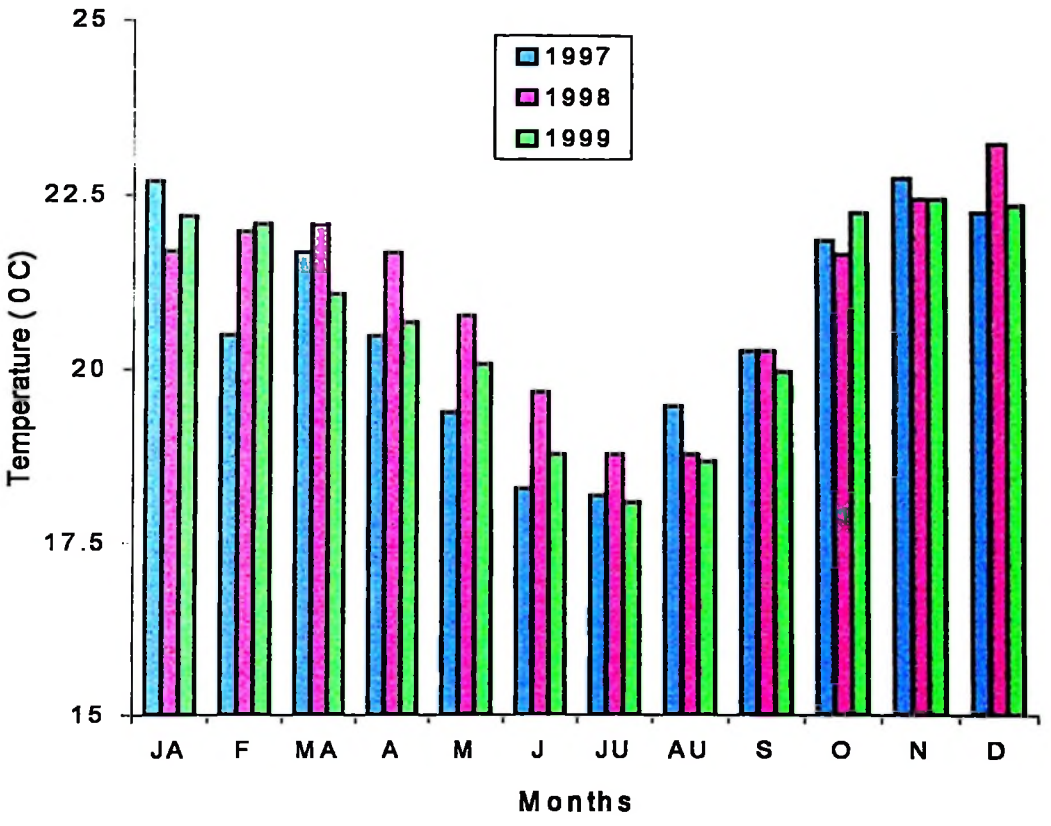


Figure 4 Temperature (^{0}C) (January 1997 to November 1999) for Nduli village, Iringa, Tanzania. (Source: Iringa Meteorology 1999).

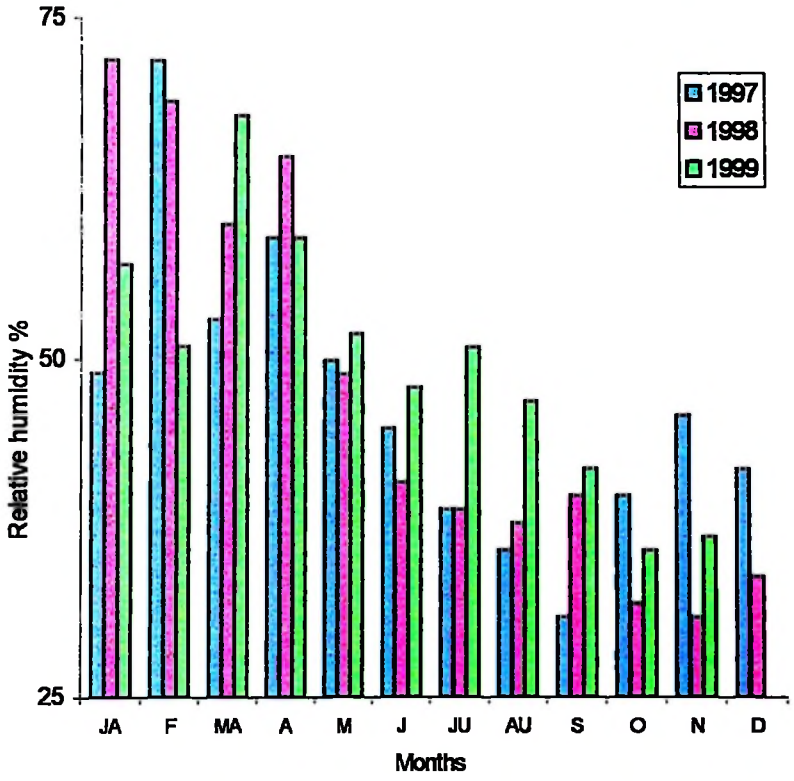


Figure 5 Relative humidity (%RH) (January 1997 to November 1999) for Nduli village, Iringa, Tanzania. (Source: Iringa Meteorology 1999).

3.2 EXPERIMENTAL LAYOUT

The experiment was divided into two major sections. Section one involved preliminary evaluation of soil, pasture, feed and animal tissue mineral concentration with emphasis on Ca, P and Zn. Section two involved Ca, P and Zn feed supplementation in forty-eight lactating cows.

3.2.1 Preliminary evaluation of soil, pasture, feed and animal tissue calcium, phosphorus and zinc status

Soil calcium, phosphorus and zinc status

Ten soil samples were analysed for calcium, phosphorus and zinc contents. Samples were collected in April 1997, September 1997, January 1998, May 1998, September 1998 and March 1999. The analysis was performed at the Department of Soil Science, Sokoine University of Agriculture, Tanzania based on standard methods using the atomic absorption spectrophotometer (See section 3.5).

Calcium, phosphorus and zinc contents in pasture and feed

Ten pasture samples were analysed for calcium, phosphorus and zinc contents. Samples were collected in April 1997, September 1997, January 1998, May 1998, September 1998 and March 1999. Analyses of Ca, P and Zn in pasture grass and feed was performed at the Departments of Animal Science and Production and Department of Soil Science at Sokoine University of Agriculture, Tanzania as well as at the Department of Animal Health and Welfare, Danish Institute of Agricultural

Sciences, Research Centre, Foulum, Denmark based on standard methods (See section 3.4)

Animal tissue calcium, phosphorus and zinc concentration

Fifty-two lactating cows, sixteen pregnant cows, nine pregnant heifers and eighteen steers were used in the preliminary evaluation of Ca, P and Zn status. The breed of the animals consisted of first, second and third crosses between Zebu, Friesian and Ayrshire. The parity of pregnant cows and lactating cows ranged from one to three, whereas age of heifers ranged from 28 to 36 months and that of steers 12 months to 24 months. Lactating cows calved between December 1996 and April 1997. These animals were grazed and supplemented with 1 to 2 kg concentrates per animal per day during milking for the lactating cows. Pregnant cows and heifers were given 2 kg of concentrate twice per week in their night sheds. The concentrate mixture was composed of maize bran and sunflower seed cake in a ratio of 4:1. Mineral contents of the concentrate are presented in Table 1. Blood sampling for this study was carried out in April 1997.

3.2.2 Effect of mineral supplementation in grazing crossbred dairy cows.

Experimental animals and treatments

Forty-eight lactating cows comprising crossbred Zebu, Ayrshire and Friesian (in their 1st, 2nd and 3rd parity) were used in the mineral supplementation trial. The cows were divided into eight groups of six cows each and were daily drenched with mineral supplement as indicated in Table 2. Allocation of cows in groups was based on

Table 1. Mineral composition of maize bran, sunflower seedcake and concentrates (based on dry matter) given to the animals during the preliminary survey. (M/S = Maizebran / sunflower seedcake).

Parameter	Maize bran (M)	Sunflower seedcake (S)	Concentrates M/S ratio (4:1)
Calcium (%)	0.13	0.38	0.12
Phosphorus (%)	0.31	1.40	0.59
Magnesium (%)	0.21	0.67	0.28
Sodium (%)	0.13	0.21	0.17
Potassium (%)	0.27	1.20	0.38
Iron (ppm)	288	593	352
Copper (ppm)	10.1	17.0	12
Zinc (ppm)	35.0	140	48
Manganese(ppm)	26.0	44.0	30

Table 2. Source and amount of calcium, phosphorus and zinc supplemented to experimental cows

GROUPS	MINERAL SUPPLEMENT		
	Ca (g)	P (g)	Zn (mg)
Group 1 (Control)	-	-	-
Group 2 (Ca)	10 ($CaCO_3$)	-	-
Group 3 (P)	-	8 ($Na_2HPO_4.2H_2O$)	-
Group 4 (Zn)	-	-	400 (ZnO)
Group 5 (Ca/P)	10 ($Ca_2HPO_4.2H_2O$)	8 ($Ca_2HPO_4.2H_2O$)	-
Group 6 (Ca/Zn)	10 ($CaCO_3$)	-	400 (ZnO)
Group 7 (P/Zn)	-	8 ($Na_2HPO_4.2H_2O$)	400 (ZnO)
Group 8 (Ca/P/Zn)	10 ($Ca_2HPO_4.2H_2O$)	8 ($Ca_2HPO_4.2H_2O$)	400 (ZnO)

KEY: $CaCO_3$ = calcium carbonate; $Na_2HPO_4.2H_2O$ = sodium monophosphate; $Ca_2HPO_4.2H_2O$ = dicalcium phosphate; ZnO = zinc oxide.

breed, lactation number, time of calving and milk yield. With these criteria each group consisted of one first, three second and two third lactating cows. Furthermore, each group consisted of one first cross cow between Zebu and Ayrshire (ZA), two crossbred cows between Zebu and Friesian (ZF), one second crossbred of Zebu and Friesian (ZFF) and two second crossbred of Zebu and Ayrshire (ZAA). Four animals in each group were synchronised so that they would calve between February and March 1998/99 whereas two cows in each group were allowed to calve between October and November. All mating were performed using bulls available at the farm. Cows were thoroughly examined clinically before commencement of the experiment and were under concentrate feeding which comprised maize bran and cotton seed cake at a ratio of 3:1 for five weeks. Mineral contents of the mixture are given in Table 3. Only cows without signs of lameness were used for follow up during the experimental period.

Mineral supplementation *per se* started at the end of May 1997 and ended late March 1999. The minerals were mixed with water and administered using a long necked bottle daily (Coca-Cola bottle). The basis for this amount of supplementation was first to replace the amount of mineral being lost in milk. Cows at this farm in their third and fourth lactation produced an average of 10 kg of milk per day where as cows in their first and second lactation produced an average of 6 – 7 kg of milk. There is approximately 1 g of Ca and 0.8 g of P in every 1 kg of milk (NAS, 1988). Second, manufacturers of mineral formulation available in markets of Tanzania recommend Ca concentration of 6 to12 g, P concentration of 5 to 12 g and 100 – 500 mg Zn per animal per day, respectively (Table 4).

Table 3. Mineral composition of concentrate mixture, alfalfa, pasture grass, green fodder and stored hay (based on dry matter) given to the experimental cows.

Parameter	Concentrate M/S ratio (3:1)	Concentrate M/S ratio (4:1)	Alfalfa (green)	Green fodder (mixture)	Pasture grass	Stored hay
Calcium (%)	0.20	0.15	1.40	0.30	0.33	0.30
Phosphorus (%)	0.71	0.43	0.30	0.21	0.27	0.36
Magnesium (%)	0.35	0.24	0.26	0.19	0.16	0.15
Sodium (%)	0.21	0.18	0.27	0.22	0.21	0.21
Potassium (%)	0.27	0.31	2.30	1.90	2.41	2.40
Iron (ppm)	364	229	98.0	69.0	154	84
Copper (ppm)	12.6	8.0	13.3	4.2	4.57	4.0
Zinc (ppm)	68.0	56.0	28.0	18.0	23.0	23.0
Manganese (ppm)	29.0	25.0	56.0	215	182	196

Table 4 Source, amount of calcium and phosphorus reccommended for dairy cows in different mineral mixture available in the Tanzanian market (1997 – 1999).

	Mineral mixture	Recommended amount per animal per day (g)	Content of Ca, P and Zn in the reccommended amount (g)		
			Ca (g)	P (g)	Zn (mg)
1.	Macklick super	40 – 80	7 - 14	4.4 – 8.8	100
2.	Unga high phosphorus	100	12	12	150
3.	Afya bora stock lick	75 - 100	2.5 – 3	?	150
4.	Afya bora super lick	100 - 250	12 - 30	12- 30	500
5.	Manca Ca	30	8.4	-	-
6.	Cattle mix	50	8.7	8.4	250

? = concentration not indicated; - not included.

General management at the farm

Animals at the farm were grazed throughout the day from 8.00 a.m – 5.00 p.m. and at night the animals were kept in kraals. The floor of these Kraals was made of concrete. Water was provided *ad libitum* in the paddocks and night kraals. Two kilograms of concentrate were given to the each milking cow during milking time. Grass hay and concentrate were supplemented to all animals during the dry period. Feeding of lactating cows was slightly different in that cows were grazed on pasture from 8.00 a.m. to 3.00 p.m. with a rest of one hour in kraals before evening milking. It was during this time when each cows was given 1 to 2 kg of mixed cultivated green fodder. The composition of these pastures depended on the availability but commonly was a mixture of *Pennicum maximum*, *Pennisetum purpureum*, and legumes such as *Medicago sativa* (alfalfa), *Desmodium intortum* and *Sesebania sesibani*. Other dominating pasture grass species for grazing included *Chloris spp*, *Hyperrhenia spp* and *Digitalis spp*. Cows were usually milked twice a day at 3.30 to 6.00 a.m. in the morning and at 4.00 p.m. to 6.00 p.m. in the afternoon. All cows were milked by hand, and each milker had to milk 8 to 10 cows at each milking time.

Feeding of experimental cows

Besides grazing, all experimental animals were supplemented with concentrates at a rate of 3 kg per animal per day during the rainy season i.e. 1998 and 1999. During the dry season the amount of concentrate was increased to 4 kg per animal per day. In addition, all animals received 3- 4 kg of hay; 2 kg of cut green fodder each per day during the dry season. During the second year of study 1998/1999 i.e. starting from March 1998 each animal received an additional 1 kg of green alfalfa mixed with

concentrate during the rainy season and 2 kg during the dry season. The concentrate mixture contained maize bran and sunflower seed cake at a ratio of 3:1 (May 1997 to July 1998) and 4:1 (August 1998 – March 1999). The mixture was provided to each cow throughout the study period during milking and also to dry cows. The mineral contents of green fodder, hay and concentrate are presented in Table 3.

Disease control measures and health monitoring

Animals were dewormed at three months intervals throughout the study period using Wormicid Plus ®, manufactured in Kenya by Cosmos limited. Wormicid Plus is a wide spectrum antihelmintic, which contains 1.5% w/v levamisole HCl B.P.Vet and 8.0% w/v Bithionol sulfoxide. Wormicid Plus is used in the control and treatment of liver flukes lungworms, stomach and intestinal strongyles, ascarides and other round worms, rumen flukes (paramphistomes) and tape worms.

Dipping was carried out weekly using Supa dip for the control of tick borne diseases and other external parasites such as fleas. The ratio of initial mixing with water was 1:2200 and for replenishment 1:1000 in the diptank after every 100 animals had been dipped. Supa dip is an acaricide containing chlorfenvinphos 110% w/v and was manufactured by Pitman Moore Limited and distributed by Henschel liaison Office, Dar es salaam, Tanzania.

Diseases condition such as the tick borne diseases (east coast fever, anaplasmosis, babesiosis and heartwater) and pneumonia were closely monitored and when suspected blood smears were taken to check for haemoparasite diagnosis. Screening

for brucellosis was also carried out, using standard laboratory techniques (Rose bengal plate test (RBPT) and serum agglutination test (SAT) as described by Brinely *et al* (1978). In addition fecal egg count and culture were determined in all experimental cows using modified McMaster and Baermann techniques as described by the Ministry of Agriculture, Fisheries and Food UK (1986).

Health status monitoring

Health status of the experimental animals was closely monitored. Body weight and body condition score were also carried out to supplement the health status. Mammary gland, hoof and reproductive organs health were also examined for any lesion(s) or anatomical abnormalities.

Body weight and condition score (BCS)

Body weight (kg) was estimated using a heart girth tape measure (We-Bo, Denmark) distributed by Kruuse (Denmark). The heart girth circumference of the chest was measured just behind the forelimbs. The estimation was carried out on the same day when blood samples were collected.

Body condition was scored as described by Wilderman *et al.* (1982) using a scale from 1 (very poor) to 5 (grossly fat) with half point intervals, determined by assessment of the degree of fatness at the tail head and loin. The same person performed the estimate at two months interval on the same day when blood samples were being collected. Body condition scoring features are summarised in Table 5 and Fig. 6 to 10.

Table 5 Body condition score points and their description in grazing dairy cows at ASAS Dairy Farm, Iringa, Tanzania.

SCORE	DESCRIPTION	FIGURE
1.0	Emaciated animal Prominent projecting hip bones, tail head and spinous processes Wasted gluteal muscles with concave appearance Severely depressed area below tailhead and pin bones	6
2.0	Emaciated animal Hip bones, tail head and spinous processes are less obvious Wasted gluteal muscles with concave appearance Depressed area below the tailhead and pin bones Bone structure have flesh cover	7a and b
3.0	Hip bones, tail head and spinous processes felt by applying slightly pressure Gluteal muscles with straight appearance Tissue cover below the tailhead and pin bones Bone structure have flesh cover without sign of fat deposition	8a and b and 9a and b
4.0	Hip bones, tail head and spinous processes felt by applying more pressure Gluteal muscles with straight appearance Fat cover below the tailhead and pin bones Bone structure have flesh cover with sign of fat deposition	10a and b
5.0	Hip bones covered with tissue no longer felt hard Tai head appears buried in fat tissue Spinous processes are not felt Bulged and convexed gluteal muscles Prominent evidence of subcutaneous fat deposition Bone structure have flesh cover with sign of fat deposition	None at the farm

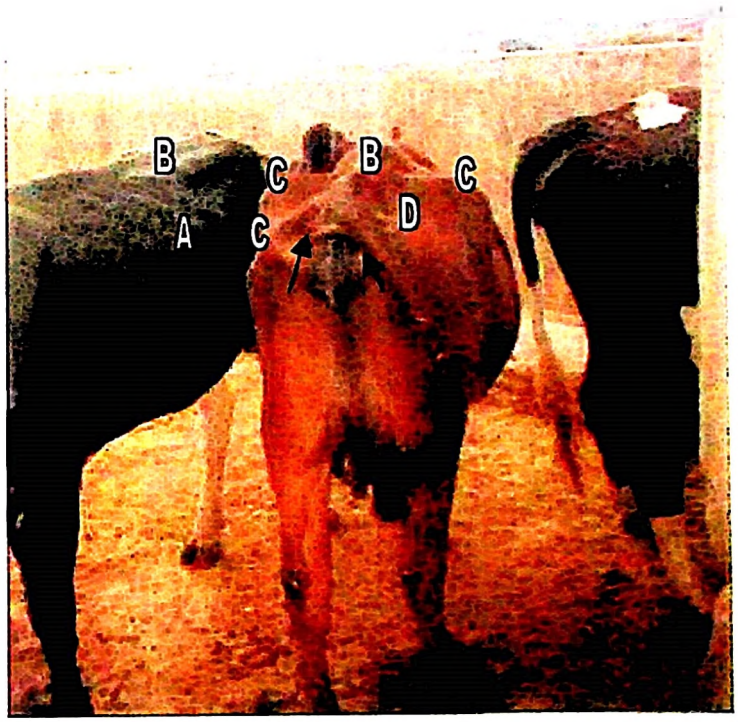


Figure 6 Back view of cows scored condition 1.0. A = Ribs can be seen; B = prominent spinous processes, C = prominent hipbones with little flesh cover; D = wasted gluteal muscles; arrow = severe depression below the tail head.

(a)

(b)

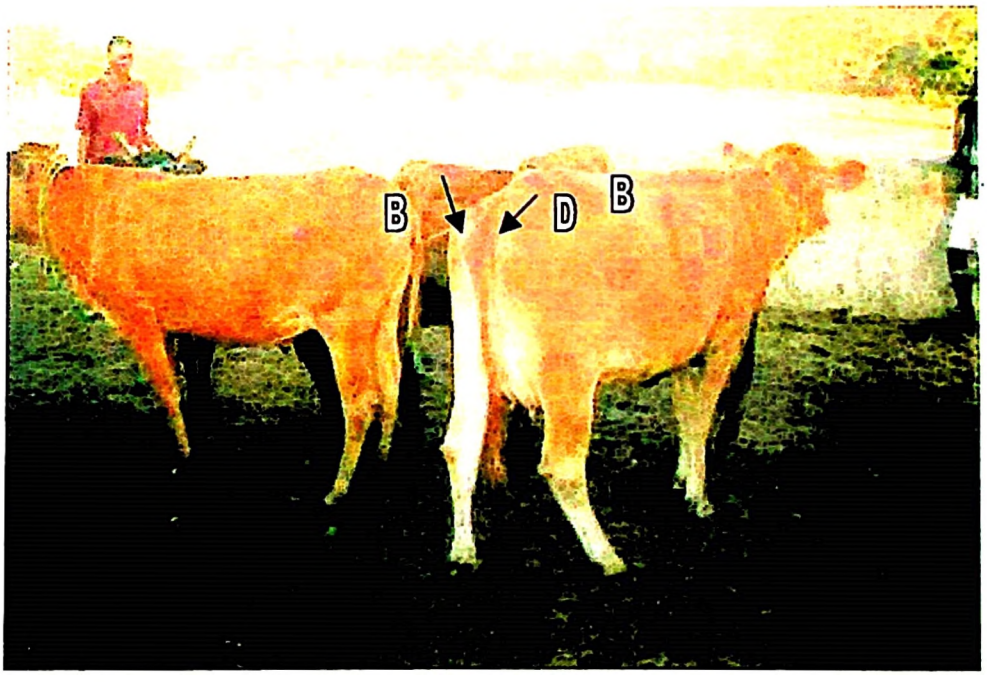


Figure 7 (a) and (b) Side view (7a) and back view (7b) of cows with body score 2.0. A = Ribs can be seen; B = hipbones are obvious but they have flesh cover; C = spinous processes can be seen; D = gluteal muscles have a concave appearance; arrow = depression below the tail head.

(a)

(b)

Figure 8 (a) and (b) Side view of cows scored condition 3.0. Note: A = Ribs can be seen; B = gluteal muscle has a straight appearance; C = spinous process are not obvious but can be palpated.

(a)

(b)

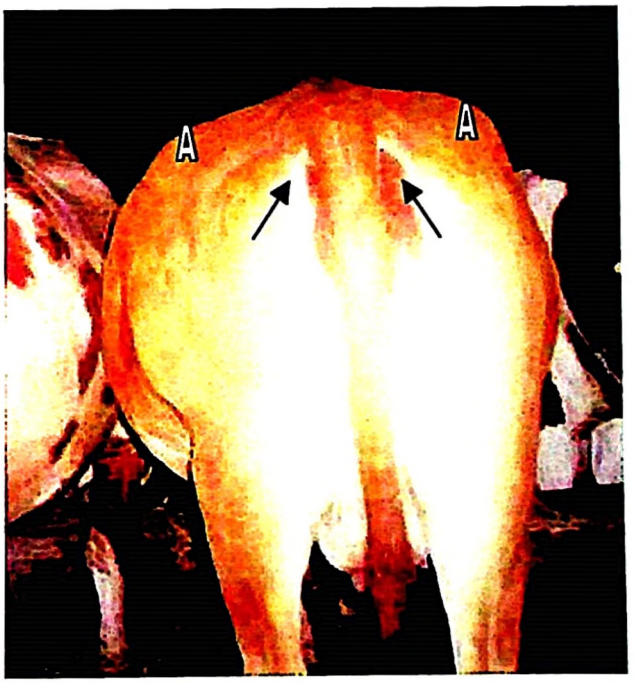
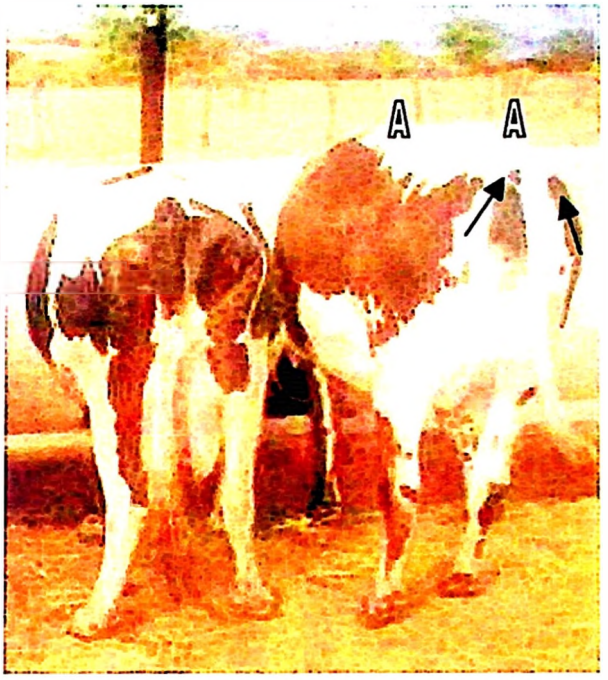


Figure 9 (a) and (b) Back view of cows with body score 3.5. A = straight gluteal muscles, with hipbones covered by tissues. Arrows indicates slightly fat cover below the tail head.

(a)



(b)

Figure 10 (a) and (b) Back view of cows with body score 3.5 (left 10 a) and 4.0 (right 10 (a) and 10 (b)). A = Hip bones are less prominent and round, ribs and spinous processes can be felt by applying slightly pressure; arrows = fat cover below the tail head can be palpated easily.

Mammary gland health

In order to evaluate the health of the mammary gland any lesion(s) or anatomical abnormalities were recorded. Digital palpation was used to assess the texture of the mammary gland with respect to indurations and fibrosis. Before milking a strip of milk was examined using the on farm California Mastitis Test kit (CMT Kruuse, Denmark) to estimate somatic cell count before the experiment started and thereafter during each blood sample collection. The pH of milk was also measured using indicator papers (BOVIVET indicator, Kruuse, Denmark) once per week throughout the study period to monitor mastitis incidence.

Hoof health and assessment for lameness

All limbs were examined visually before and during the experimental period as described by Greennough *et al.*, (1981) i.e. visual examination as well as palpation to detect limb lesions. Limb lesions were recorded by site, type of lesion (i.e. normal overgrown hoof, abnormal overgrown hoof (corkscrew claw or deviation of the claw from normal in two planes, beak claw or scissors foot), superficial (skin or subcutaneous) swelling and deep (muscle or bone) swelling. Detailed procedure of examination is provided in Appendix 1.

Radiological pictures of the limb bones were taken at the beginning of the experiment and thereafter at 4 months interval for the whole period of study. The radiographs were examined for alteration in position, size, contour, architecture and density of osseous structures to detect disturbance in mineralization and osteoid formation. To maintain uniformity the left front and hind digit from two cows from

each group was radiologically photographed. Dorsopalmer and lateral medial views were taken using normal x – ray film (Fuji RX universal) and standard intensifying screen in cassettes at focus film of one meter. Exposure of 70 kV and 25 mAs were employed using a portable x ray machine.

Reproductive performance examination

Reproductive performance was based on detection of oestrus using both heat signs and heat detector instrument (KAMAR) from USA (KAMAR, Colorado). Dates of conception determined by date of service and rectal palpation were recorded. Pregnancy was confirmed by subsequent rectal palpation at 35 days after the last service. In addition milk progesterone assay was carried out weekly for twelve weeks starting day 14 postpartum to detect oestrus, embryonic death and pregnancy. Other record included days from calving to subsequent conception, number of services per conception, embryonic deaths and abortions, stillbirths and retained placenta.

Milk production

Individual cow records were used and it included identification number, mean monthly milk yield (MMY), days in lactation (LAD) and total milk yield per lactation (TML).

3.3 SAMPLING

Ten soil and pasture grass samples were taken in April 1997, September 1997 and January 1998, May 1998, September 1998 and March 1999. A total of 93 and 48 animals were involved in blood sampling for preliminary mineral evaluation and

mineral supplementation trials, respectively. Furthermore 48, cows in mineral supplementation trials were used for milk sample collection.

3.3.1 Soil pasture and feed concentrates

Soil samples were collected using systematic sampling procedures recommended by Dick *et al.* (1996) with slight modification. In this method, two field samples taken at one sub area were thoroughly mixed to make one sample or bulk sample. In this study ten sub areas were selected for the whole farm. With each soil sample taken a sample of pasture grass was also taken.

Soil samples

All soil samples were collected from 0 – 20 cm of the topsoil. The sample were put into polyethylene bags and dried at room temperature (28°C). Thereafter they were sieved using a 2 mm sieve to remove roots and stones, as recommended by Dick *et al.* (1996). To avoid contamination, locations close to the roads were avoided for sampling and clean and rust free tools were used.

Grass samples

Upper part of the grasses, 5 cm from the ground was cut and washed with distilled water to remove soil contamination (lower parts of the plant were avoided because of contamination with soil caused by rain spattering). Paper bags were used for packing grass samples to avoid bacterial and fungal growth during transportation. Drying of samples was carried out at 35°C and later at 50°C to stop biological processes. The

samples were ground and sieved using a 2 mm sieve as recommended by Sillanpää (1982).

3.3.2 Blood

Blood samples were collected from jugular vein from each animal throughout the study period. Blood samples for mineral trials were collected on the initial day of mineral supplementation and then subsequently at an interval of two months for a period of two years (i.e. May 1997 - March 1999). Blood samples were collected between 7.00 - 9.00 a.m. using plain, heparinised and EDTA vacutainer tubes.

Treatment of blood samples

Whole blood sample in EDTA tubes was divided into three portions. One portion was used for glutathione peroxidase (GSH.Px) determination and was kept at a temperature between -15 and -20°C before analysis. Another aliquot was used immediately for determination of PCV %, Hb, WBC and differential WBC count. Into the third aliquot sodium fluoride (NaF) was added to arrest cellular glycolysis and was for glucose analysis. Whole blood in plain vacuitaner tubes was used for harvesting serum for immunoglobulin determination. Whole blood samples collected in heparinised tubes as well as the sample for glucose determination were centrifuged within one hour after collection at 5000 x G for 10 minutes to obtain plasma. The plasma from heparinised whole blood vacutainer tubes was used for Ca, Pi. Zn, ALP, ASAT, Cp, TP, Glu and Plu determinations.

Plasma for determination of plasma Ca, Zn and Pi was harvested into tubes washed in 6N nitric acid to prevent exogenous mineral contamination. The plasma and serum samples were stored at temperature between -15°C and -20°C pending analysis.

3.3.3 Milk samples

Milk samples for progesterone test was taken weekly for 12 weeks from all cows, starting 14 days post partum. Milk samples were preserved using potassium dichromate tablets. The samples were placed in a cool box during collection. The samples were centrifuged at 2000 x G for 10 minutes in order to separate the milk fat. The skim milk thus obtained was then stored at temperature between -15°C and -20°C pending analysis. Another set of milk samples was also taken for milk protein and fat analysis. Skimmed milk for progesterone and whole milk for milk protein and fat analyses were frozen between -15°C and -20°C pending analysis.

3.4 LABORATORY ANALYSIS

Soil, pasture and feed mineral analyses included Ca, P and Zn. In addition Mg, K, Na, Fe, Cu and Mn concentration were analysed in order to understand the relationship of Ca, P and Zn to these minerals. Blood mineral analyses included plasma Ca, Pi and Zn and plasma ALP activity as a supportive indicator of adequacy of Ca, P and Zn. In addition, Cp and GSH.Px activities were measured to estimate blood Cu and Se. To evaluate the health status of the animals in relation to mineral supplementation PCV %, Hb, ASAT, total and differential WBC count, as well as

SIM were measured. To assess the effects of mineral supplementation on energy and protein metabolism, plasma Glu, TP and Plu were determined. Milk was analysed for milk protein (MP) and fat (MF). Milk progesterone was determined as an aid in the assessment of return to oestrus, embryonic death and abortions.

Most analyses were carried out at the Sokoine University of Agriculture, Morogoro, Tanzania and a few items were analysed in Denmark at the Danish Institute of Agricultural Sciences. Analyses of blood plasma parameters were carried out in the Department of Veterinary Physiology, Biochemistry, Pharmacology and Toxicology. Milk progesterone determination was carried in the Department of Veterinary Surgery and Theriogenology, whereas milk protein and fat determination were performed at the Department of Animal Science and Production. Pasture grass and concentrate analyses were performed at the Department of Animal Science and Production SUA as well as the Department of Animal Health and Welfare, Danish Institute of Agricultural Sciences, Denmark. Soil, grass pastures and plasma Zn samples analyses were carried out in the Department of Soil Science, SUA.

3.4.1 Soil calcium, phosphorus and zinc status

Soil calcium, phosphorus and zinc

The amount of exchangeable calcium was determined using atomic absorption spectrophotometer, following extraction with an excess of 1M NH_4OAC (ammonium acetate), as described by Okalebo *et al* (1993). In this method the element in question, which must be presenting ionic form is atomised in a flame at a

temperature sufficiently enough for the ions to be converted to excited state. The atom absorbs light energy of specific wavelength as it enter excited state. As the number of atoms in the light path increases, the amount of light absorbed also increases. By measuring the amount of light absorbed, a quantitative determination of the amount of analyte can be made.

Extractable phosphorus was determined according to methods described by Black (1965), Bray and Kurtz (1945) using ammonium fluoride for extraction. Determination of soil Zn was based on DTPA extracting method as described by Lindsay and Narvell (1969). In this method diethylene triamine penta acetic acid is used for extraction of Zn from the soil before reading using atomic absorption spectrophotometer.

3.4.2 Calcium, phosphorus and zinc content in pasture and feed concentrates

Calcium

Calcium was determined using methods recommended by Artificial Official Analytical Chemistry (AOAC), (1975) as described by Milner and Whiteside (1984). In this method the feedstuff is ashed at 550 ° C for three to five hours and the ash digested using concentrated hydrochloric acid at a ratio of 1:1 for 24 hours to liberate the mineral. The solution of the sample is then analysed using atomic absorption spectrophotometer after further dilution and using suitable standard solution to prepare a curve.

Phosphorus

Phosphorus was determined colorimetrically using spectrophotometer set at 420 nm as recommended by AOAC (1972) and described by Helrich (1990). In this method phosphorus is brought into the form of orthophosphate, which is determined colorimetrically after complex formation with Vanado – molybdate.

Zinc

Zinc concentration was determined according to method described by Smith and Schenk (1972), Basson and Bohmer (1972) and Runhel and Baak (1972) as described by Milner and Whiteside (1984). The same principle is used as in calcium determination but no further dilution is done.

3.4.3 Plasma mineral concentration

Total calcium

The analysis was done according to modified methods of Gitelman (1967) and Kessler and Wolfman (1964) using a Cecil spectrophotometer. In this method plasma is added to the acidic medium and incubated for a period of four minutes to ensure the release of protein bound calcium. Thereafter, the acidic medium is made alkaline and then cresolphthalein is added forming a coloured complex with calcium ions, the absorption of the complex is measured at 574 nm. Intra and inter assay precision were 1% and 2%, respectively. To assure precision of analytical level and accuracy of the measurements at least one control sample (2.50 mmol Ca/l) was analysed together with a test sample batch. Detailed procedure is provided in Appendix 2.

Inorganic phosphate

Plasma Pi was assayed by measurement of vanado- phospho- molybdate complex formed in acid as described by Fiske and Subarrow (1925). Absorbance of this complex is read at 420 nm. Absorbance data are converted into concentration values using a standard curve. Intra and inter assay precision were 1% and 2%, respectively. To assure further precision of analytical level and accuracy of the measurements at least one control sample (2.0 mmol Pi/ l) was analysed together with a test sample batch. Detailed procedure is presented in Appendix 3.

Zinc

Plasma Zn concentration was determined by atomic absorption spectrophotometer as described by Milner and Whiteside (1984). In this method Zn was liberated from protein by using trichloroacetic acid after centrifugation at 3000G for 10 minutes. Zinc from the supernatant was then determined by atomic absorption spectrophotometer using prepared standards. The Intra assay and inter assay coefficients of variation was 2% and 3%, respectively. Detailed procedure is provided in Appendix 4.

Copper

Plasma Cu concentration was estimated by determining the activity of ceruloplasmin activity as described by Schosinky *et al.* (1974). In this method O - diasidin dihydroxychrolide (4-4'- diamino –3-3 dimethoxybiphenly) was used as substrate and acetate as a buffer at pH 5.5. Ceruloplasmin acts as a diamine oxidase and the quantitative determination is based on this activity. The intra and inter assay

coefficient of variation was 2% and 3%, respectively. Detailed procedure is provided in Appendix 5.

Selenium

Selenium estimation was done by determining the activity of glutathione peroxidase (GSH.Px) in whole blood. The activity of (GSH.Px) in whole blood was determined using RANSEL reagent kit from RANDOX laboratories, United Kingdom. Glutathione peroxidase (GSH.Px) catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH the oxidised glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$ as shown in the equation below. The decrease is measured by absorbency at 340nm.

$$2GSH + ROOH \xrightarrow{GPX.Px} ROH + GSSG + H_2O$$

$$GSSG + NADPH + H^+ \xrightarrow{GR} NAPD^+ + 2GSH$$

For control of precision and accuracy a RANSEL control of whole blood provided was analysed together with the test samples for each batch of analysis. The intra assay and inter assay coefficients of variation was 2% and 3%, respectively. Detailed procedure is provided in Appendix 6.

3.4.4 Haematological parameters

Packed cell volume %

Packed cell volume (PCV %) was measured using the microhaematocrit method as described by Baker and Silverton (1976). In this method the blood in capillary tube was spun at 10,000 rpm for 5 minutes in the microcentrifuge. The spurned tube was placed into the designed scale and the PCV was read as percentage. Intra assay coefficient of variation was 1%. Detailed procedure is provided in Appendix 7.

Haemoglobin (Hb)

The cyanmethaemoglobin method using Drabkin's diluent as described by Baker and Silverton (1976) measured haemoglobin. The haemoglobin was converted by the action of ferricyanide to methaemoglobin. This then was converted to cyanmethaemoglobin by the action of potassium cyanide. The concentration of cyanmethaemoglobin was then measured by spectrophotometer at a wavelength of 540 nm. Intra assay coefficient of variation was 1%. Detailed procedure is provided in Appendix 8.

Total white blood cell (TWBC) and differential WBC counts (DWBC)

Whole blood sample for total WBC and differential WBC counts was diluted 1:20 with diluting fluid and transferred to a haemocytometer. White blood cells were counted in each of the four representative squares as described by Baker and Silverton (1976). Blood slides for differential WBC were fixed in absolute methyl alcohol before staining them with giemsa stain as described by Schalm *et al.* (1975).

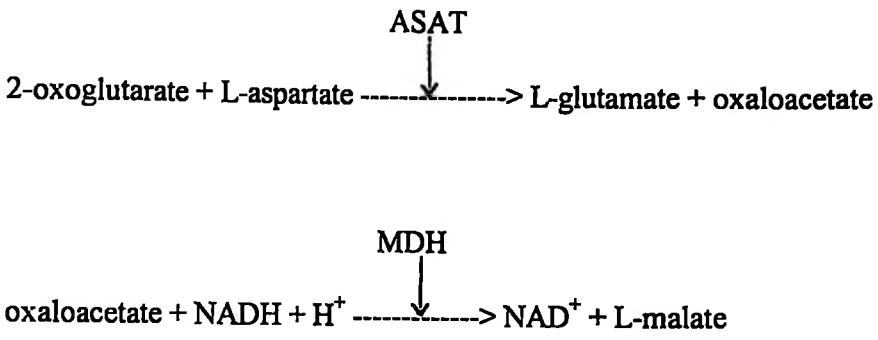
The stained slides were left in giemsa for 15 – 20 minutes and then dried. Two hundred cells were counted and the results were expressed for each cell type as a proportion of the total WBC number counted. Detailed procedure is provided in Appendix 9.

Serum immunoglobulins (SIM)

Serum immunoglobulin concentration was determined using a selective turbidity produced by zinc sulphate solution (208 mg /l) as described by McEwan *et al.*, (1970). Intra assay and inter assay coefficients of variation was 2% and 3%, respectively. Detailed procedure is provided in Appendix 10.

Plasma aspartate aminotransferase (ASAT)

Aspartate aminotransferance activity in plasma was determined using commercial reagent kit (RANDOX laboratories, Northern Ireland). The method is based on continuous monitoring assay obtained by coupling the transaminase reactions to specific dehydrogenase reactions. The oxoacids formed in the transaminase reaction are measured indirectly by enzymatic reduction to the corresponding hydroxyacids; the accompanying change in NADH concentration is monitored spectrophotometrically. Thus oxaloacetate, formed in the ASAT reaction is reduced to malate in the presence of malate dehydrogenase (MDH) as shown in the equation below. As the reaction proceed, NADH is oxidised to NAD^+. Measuring the decrease in absorbency at 340 nm follows the disappearance of NADH per unit time.



For precision and accuracy ASAT control sample was analysed together with the test samples for each analysis run. The intra assay and inter assay coefficient of variation were 4% and 5%, respectively. Detailed procedure is provided in Appendix 11.

Plasma alkaline phosphatase (ALP)

The analysis was carried out according to methods of Bower and McComb (1975). Alkaline phosphatase liberates ortho phosphate from organic and inorganic substrates. Alkaline phosphatase liberates ortho phosphate from p-nitrophenyl phosphate in alkaline solution. The absorbency of ortho phosphate (highly yellow coloured product) is monitored spectrophotometerically at a wavelength of 405 nm. Detailed procedure is presented in appendix 12.

3.4.5 Plasma metabolites

Plasma total proteins (TP)

Total plasma protein was measured using a commercial kit (RANDOX laboratory Northern Ireland). The method is based on the biuret reaction in which serum or plasma protein is reacted with an alkaline copper sulphate solution producing a

violet coloured complex. The absorbency of this complex can be measured by spectrophotometer at 540 nm. The intra assay and inter assay coefficients of variation was 1% and 2%, respectively. Detailed procedure is presented in Appendix 13.

Plasma urea (Plu)

Plasma urea was determined using commercial reagent kit (Boehringer, Mannheim, Germany). In this method urea is hydrolysed to ammonium ions by the enzyme urease, the ammonium ions formed react with salicylate and hypochloride to give a green dye which can be monitored spectrophotometrically at 540 nm. The intra assay and inter assay coefficients of variation was 1% and 2%, respectively. Detailed procedure is provided in Appendix 14.

Plasma glucose (Glu)

Plasma glucose was determined using commercial reagent kit (RANDOX, labotatories, Northern Ireland). In this method the enzyme glucose oxidase catalyses the conversion of glucose in plasma filtrate to gluconic acid. The equivalent quantity of hydrogen peroxide formed oxidises ortho – dianisidine in the presence of the enzyme peroxidase to a red brown colouring complex. The colour intensity is proportional to the glucose concentration and is measured spectrophotometrically at 540 nm. The intra and inter assay coefficient of variation was 1% and 2%, respectively. Detailed procedure is provided in Appendix 15.

3.4.6 Milk analysis

Milk Fat (MF)

Milk fat was determined using the Gerber method as described by British Standards Institution (1989). The Gerber method is based on the use of sulphuric acid (H_2SO_4) and amyl alcohol to free the fat from its emulsified state and the use of centrifugal force to separate the fat. Mixing milk with concentrated sulphuric acid cause destruction and dissolving of milk components, releasing the fat. Addition of amyl alcohol has the function of separating fat from other components. The mixture of milk, sulphuric acid and amyl alcohol was centrifuged for 4 – 5 minutes at 1100 revolutions per minute (r.p.m.) and after adjusting the temperature to 65°C the result of the test was read directly. The intra and inter assay coefficients of variation was 1% and 2%, respectively. Detailed procedure is presented in Appendix 16

Milk Protein (MP)

Milk protein was determined based on measurement of total nitrogen concentration in test samples by Kjeldahl analysis as described by (AOAC, 1995). Milk samples were digested with sulphuric acid in the presence of potassium sulphate and a low concentration of copper catalyst to form ammonium sulphate. Ammonia was released from the acid digested by addition of sodium hydroxide, then distilled and collected in a boric acid solution. Hydrochloric acid was used for titration; the amount of hydrochloric acid used was proportional to the amount of nitrogen originally present in the milk tested. The final (N) answer was multiplied by 6.38 to express the result as total protein. The intra assay and inter assay coefficients of

variation was 2% and 3%, respectively. Detailed procedure is presented in Appendix 17.

Milk progesterone

Milk progesterone was determined using radioimmunoassay (Coat-A-Count, Diagnostic Products Corporation (DCP), Los Angeles, U.S.A). The international Atomic Energy Agency (IAEA), Vienna, Austria, supplied the milk progesterone standards. The intra assay and inter assay coefficients of variation were 5% and 13%, respectively. The following parameter definitions were used

(a) Interval between parturition and resumption of oestrous activity (PRO) and was defined as the interval from parturition until the sampling day before the milk progesterone exceeded 2.5 nmol/l on 3 consecutive occasions.

(b) Interval between parturition and next conception (PCO) was defined as the interval from parturition until the sampling day before the milk progesterone exceeded 15 nmol/l for 3 consecutive sampling.

c) Early embryonic death (EED) occurred when the animal returned to heat after sustaining more than three elevated levels of milk progesterone.

3.5 STATISTICAL ANALYSIS

Data was analysed statistically using SAS general linear model (SAS Institute Inclusion, 1990). Differences between observed mean were estimated by the least significant difference test as described by Box et al., (1978). Furthermore, a multiple regression analysis was performed for the data for whole study period, dry and rainy

seasons. In summary the treatment effects were evaluated in a combined model as follows: -

$$R = \mu + B_1X_1 + B_2X_2 + \ldots B_mY_m + e$$

Where:

R	=	Response or dependent variable.
μ	=	General common mean to all observations in the study.
B_1, B_2, B_m	=	Unknown effects of the mineral supplements
$X_1\ X_2\ X_m$	=	Independent variables (month, year, season, concentrate)
e	=	Refers to the random effects peculiar to each observation on the variables studied.

CHAPTER FOUR

RESULTS

4.1 PRELIMINARY MINERAL STATUS EVALUATION

4.1.1 Soil mineral concentrations

Calcium

Mean soil calcium is presented in Table 6. Mean soil Ca was adequate at all sampling periods when compared to normal levels (2.50 me\ 100 g) given by Okalebo *et al* (1992). No significant variation was observed ($P > 0.05$) between sampling periods. However, there was a tendency towards more Ca in the dry season (5.06 – 5.67 me/ 100g) than in the rainy season (4.46 – 5.57 me/ 100 g). Soil calcium was positively correlated to sodium ($r = 0.44$, $P < 0.001$), potassium ($r = 0.32$, $P < 0.01$), copper ($r = 0.35$, $P < 0.01$) and magnesium ($r = 0.61$, $P < 0.001$).

Phosphorus

Mean soil phosphorus is presented in Table 6. A significant variation was observed ($P < 0.05$) between sampling periods. Phosphorus was low during the rainy season (April 1997 and January 1998) when compared to normal levels (> 15 ppm) given by Okalebo *et al* (1992) but was adequate in other sampling periods including March 1999. Soil phosphorus was negatively correlated to iron ($r = - 0.38$, $P < 0.001$) and copper ($r = -0.38$, $P < 0.01$).

Zinc

Mean soil zinc is presented in Table 6. No significant variation was observed ($P > 0.05$) between sampling periods. However there was a tendency towards more Zn in the dry season (0.99 – 1.02 ppm) than in the rainy season (0.81– 0.98 ppm). Zinc

Table 6. Soil concentration of calcium, phosphorus and zinc at ASAS Dairy Farm, Iringa, Tanzania. (Means with the same superscript letter within the same column and parameter do not differ significantly at $P > 0.05$, (n =10), () = range).

Parameter	Calcium (me/100 g)	Phosphorus (ppm)	Zinc (ppm)	Season
Mean critical level	< 2.50[1]	< 15[1]	< 1.00[2]	
April 1997	5.57 ± 1.05[a] (1.66 – 10.9)	11.9 ± 5.9[b] (0.07 – 38.7)	0.93 ± 0.19[a] (0.25 – 2.22)	Late rainy
September 1997	5.06 ± 1.05[a] (0.84 – 12.8)	31.8 ± 5.9[a] (1.4 – 63.4)	0.99 ± 0.19[a] (0.14 – 2.47	Late dry
January 1998	4.46 ± 1.05[a] (0.84 – 9.35)	14.8 ± 5.9[b] (0.10 – 39.7)	0.81 ± 0.19[a] (0.35 – 2.05)	Early rainy
May 1998	5.64 ± 1.05[a] (1.92 – 10.3)	28.1 ± 5.9[a] (4.21 – 50.4)	1.02 ± 0.19[a] (0.35 – 2.02	Early dry
September 1998	5.67 ± 1.05[a] (0.84 – 10.8)	30.8 ± 5.9[a] (2.4 – 53.4)	1.02 ± 0.19[a] (0.34 – 2.07	Late dry
March 1999	5.13 ± 1.05[a] (1.84 – 11.8)	34.6 ± 5.9[a] (1.4 – 63.4)	0.98 ± 0.19[a] (0.20 – 2.47)	Mid rainy

Source for mean critical level, [1] = Okalebo *et al* (1992), [2] = Landon (1991)

was deficient when compared to normal values (> 1.00 ppm) given by Landon, (1991) in all soil samples analysed except May 1998 and September 1998. Soil zinc was positively correlated to soil copper (r =0.37, P =0.0078) and iron (r = 0.32, P = 0.0056).

Other minerals

Sodium, potassium, magnesium, copper and iron concentration were adequate in the soil when compared to normal values given by Landon (1991) and Okalebo *et al* (1992) (Table 7).

4.1.2 Mineral content in forage and feed concentrates

Calcium

Means calcium contents in forage and feed concentrates are presented in Tables 1, 3 and 8. No significant variation on forage Ca was observed (P > 0.05) between sampling periods. However, there was a tendency towards more Ca in the dry season (0.38 – 0.39 %) as compared to the rainy season (0.33 – 0.36 %). A positive correlation was observed between Ca in the soil and Ca in the pastures (r = 0.39, P = 0.0018). Maize bran contained 0.13% Ca whereas sunflower seedcake had 0.38% Ca. Concentrate mixture containing maize bran and sunflower seedcake at a ratio of 4 to 1 had 0.15% Ca whereas at a ratio of 3 to 1 had 0.20 % Ca.

Table 7. Soil concentration of magnesium, sodium, potassium, iron and copper at ASAS Dairy Farm, Iringa, Tanzania. (Means with the same superscript letter within the same column and parameter do not differ significantly at $P > 0.05$, (n =10), () = range).

Parameter	Magnesium (me/100 g)	Potassium (me/100 g)	Sodium (me/100 g)	Iron (ppm)	Copper (ppm)
Mean critical level	< 0.17[1]	< 0.13[1]	< 0.1[2]	< 4.50[2]	< 0.20[2]
April 1997	1.57 ± 0.21[a] (0.46 – 2.74)	0.42 ± 0.06[a] (0.29 – 1.11)	0.31 ± 0.04[a] (0.19 – 0.48)	87.7 ± 21.6[a] (37.5 – 185)	2.24 ± 0.45[a] (0.22 – 6.10)
September 1997	0.99 ± 0.21[b] (0.32 – 1.68)	0.40 ± 0.06[a] (0.25 – 0.76)	0.37 ± 0.04[a] (0.23 – 0.64)	75.8 ± 21.6[a] (19.7 – 268)	1.55 ± 0.45[a] (0.18 – 4.07)
January 1998	1.25 ± 0.21[a] (0.32 – 2.54)	0.36 ± 0.06[a] (0.23 – 0.54)	0.33 ± 0.04[a] (0.24 – 0.52)	79.4 ± 21.6[a] (24.1 – 260)	1.71 ± 0.45[a] (0.14 – 3.48)
May 1998	1.55 ± 0.21[a] (0.56 – 2.70)	0.42 ± 0.06[a] (0.23 – 1.15)	0.32 ± 0.04[a] (0.19 – 0.45)	88.9 ± 21.6[a] (40.5 – 195)	2.17 ± 0.45[a] (0.32 – 5.10)
September 1998	1.15 ± 0.21[a] (0.74 – 1.58)	0.40 ± 0.06[a] (0.30 – 0.66)	0.34 ± 0.04[a] (0.22 – 0.56)	76.4 ± 21.6[a] (21.1 – 268)	1.38 ± 0.45[a] (0.22 – 4.70)
March 1999	1.17 ± 0.21[a] (0.32 – 2.58)	0.37 ± 0.06[a] (0.25 – 0.64)	0.37 ± 0.04[a] (0.25 – 0.64)	101 ± 21.6[a] (19.7 – 238)	1.92 ± 0.45[a] (0.68 – 4.07)

Source for mean critical level: [1] = Okalebo *et al* (1992), [2] = Landon (1991).

Table 8. Forage concentration of calcium, phosphorus and zinc concentration (bases on % dry weght) at ASAS Dairy Farm Iringa, Tanzania. (Means with the same superscript letter within the same column and parameter do not differ significantly at P > 0.05, (n =10), () = range).

Parameter	Calcium (%)	Phosphorus (%)	Zinc (ppm)	Ca:P ratio
Mean critical level	< 0.30[1]	< 0.25[1]	< 40[1]	
April 1997	0.33 ± 0.03[a] (0.20 – 0.58)	0.27 ± 0.03[a] (0.16 – 0.37)	23.0 ± 2.0[a] (12.0 – 35.0)	1.22:1
September 1997	0.39 ± 0.03[a] (0.27 – 0.52)	0.31 ± 0.03[a] (0.11 – 0.52)	24.1 ± 2.0[a] (14.0 – 35.0)	1.26:1 (1:1 – 2.2:1)
January 1998	0.33 ± 0.03[a] (0.24 – 0.39)	0.34 ± 0.03[a] (0.20 – 0.39)	21.2 ± 2.0[a] (12.0 – 29.0)	1:1 (0.9:1 – 2:1)
May 1998	0.31 ± 0.03[a] (0.18 – 0.63))	0.27 ± 0.03[a] (0.20 – 0.33)	25.2.0 ± 2.0[a] (17.0 – 39.0)	1.15:1 (0.8:1–2.5:1)
September 1998	0.38 ± 0.03[a] (0.20 – 0.62)	0.30 ± 0.03[a] (0.15 – 0.58)	25.2 ± 2.0[a] (14.0 – 35.0)	1.27:1 (1:1- 2:1)
March 1999	0.36 ± 0.03[a] (0.24 – 0.56)	0.25 ± 0.03[a] (0.12 – 0.42)	27.0 ± 2.0[a] (21.0 – 37.0)	1.44:1 (1.1 – 2.1)

Source for mean critical level: [1] = McDowell *et al.*, (1983).

Phosphorus

Phosphorus concentrations in forage and feed concentrates are presented in Table 1, 2 and 8. Significant variation ($P < 0.05$) was observed in forage P between sampling periods. Phosphorus level was on borderline to normal levels (> 0.25%) in April 1997 (0.27%), September 1997 (0.27%) and March 1999 (0.25%) but was adequate in other sampling periods. Maize bran contained 0.31% P whereas sunflower seedcake had 1.40% P. Concentrate mixture containing maize bran and sunflower seedcake at a ratio of 4 to 1 had 0.43% P whereas at a ratio of 3 to 1 had 0.71 % P.

Zinc

Mean zinc content in forage and feed concentrates are presented in Table 1, 2 and 8. No significant variation was observed ($P > 0.05$) between sampling periods. Zinc was deficient at all sampling periods (21.2 – 27.0 ppm) when compared to normal levels (> 40 ppm). Maize bran contained 35 ppm Zn whereas sunflower seedcake had 140 ppm. Concentrate mixture containing maize bran and sunflower seedcake at a ratio of 4 to 1 had 56 ppm Zn whereas at a ratio of 3 to 1 had 68 ppm Zn.

Other minerals

Sodium, potassium, magnesium and iron concentrations in forage and feed concentrates were adequate in the forages, except for copper, which was low when compared to normal values given by McDowell, (1983) (Table 9).

Table 9. Forage concentration of magnesium, sodium, potassium, iron and copper (based on % dry weight) at ASAS Dairy Farm Iringa, Tanzania. (Means with the same superscript letter within the same column and parameter do not differ significantly at $P > 0.05$, (n =10), () = range).

Parameter	Magnesium (%)	Potassium (%)	Sodium (%)	Iron (ppm)	Copper (ppm)
Mean critical level	< 0.20[1]	< 0.60[2]	< 0.18[2]	< 50[1]	< 8[1]
April 1997	0.16 ± 0.01[a] (0.11 – 0.21)	2.41 ± 0.68[a] (1.55 – 3.95)	0.21 ± 0.02[a] (0.16 – 0.33)	154 ± 28[a] (69 – 362)	4.57 ± 0.46[a] (1.90 – 10.1)
September 1997	0.18 ± 0.01[a] (0.12 – 0.22)	2.21 ± 0.68[a] (1.08 – 3.80)	0.23 ± 0.02[a] (0.13 – 0.38)	190 ± 28[a] (93 – 564)	3.25 ± 0.46[a] (1.30 – 5.40)
January 1998	0.15 ± 0.01[a] (0.11 – 0.22)	2.67 ± 0.06[a] (1.75 – 3.87)	0.21 ± 0.02[a] (0.16 – 0.28)	144 ± 28[a] (54 – 294)	3.88 ± 0.46[a] (2.20 – 5.10)
May 1998	0.16 ± 0.01[a] (0.12 – 0.20)	2.27 ± 0.06[a] (1.65 – 3.32)	0.20 ± 0.02[a] (0.15 – 0.31)	154 ± 28[a] (89 – 304)	3.33 ± 0.46[a] (1.20 – 8.10)
September 1998	0.17 ± 0.01[a] (0.12 – 0.20)	2.05 ± 0.06[a] (1.38 – 2.82)	0.21 ± 0.02[a] (0.13 – 0.33)	144 ± 28[a] (110 –182)	2.53 ± 0.46[a] (1.30 – 3.40)
March 1999	0.17 ± 0.01[a] (0.12 – 0.26)	2.25 ± 0.06[a] (1.68 – 3.12)	0.22 ± 0.02[a] (0.13 – 0.31)	167 ± 28[a] (93 – 282)	2.94 ± 0.46[a] (1.00 – 4.90)

Source for mean critical level: [1] = McDowell *et al.*, (1983) and [2] = Landon (1991)

4.1.3 Calcium, inorganic phosphate and zinc concentration in plasma

Plasma calcium concentration

The levels of plasma calcium in lactating cows, pregnant cows, pregnant heifers and steers are presented in Table 10. A significant variation was observed ($P < 0.05$) between groups of animals. Plasma Ca concentration in lactating cows (2.20 mmol/l) was within the normal suggested ranges (2.17 – 2.84 mmol/l). Low plasma Ca was recorded for non lactating pregnant cows (2.14 mmol/l), pregnant heifers (2.14 mmol /l) and steers (1.90 mmol/l).

Plasma inorganic phosphate concentration

The levels of plasma inorganic phosphate in lactating cows, pregnant cows, pregnant heifers and steers are presented in Table 10. A significant variation was observed ($P < 0.05$) between groups of animals. Plasma Pi concentration in steers was 1.80 mmol/l which was borderline to the suggested normal level (> 1.45 mmol/l). Low plasma Pi was recorded for the lactating cows (0.94 mmol/l), non lactating pregnant cows (1.24 mmol/l) and pregnant heifers (1.24 mmol /l).

Plasma zinc concentration

The levels of plasma zinc in lactating cows, pregnant cows, pregnant heifers and steers are presented in Table 10. Plasma Zn concentration was deficient in all groups except lactating cows (12.2 μmol Zn /l). The rest of the animal groups i.e. steers

Table 10. Plasma calcium, phosphorus and zinc for lactating and pregnant cows, pregnant heifers and steers at ASAS Dairy Farm Iringa, Tanzania. (Means with the same superscript letter within the same row and parameter do not differ significantly at P > 0.05, () = range).

Parameter	Lactating cows (n = 52)	Pregnant cows (n = 16)	Pregnant heifers (n = 9)	Steers (n = 17)	Critical level
Plasma Ca (mmol/l)	2.20 ± 0.31^{a} (1.56 – 3.05)	2.14 ± 0.07^{a} (1.71 – 2.35)	2.14 ± 0.07^{a} (2.01 – 2.24)	1.90 ± 0.21^{b} (1.35 – 2.09)	>2.17[1]
Plasma Pi (mmol/l)	0.94 ± 0.43^{a} (0.23 – 1.65)	1.28 ± 0.27^{a} (0.78 – 1.61)	1.23 ± 0.17^{a} (1.04 – 1.49)	1.80 ± 0.34^{a} (1.14 – 2.52)	>1.80[2]
Plasma Zn (µmol/l)	12.2 ± 0.4^{a} (10.7 – 13.2)	11.4 ± 0.4^{a} (10.5 –12.2)	11.8 ± 0.4^{a} (8.20 – 12.9)	8.8 ± 0.4^{b} (6.10 – 10.2)	>12.0[3]

Source for mean critical level: [1] = Rosol *et al.*, (1995), [2] = Ternouth, 1990, [3] = Underwood and Suttle, (1999).

(8.8 μmol Zn /l), pregnant cows (11.4 μmol Zn /l) and heifers (11.8 μmol Zn /l) had low plasma Zn concentation when compared to normal range of plasma Zn concentration (12.0 – 18.5 μmol Zn /l) (Underwood and Suttle, 1999).

4.2. EFFECTS OF CALCIUM, PHOSPHORUS AND ZINC SUPPLEMENTATION ON PLASMA MINERAL CONCENTRATION

4.2.1. Plasma total calcium concentration

The means and ranges of plasma Ca concentration are presented in Fig. 11 and Appendix 18. Significant variation ($P < 0.05$) on plasma Ca concentration between control and Ca, P and Zn supplemented cows was observed between sampling periods except May 1997, and July 1998. Cows supplemented with Ca tended to have low plasma Ca in July 1997 and 1998, March 1998 and May 1998 compared to control cows. Season had an influence ($P < 0.001$) on plasma Ca. High levels of plasma Ca was recorded during the wet season. High plasma Ca was observed in Ca supplemented cows (Group 2) in November 1997 (2.88 mmol /l) and the lowest plasma Ca was observed in July 1997 in the same group (1.39 mmol /l). Plasma Ca was positively correlated to plasma Pi during the wet season in the control cows .($r = 0.42$, $P = 0.0194$), Ca ($r = 0.38$, $P = 0.0393$) and Zn supplemented cows ($r = 0.48$, $P = 0.0076$). No correlation was observed between plasma Ca and Pi in the P supplemented cows. Furthermore, plasma Ca was positively correlated to plasma Zn

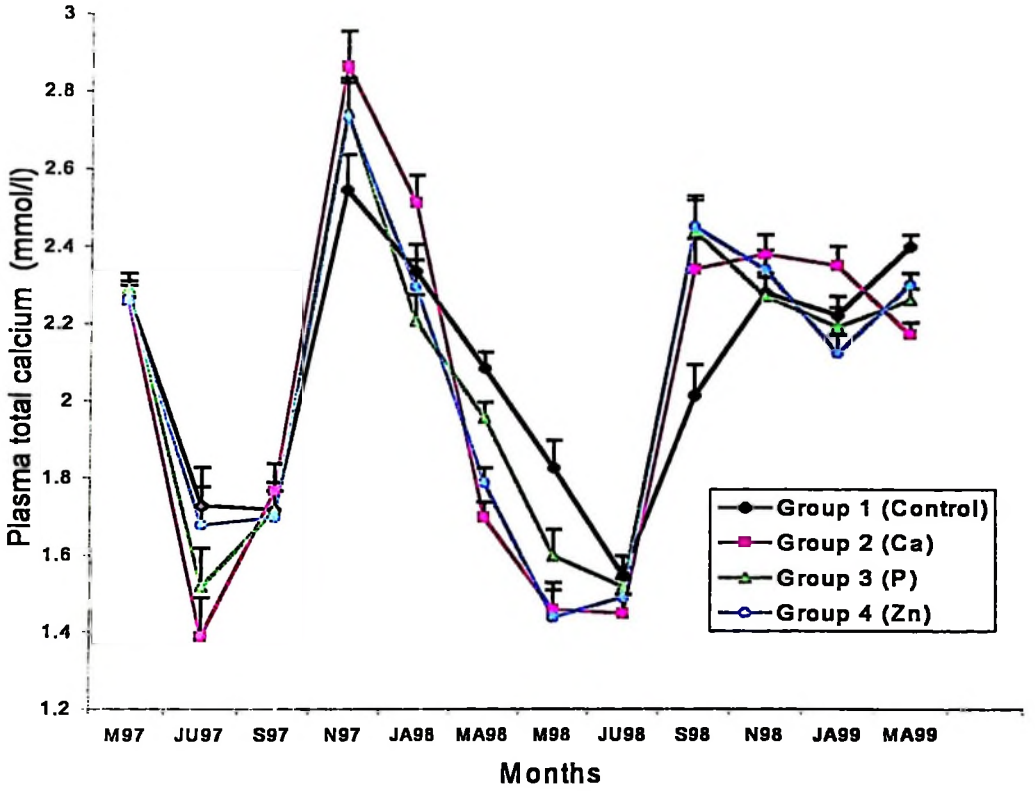


Figure 11 Plasma total calcium (mmol/l) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania.

in the dry season in the control (r = 0.30, P = 0.0518), Ca (r = 0.42, P =0.0194) and P supplemented cows (r = 0.39, P =0.0110). However during the rainy season plasma Ca was negatively correlated to plasma Zn in the P supplemented cows (r = - 0.42, P =0.0201). No significant correlation was observed in Zn supplemented cows.

4.2.2 Plasma inorganic phosphate (Pi)

The means and ranges of plasma Pi concentration are presented in Fig. 12 and Appendix 19. No significant variation (P > 0.05) in mean plasma Pi between groups within sampling periods was observed except for September, 1997, September 1998 and March 1999 in which Ca supplemented cows had high plasma Pi compared to the control cows. The highest plasma Pi was observed in September 1997 and November 1997 (2.43 mmol Pi / l) in cows supplemented with Ca (Group 2). During the dry season plasma Pi was positively correlated to plasma Zn in Ca Supplemented cows only (r = 0.43, P =0.0041). However, in the rainy season plasma Pi was negatively correlated to plasma Zn in the control (r = - 0.37, P =0.0467) and P supplemented cows (r = - 0.40, P =0.0285). No significant correlation (P > 0.05) was observed in the Zn supplemented cows.

4.2.3 Plasma zinc concentration

The means and ranges of plasma Zn concentration are presented in Fig. 13 and Appendix 20. Significant variation (P < 0.001) on plasma Zn between the control, Ca, P and Zn supplemented cows was observed between sampling periods. Cows supplemented with Ca had high plasma Zn except in July 1997 and July 1998 in which, the level was low compared to the control, P and Zn supplemented cows.

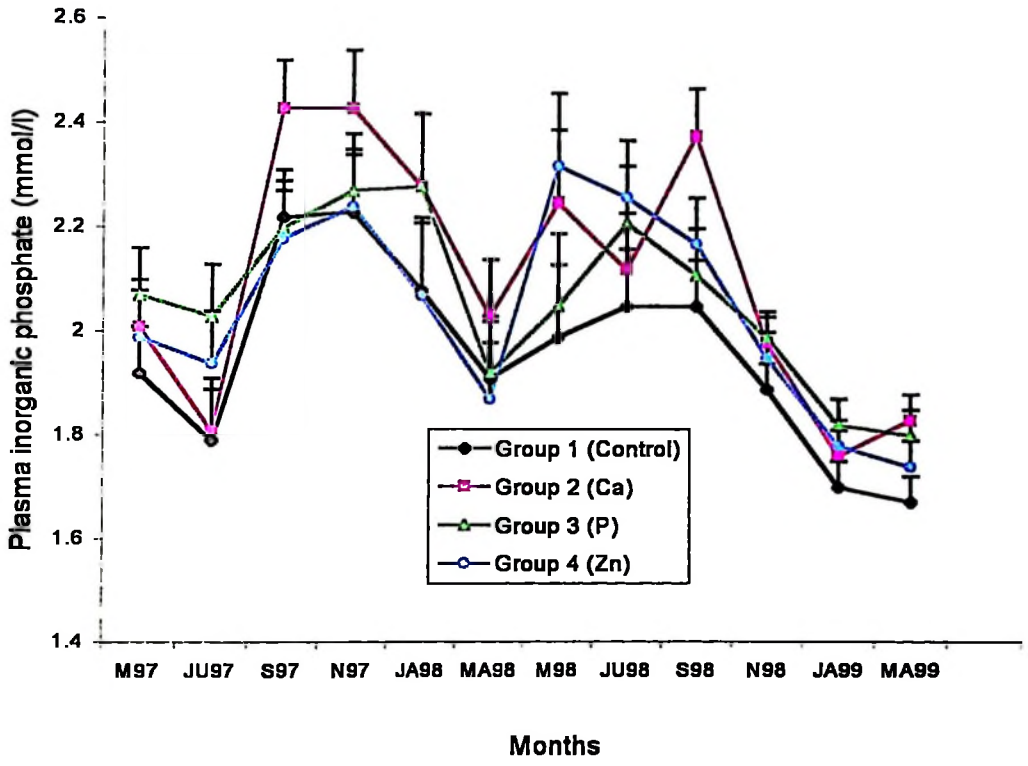


Figure 12 Plasma inorganic phosphate (mmol/l) in both non and mineral supplemented grazing crossbred zebu cows at ASAS Dairy Farm, Iringa, Tanzania.

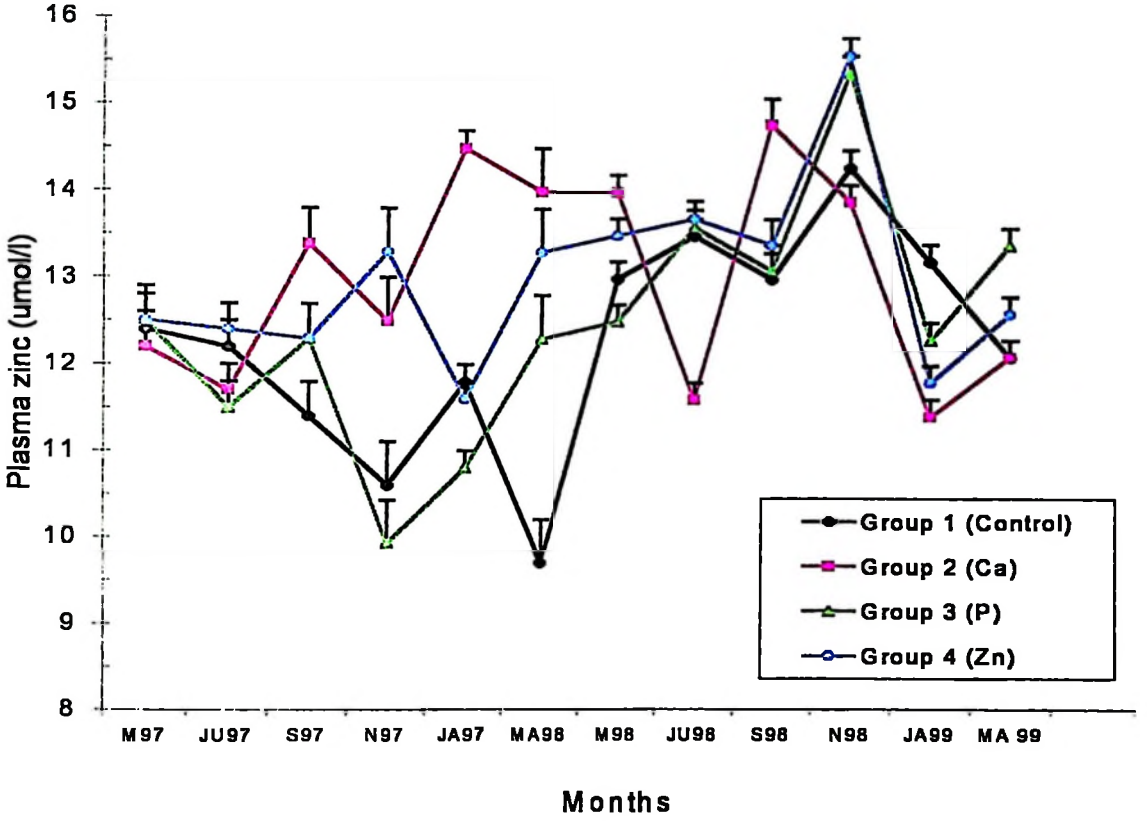


Figure 13 Plasma zinc concentration (µmol/l) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania.

4.3 EFFECT OF CALCIUM, PHOSPHORUS AND ZINC SUPPLEMENTATION ON HEALTH AND IMMUNE STATUS

4.3.1 Disease conditions in the experimental cows

Disease conditions encountered in the experimental cows during the study period are presented in Table 11, Appendices 23 and 24. High numbers of mastitis cases were recorded in the control cows (15) and P supplemented cows (16) as compared to Ca and Zn supplemented cows which had 7 cases each. Cases of mastitis were not time specific in the control, P and Zn supplemented cows, however for cows supplemented with Ca two cases each were recorded in month of July 1997 and 1998. In Zn supplemented cows only one case was observed per month except for May 1997, September 1997, July 1998, January and March 1998 in which no cases of mastitis was observed (Appendix. 23).

Six and seven cases of anaplasmosis incidences were recorded in the control cows and P supplemented cows, respectively. While only three cases each was recorded in the Ca and Zn supplemented cows. In addition three cases of udder parakeratosis were observed in the control cows only. No cases of milk fever or clinical lameness were observed in all cows in the study groups.

No cases of reproductive problems were observed in cows supplemented with Ca. However, case of embryonic death, abortion, stillbirth, retained placenta and cystic ovary was observed in the control, P and Zn supplemented cows. Number of cases, date when the problem occurred in each group are indicated in Appendix 25.

Table 11 Number of cases for different disease conditions observed in the experimental cows at ASAS Dairy Farm, Iringa, Tanzania.

	DISEASE/ CONDITION	GROUP 1 (Control)	GROUP 2 (Ca)	GROUP 3 (P)	GROUP 4 (Zn)
1.	Mastitis	15	7	16	7
2.	Anaplasmosis	6	3	7	3
3.	East coast fever	-	-	-	1
4.	Parakeratosis	3	-	-	-
5.	Others:				
	Cytic ovaries	1	-	-	-
	Pyometra	1	-	-	-

4.3.2 Hoof health and lameness assessment

No fractures and abnormal hoof growth which, could lead to clinical lameness, was observed during the whole experimental period. Furthermore, no claw abnormalities were observed in both non and mineral supplemented cows before and after 18 months of mineral supplementation as indicated by radiological pictures presented in Fig. 14, 15, 16 and 17.

4.3.3 Body weight (BW) and body condition scores (BCS)

The mean body weights during the experimental period are presented in Fig. 18 and Appendix 26. There was no significant differences ($P > 0.05$) in live body weight between the control and Ca, P and Zn supplemented cows however, there was a tendency towards high live body weight for cows supplemented with Ca. No correlation was observed between body weight and plasma Ca, Pi or Zn in all experimental groups of cows (Appendix 22 and 27).

The mean body condition scores are presented in Fig. 19 and Appendix 28. There was a significant difference ($P < 0.05$) in BCS between groups except in May 1997, September 1997, March 1998, May 1998, September 1998, November 1998 and March 1999. Cows supplemented with Ca tended to have high BCS compared to the control, P and Zn supplemented cows.

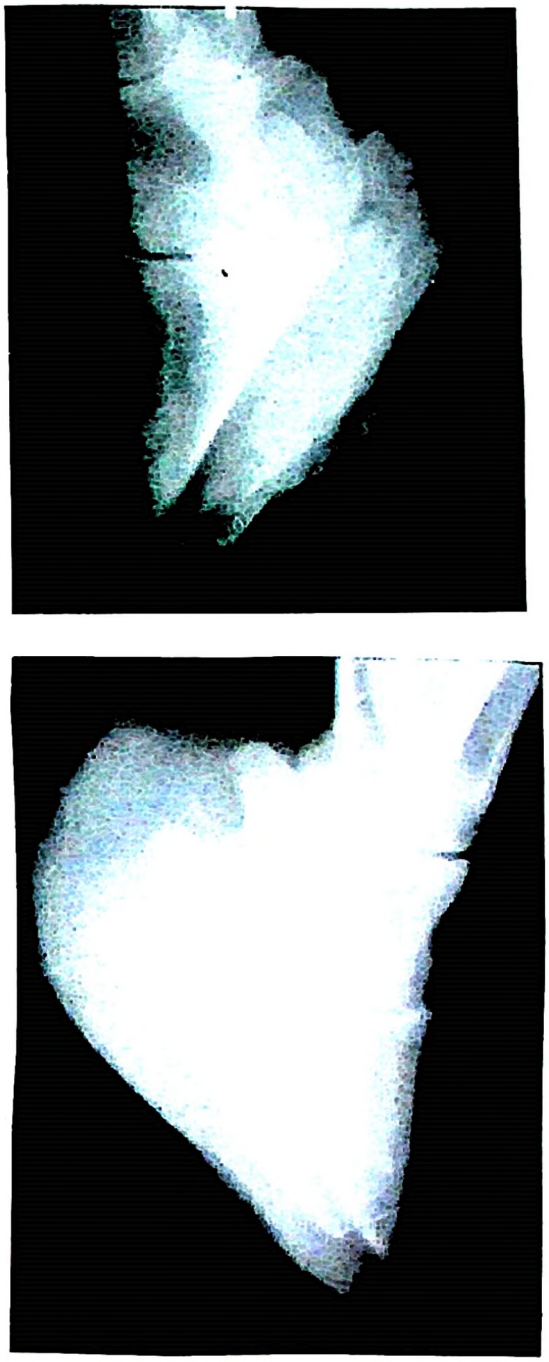


Figure 14 Lateromedial radiographic pictures of digital claws of a cow in the control group after 18 months without mineral supplementation. The bone contours are smooth, the basal surface is slightly concave.

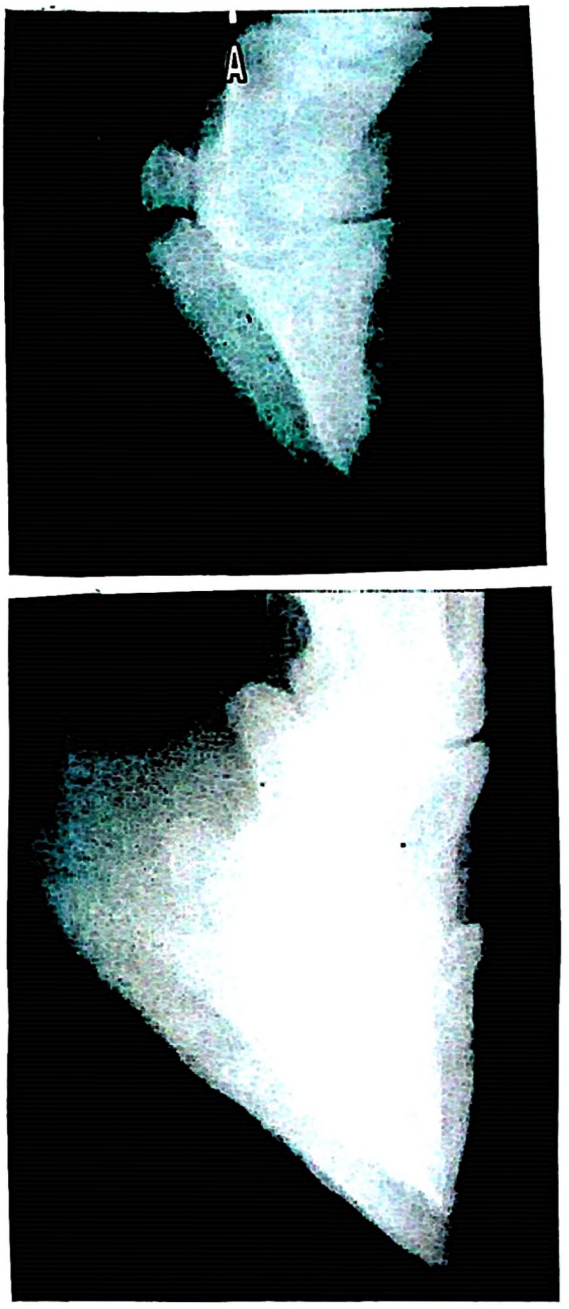


Figure 15 Lateromedial radiographic pictures of digital claws of a cow supplemented with Ca (Group 2) after 18 months of Ca supplementation. The bone contours are smooth, the basal surface is slightly concave.

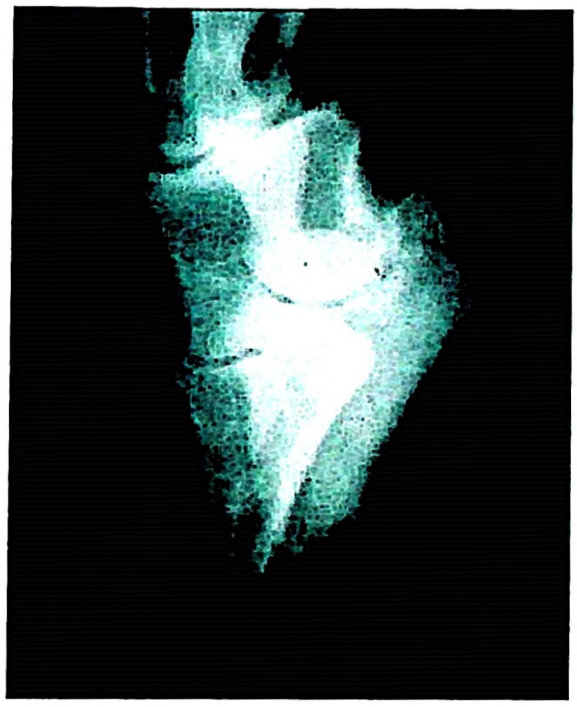


Figure 16 Lateromedial radiographic picture of a digital claw of a cow supplemented with P after 18 months of P supplementation. The bone contours are smooth, the basal surface is slightly concave.

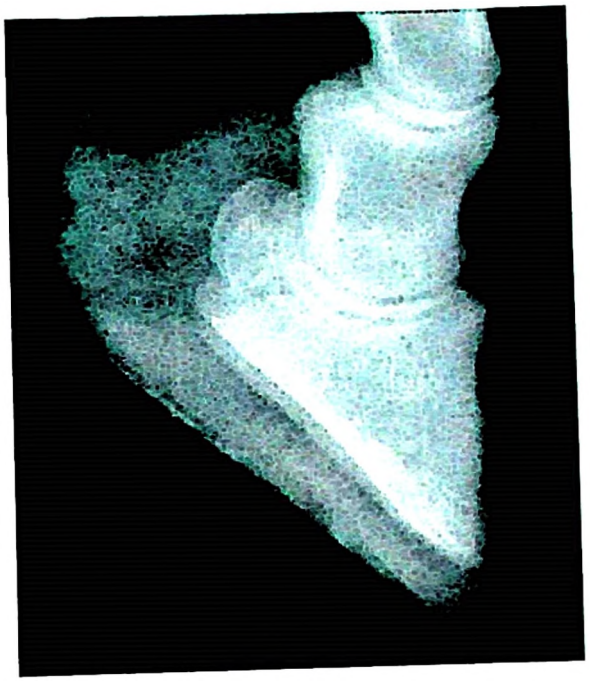


Figure 17 Lateromedial radiographic picture of a digital claw of a zinc supplemented cow after 18 months of Zn supplementation. The bone contours are smooth, the basal surface is slightly concave.

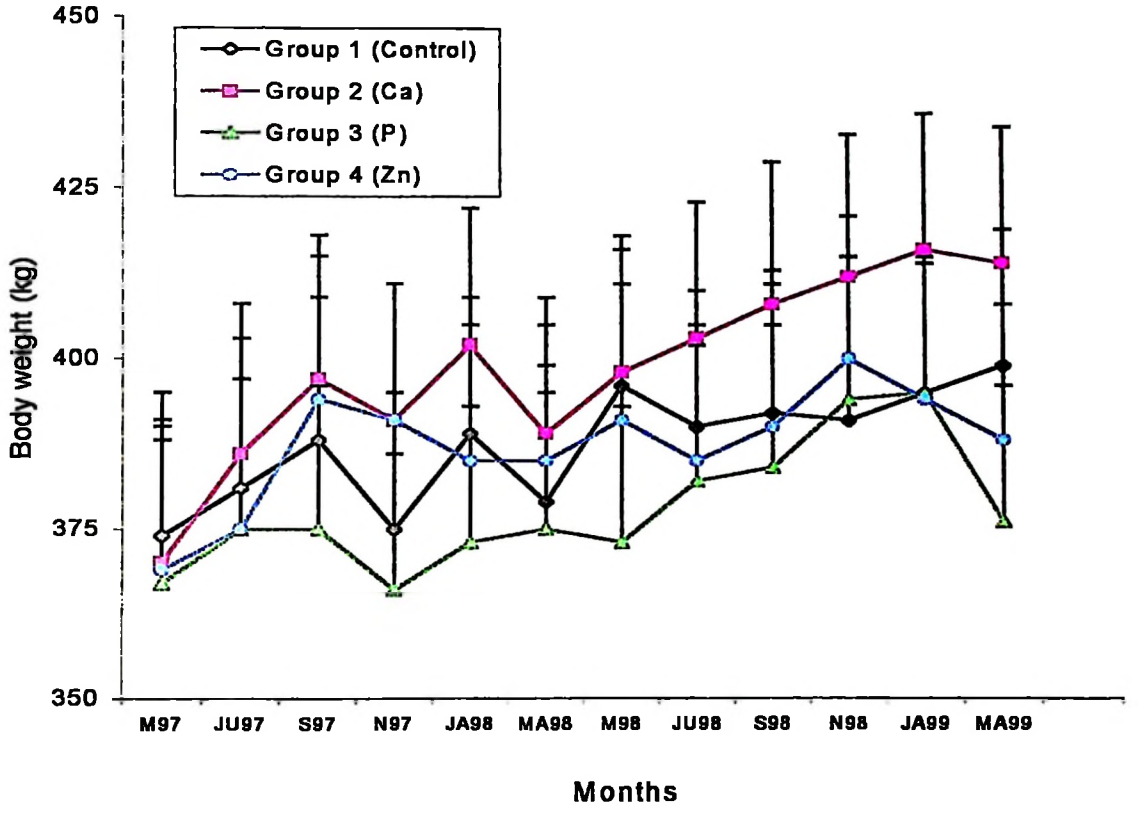


Figure 18 Live body weight (Kg) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania.

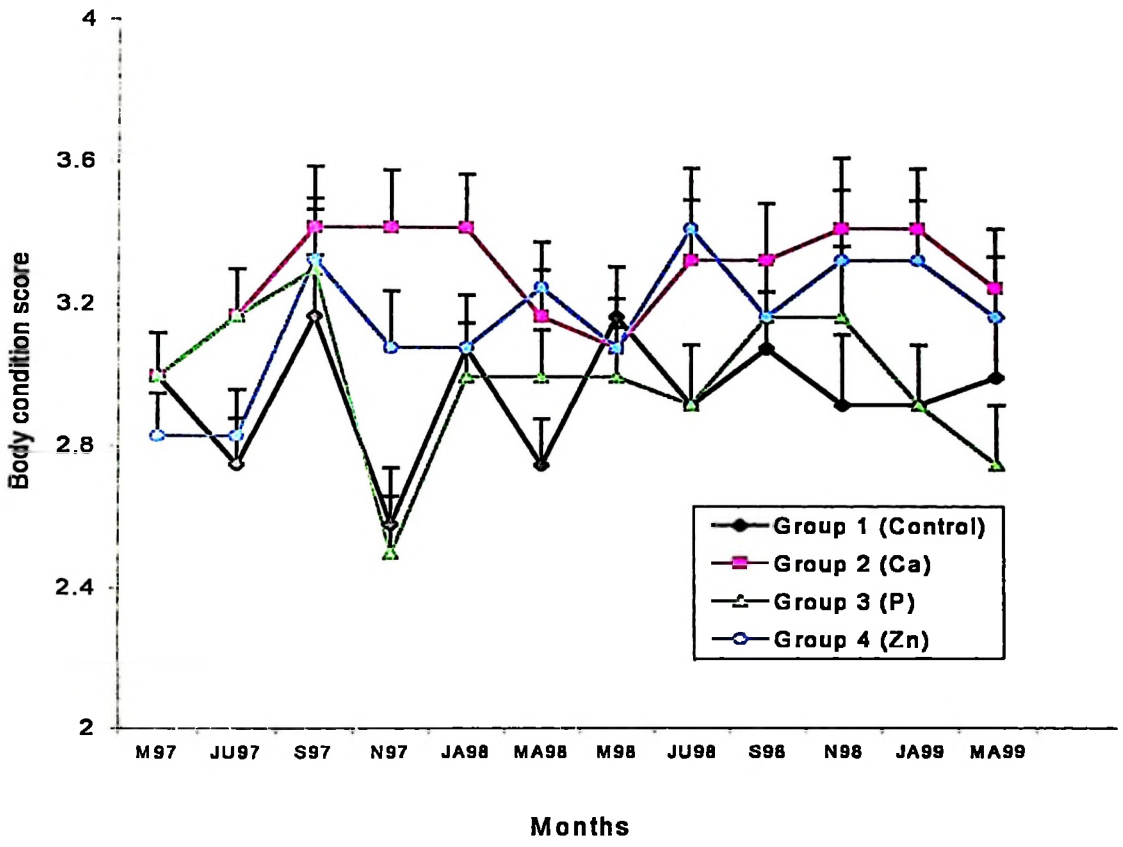


Figure 19 Body condition score in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania.

Body condition score was positively correlated to body weight in wet season in both group of cows, however cows supplemented with Ca had stronger relation (r = 0,54, P = 0.0020) than control cows (r = 0.42, P = 0.0217). Furthermore, BCS was positively correlated to plasma Pi in the dry season in Ca supplemented cows only (r = 0.32, P = 0.0383) whereas BCS was negatively correlated to plasma total protein in control cows (r = 0.35, P = 0.0246). Body condition score was negatively correlated to plasma Ca in the wet season in P supplemented cows only (r = - 0.45, P = 0.0122). No correlation was observed between BCS and plasma Ca, Pi or Zn in the Zn supplemented cows.

4.3.4 Haematological parameters

Packed cell volume (PCV%)

Means and ranges of packed cell volume percentages for the experimental cows are presented in Fig. 20 and appendix 29. No significant variation (P > 0.05) was observed in the PCV% between the control cows and Ca. P and Zn supplemented cows in most sampling periods except for July 1997, September 1997, November 1998 and January 1999. Control cows had high PCV% compared to other groups of cows in these sampling periods. Packed cell volume was negatively correlated to plasma Ca (r = -0.37, at P = 0.0416) and Pi (r = -0.34, at P = 0.0287) in Ca supplemented cows during the rainy and the dry season respectively. No significant correlation (P > 0.05) was observed between PCV and plasma Ca, Pi or plasma Zn in the control, P and Zn supplemented cows (Appendix 22, 27).

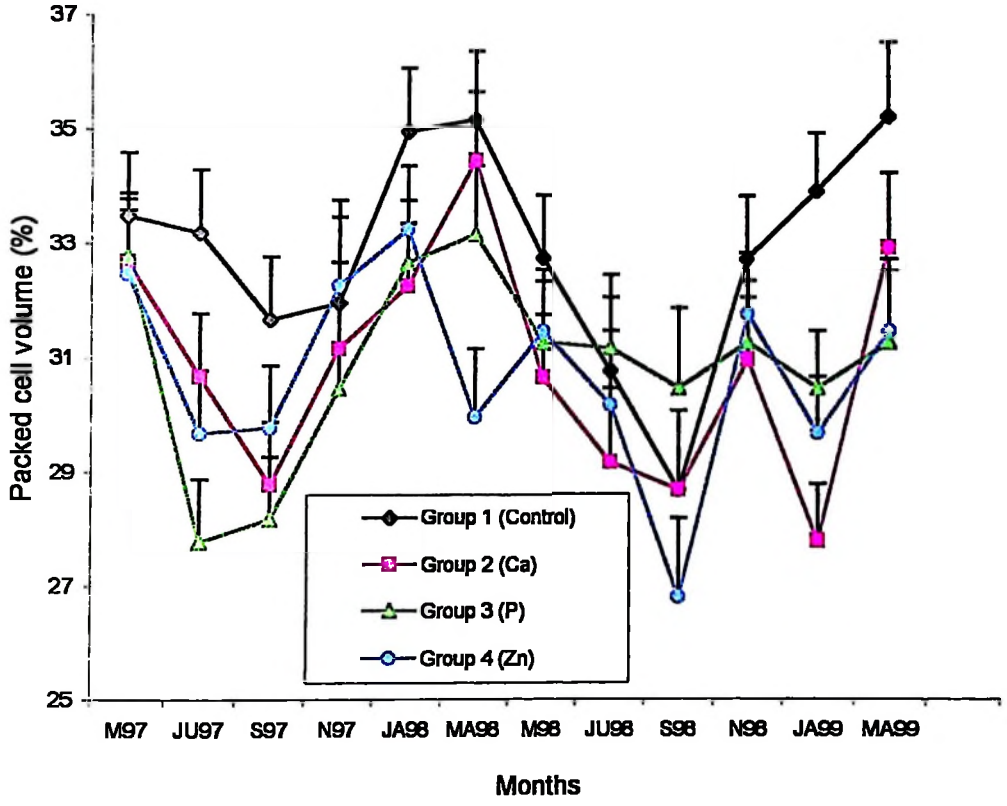


Figure 20 Packed cell volume (%) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania.

Erythrocyte haemoglobin concentration

The means and ranges of erythrocyte haemoglobin concentration are presented in Fig. 21 and Appendix 30. Significant variation ($P < 0.05$) was observed between sampling periods except for May, 1997, July 1997, November 1997, May 1998 and March 1999. High erythrocyte Hb concentration was observed in the control cows in most sampling periods when compared to cows supplemented with Ca, P or Zn. Haemoglobin concentration was positively correlated to plasma Ca ($r = 0.37$, $P < 0.0162$) in Ca supplemented cows during the dry season but was negatively correlated during the rainy season ($r = -0.35$, $P < 0.0501$). Furthermore erythrocyte Hb concentration was negatively correlated to Zn in the control cows ($r = -0.38$, $P = 0.0136$) and Zn supplemented cows ($r = -0.36$, $P = 0.0193$) during the dry season and Hb was positively correlated to plasma Zn in Ca supplemented cows during the wet season ($r= 0.46$, $P = 0.0111$). No significant correlation ($P > 0.05$) was observed between Erythrocyte Hb and Ca, Pi or Zn concentration in P supplemented cows in both the dry and the rainy season.

Total white blood cell (TWBC) and differential counts (DWBC)

The means and ranges of TWBC counts are presented in Fig. 22 and Appendix 31. There was no significant variation between groups ($P > 0.05$) in the TWBC at each sampling period except in May 1998 and September 1998. In May 1998 control cows had high TWBC whereas Ca supplemented cows had high TWBC in September 1998. Generally there was a tendency towards high TWBC in the Ca supplemented cows. Total WBC counts were positively correlated to plasma Ca in the dry season in all groups of cows. However cows supplemented with Ca had

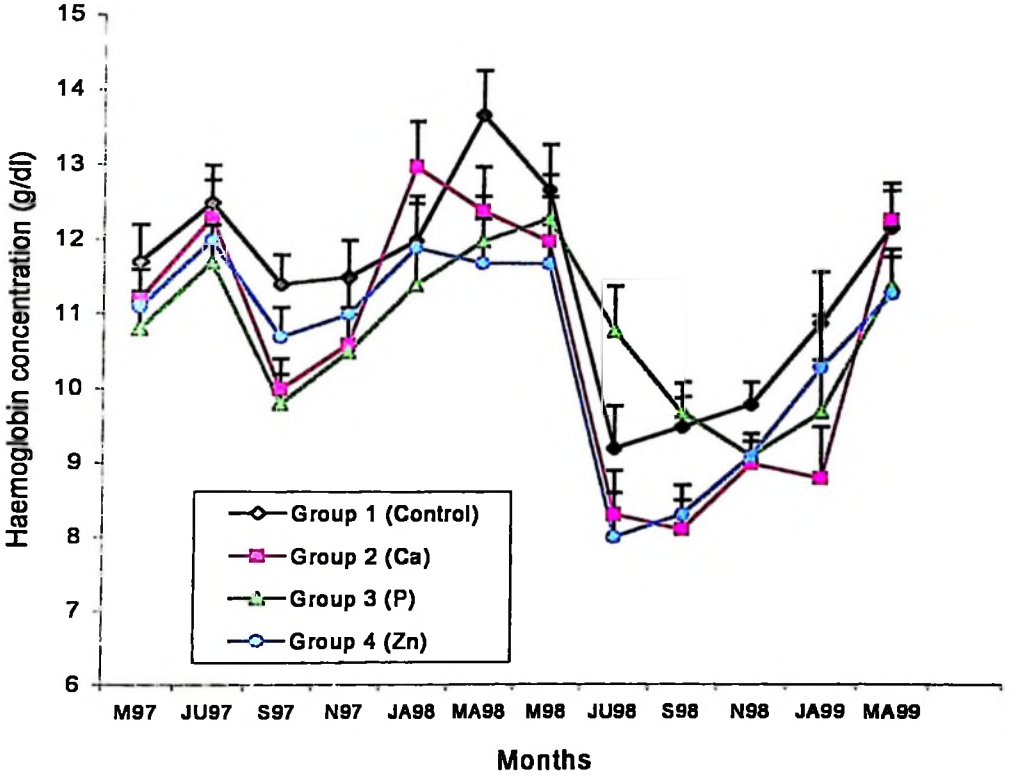


Figure 21 Haemoglobin concentration (g/dl) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania.

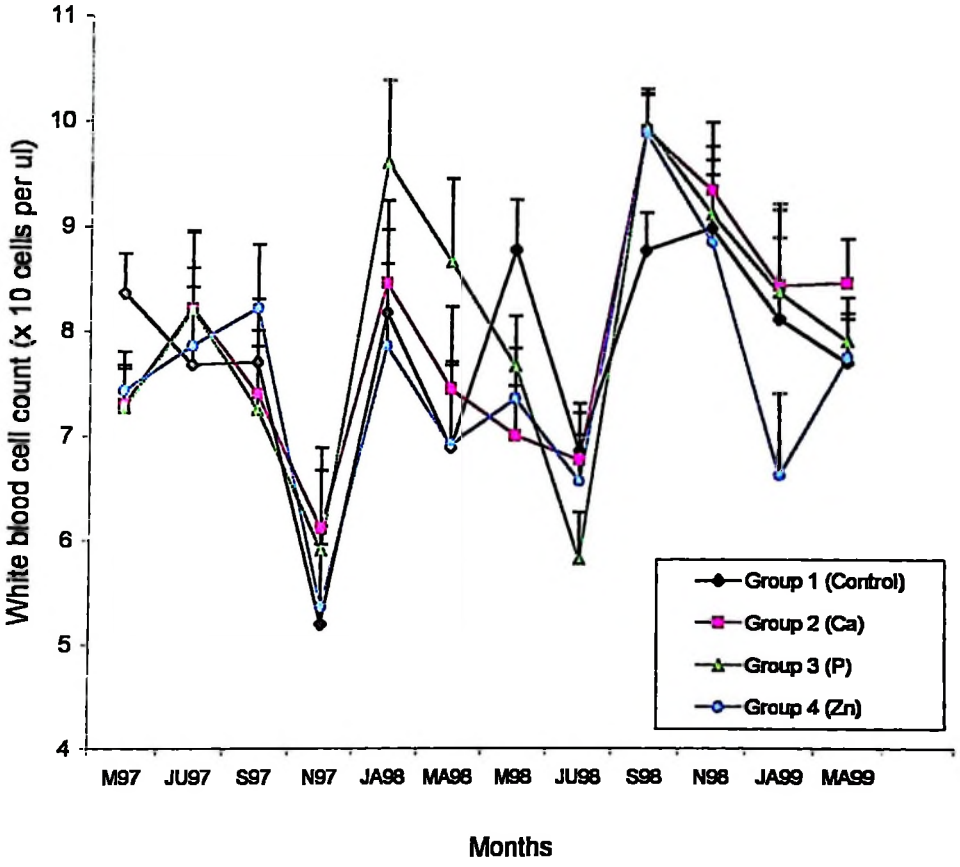


Figure 22 Total white blood cell counts (x 10^3 cells per µl) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania.

stronger correlation (r = 0.53, P = 0.0003) than P (r = 0.48, P = 0.0013), Zn supplemented (r = 0.44, P = 0.0040) and the control cows (r = 0.38, P = 0.0123). TWBC was positively correlated to plasma Ca in the rainy season in the control cows (r = 0.37, P = 0.0439) whereas a negative correlation was observed in P supplemented cows (r = -0.43, P = 0.0185). Furthermore, the TWBC was positively correlated to plasma Zn in the dry season in the Ca supplemented cows only (r = 0.46, P = 0.0023) whereas TWBC was positively correlated to plasma Zn in the rainy season in P supplemented cows (r = 0.37, P = 0.0424) (Appendix 32, 33 and 34).

Lymphocyte counts (LC)

The means and ranges for lymphocyte counts for each group are presented in Fig. 23 and Appendix 35. No significant variation (P > 0.05) in the LC was observed between sampling periods except for March 1998, May 1998 and July 1998. Lymphocyte counts were positively correlated to plasma Ca in the dry season in cows supplemented with Ca only (r = 0.52, P = 0.0004). However, plasma Pi was positively correlated to lymphocyte count in the control cows (r = 0.40, P = 0.0082) during the same period. Furthermore, LC was positively correlated to plasma Zn in the dry season in Ca supplemented cows only (r = 0.39, P = 0.0101) whereas LC was positively correlated to plasma Zn in the rainy season in the control cows (r = 0.36, P = 0.0477) and Zn supplemented cows (r = 0.48, P = 0.0067). No correlation was observed between LC and plasma Ca, Pi and Zn in the P supplemented cows.

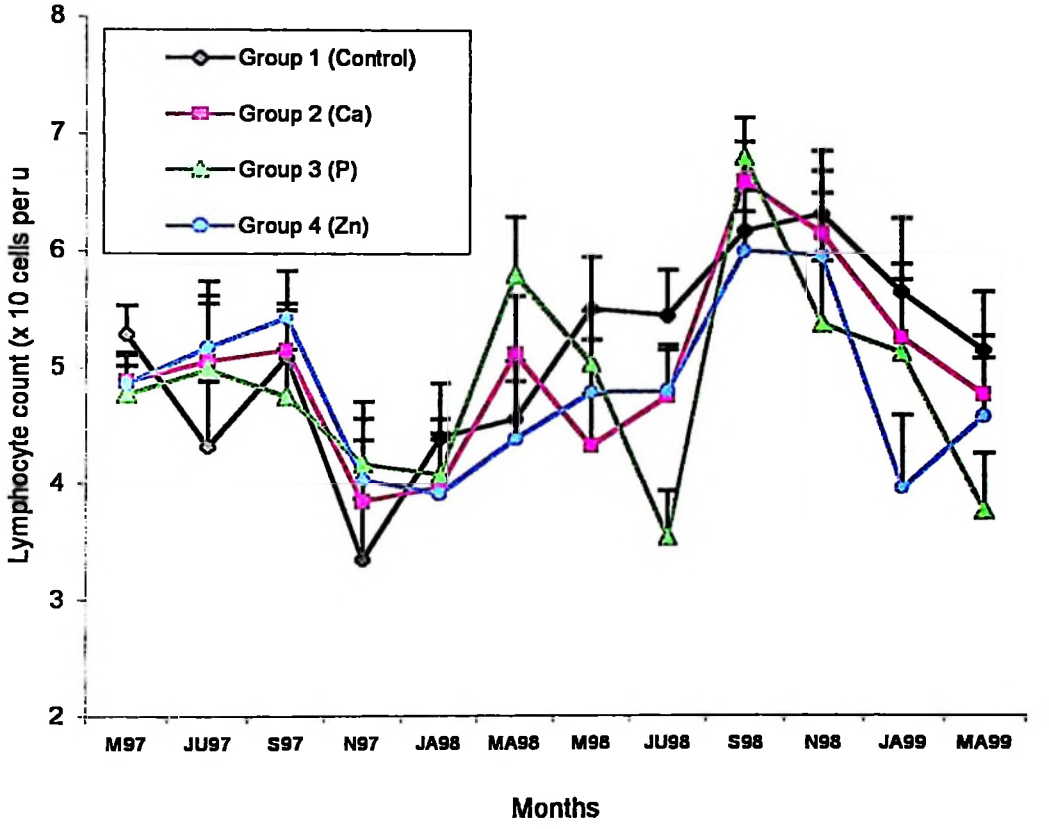


Figure 23 Lymphocyte count (x 10^3 cells per µl of blood) in both non and mineral supplemented grazing crossbred zebu cows at ASAS Dairy Farm, Iringa, Tanzania.

Neutrophil counts (NC)

The means and ranges for neutrophil counts are presented in Fig. 24 and Appendix 36. There was no significant variation between the groups ($P > 0.05$) except in November 1997, September, 1998, November, 1998, January 1999 and March 1999. In most sampling period Ca supplemented cows had high NC when compared to the control cows. Neutrophil counts were positively correlated to plasma Ca in the dry season in Ca supplemented ($r = 0.30$, $P = 0.0494$), P supplemented cows ($r = 0.32$, $P = 0.0424$) and Zn supplemented cows ($r = 0.43$, $P = 0.0044$) but the correlation was weak and non significant ($P > 0.05$) in the control cows ($r = 0.27$, $P = 0.0789$). Plasma Pi was negatively correlated to NC in control cows ($r = -0.32$, $P = 0.0353$) during the same period. Furthermore, NC was positively correlated to plasma Zn in the dry season in Ca supplemented cows ($r = 0.43$, $P = 0.0039$) and P supplemented cows ($r = 0.33$, $P = 0.0325$).

Eosinophil counts (EC)

The means and ranges for eosinophil counts are presented in Fig. 25 and Appendix 37. There was no significant variation between the control and Ca, P and Zn supplemented cows ($P > 0.05$) in EC at each sampling period except in November 1997, May 1998 and September 1998. Cows in the control group had higher mean EC 235 cells / μl and 120 cells / μl of blood in November 1997 and May 1998 whereas P supplemented cows had high EC 289 cells / μl in September 1998 compared to other groups. A negative correlation between EC and plasma Ca was observed in Ca supplemented cows ($r = -0.66$, $P = 0.0001$) and Zn supplemented cows ($r = -0.49$, $P < 0.0060$) in the rainy season.

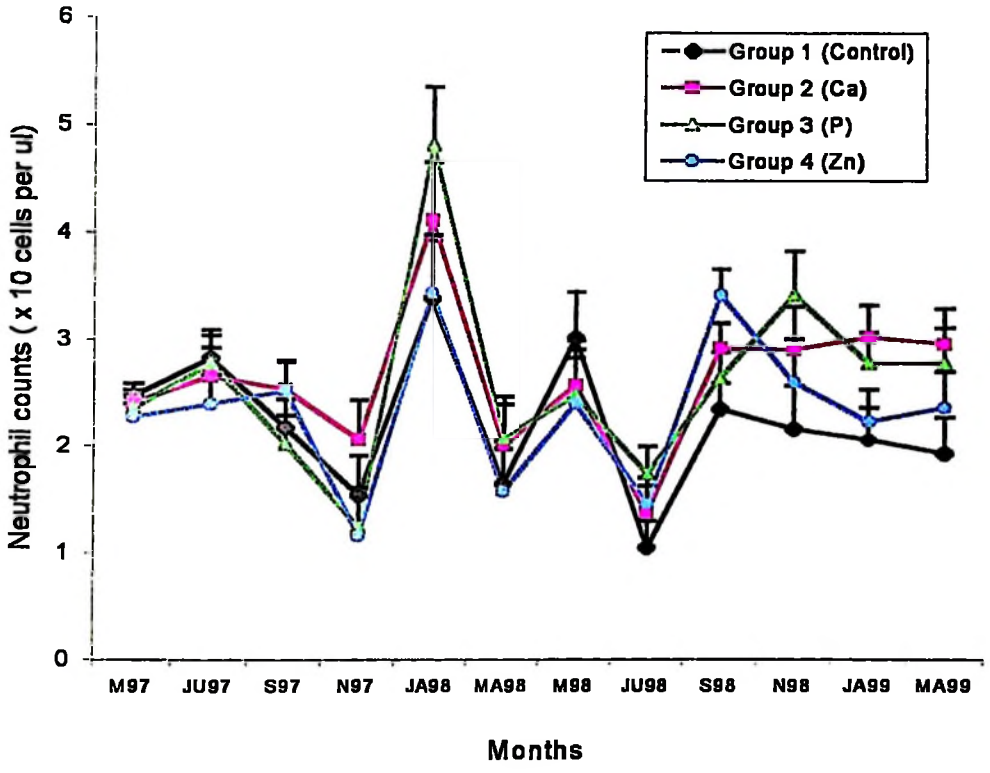


Figure 24 Neutrophil counts (x 10^3 cells per µl of blood) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania.

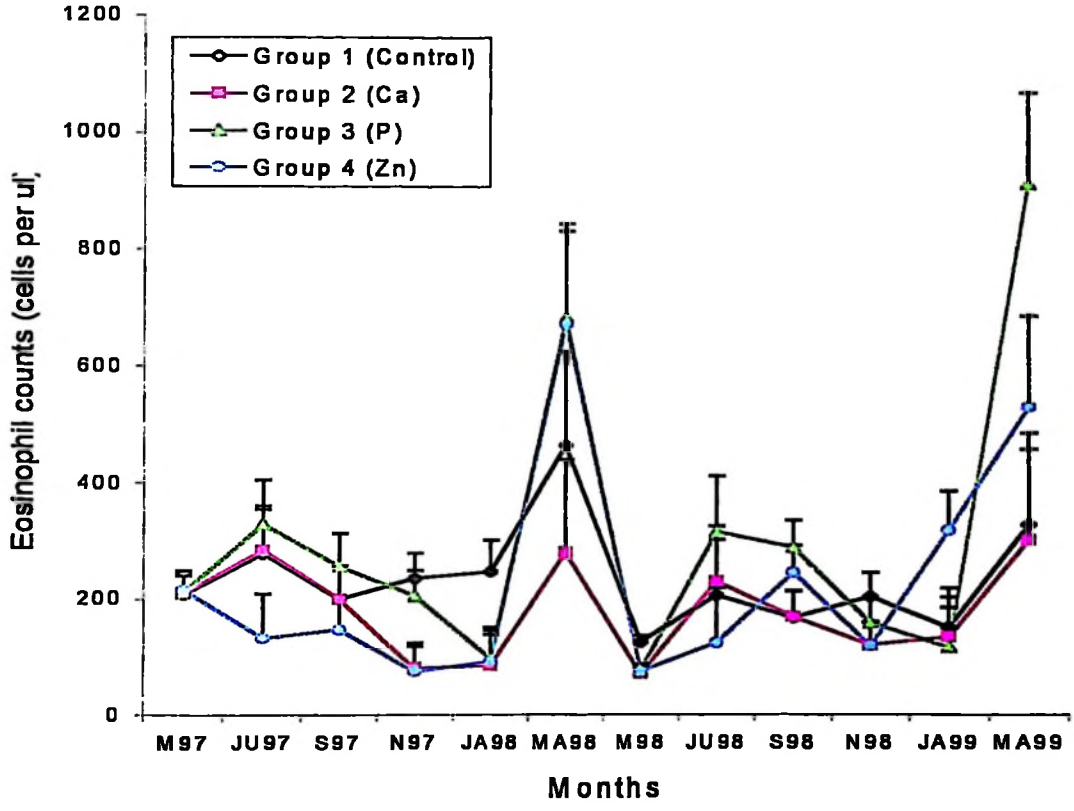


Figure 25 Eosinophil counts (cells per µl of blood) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania.

Furthermore, plasma Pi was negatively correlated to EC at the same period in Ca supplemented cows ($r = -0.53$, $P < 0.0024$) and the Zn supplemented cows plasma Pi ($r = -0.47$, $P < 0.0084$). Plasma Zn was negatively correlated to EC in the dry season in both control cows ($r = -0.38$, $P < 0.0133$) and Ca supplemented cows ($r = -0.37$, $P = 0.0157$) but it was positively correlated to EC in the P supplemented cows Pi ($r = 0.44$, $P < 0.0146$).

Monocyte counts (MC)

The means and ranges for MC are presented in Fig. 26 and Appendix 38. There was no significant variation ($P > 0.05$) between the control and Ca supplemented cows in MC at each sampling period except September 1997, November 1997, January 1998 and March 1999. Cows Supplemented with P had higher mean MC (294 cells / μl, 605 cells / μl and 342 cells / μl of blood) in November 1997, January 1998 and March 1999 respectively compared to other groups. A positive correlation ($r = 0.44$, $P = 0.0034$) between MC and plasma Ca was observed in the dry season in Zn supplemented cows only. No correlation was observed between NC and plasma Ca, Pi or Zn in the control, Ca and P supplemented cows (Appendix 32, 33 and 34).

Serum immunoglobulin (SIM)

The means and ranges for serum immunoglobulins are presented in Fig. 27 and Appendix 39. There was significant variation between groups ($P < 0.05$) for SIM at each sampling period except for November 1997 and March 1999. Serum immunoglobulin was high in the Ca supplemented cows in most of the sampling periods. Positive correlation were observed between SIM and plasma Ca in the dry

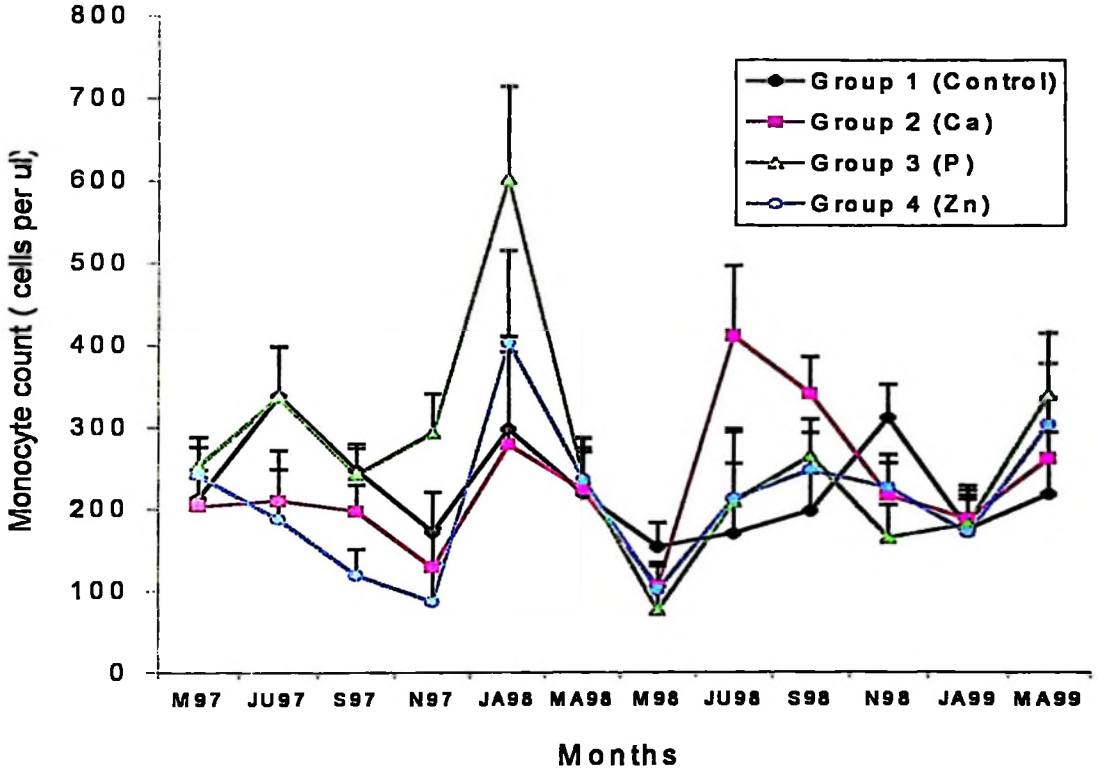


Figure 26 Monocyte count (cells per µl of blood) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania.

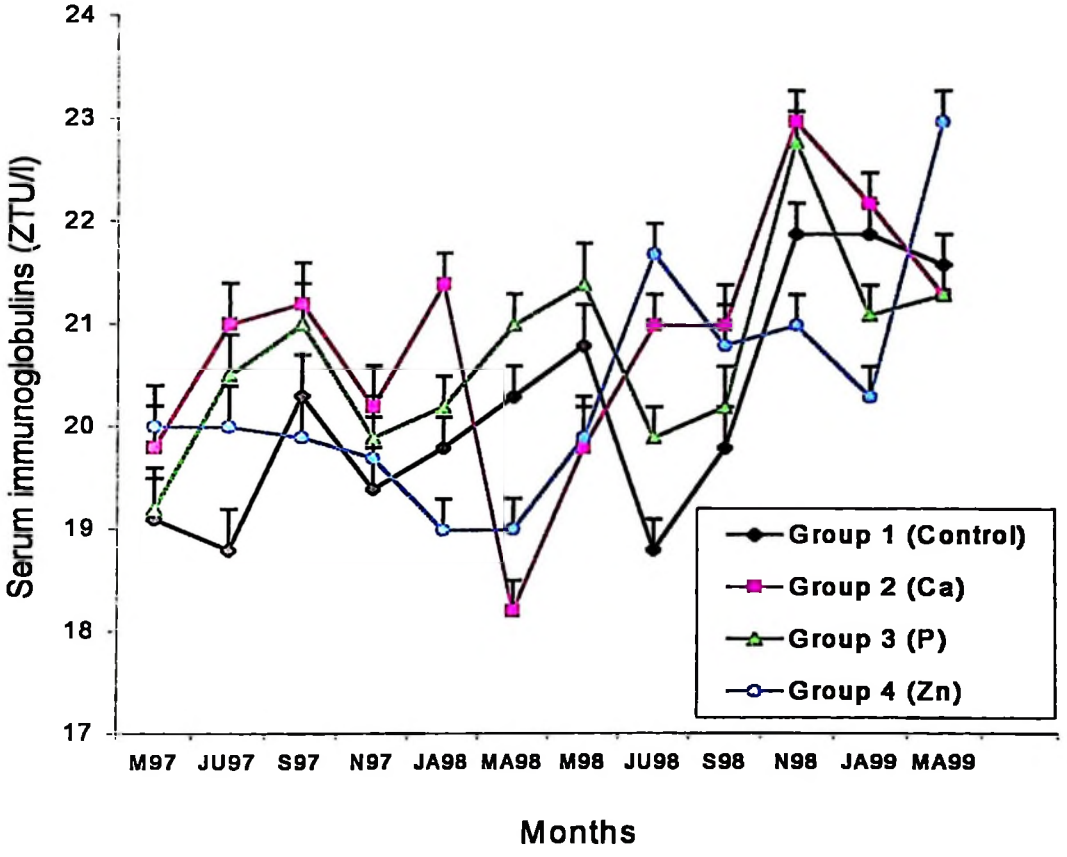


Figure 27 Serum immunoglobulin (ZTU/l) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania.

season (r = 0.36, P = 0.0209) and plasma Zn in the rainy seasons in the control cows (r = 0.45, P = 0.0124). Negative correlation between SIM and plasma Ca (r = - 0.43, P = 0.0177) and plasma Zn (r = -0.40, P = 0.0284) in the rainy season were observed in Ca supplemented cows. In the dry season SIM was negatively correlated to plasma Pi in P supplemented cows (r = -0.43, P = 0.0177) and Zn supplemented cows (r = - 0.37, P = 0.0463). Furthermore, SIM was negatively correlated to plasma Ca (r = - 0.42, P = 0.0198) and plasma Zn (r = - 0.64, P = 0.0001) in P supplemented cows during the rainy season.

Plasma aspartate aminotransferase (ASAT)

The means and ranges for plasma aspartate aminotransferase are presented in Fig. 28 and Appendix 40. There was significant variation (P < 0.05) between groups in ASAT at each sampling period except in March 1998 and May 1998. Cows supplemented with Zn tended to have high ASAT in most sampling periods compared to other groups. Positive correlation between ASAT and plasma Ca in the dry season was observed in Ca (r = 0.58, P = 0.0001), P (r = 0.51, P = 0.0001) and Zn (r = 0.32, P = 0.0383) supplemented cows. In the rainy season a positive correlation was observed in Ca supplemented cows only (r = -0.39, P= 0.0319). Furthermore, plasma Zn was positively correlated to ASAT in the dry season in control cows (r = 0.49, P = 0.0009), Ca supplemented cows (r = 0.32, P = 0.0340) and P supplemented cows (r = 0.58, P = 0.0001). Negative correlation between ASAT and plasma Pi was observed in the control cows (r = - 0.55, P = 0.0018), Ca supplemented cows (r = -0.74, P = 0.0001) and Zn supplemented cows (r = - 0.46, P = 0.0107) in the rainy season.

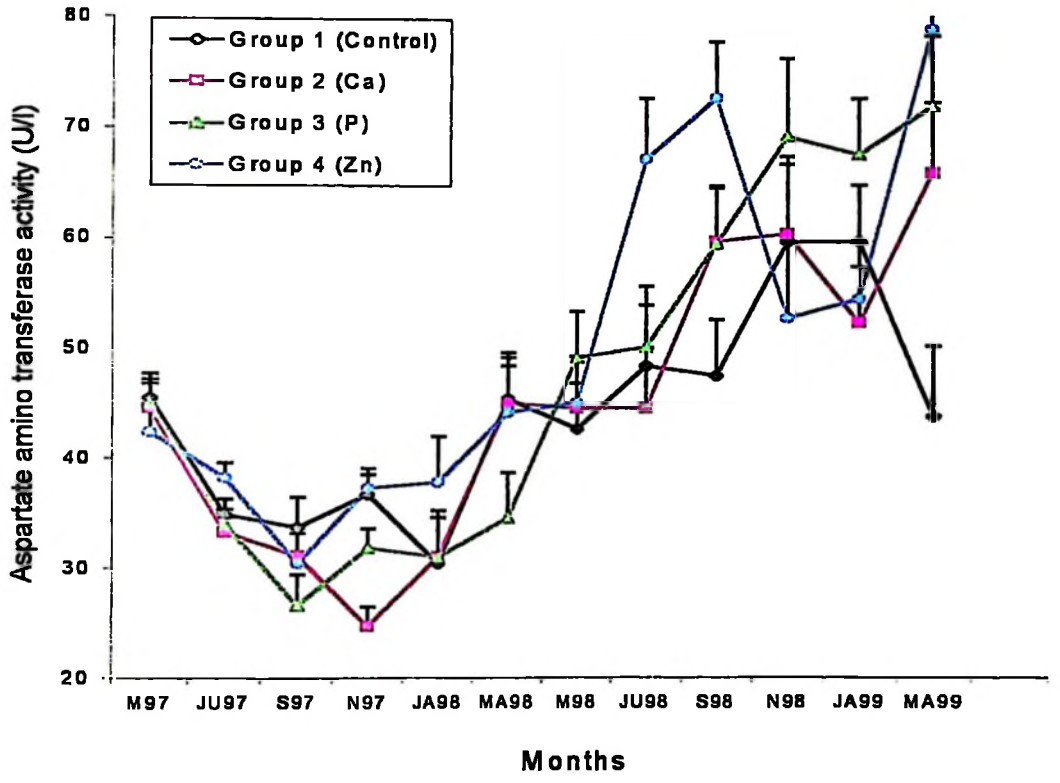


Figure 28 Aspartate aminotransferase activity (IU/l) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania.

Plasma alkaline phosphatase (ALP)

The means and ranges for plasma ALP activity are presented in Fig. 29 and Appendix 44. Mean plasma ALP between groups varied within sampling periods ($P < 0.05$), high plasma ALP was recorded in the control cows. A positive correlation was observed between ALP and plasma Ca in the Ca ($r = 0.47$, $P = 0.0018$), P ($r = 0.68$, $P = 0.0018$) and Zn ($r = 0.58$, $P = 0.0018$) supplemented cows in the dry season. However, ALP was negatively correlated to plasma Ca in the rainy seasons in Ca supplemented cows only ($r = -0.54$, $P = 0.0019$). Positive correlation between ALP and plasma Zn was observed during the rainy season in both the control cows ($r = 0.38$, $P = 0.0388$) and Ca supplemented cows ($r = -0.42$, $P = 0.0206$) whereas a positive correlation was observed during the dry season in the P supplemented cows ($r = 0.42$, $P = 0.0069$). No significant correlation was observed between plasma Pi and ALP in all groups of cows.

4.3.5 Protein and energy metabolism

Plasma total proteins (TP)

The means and ranges for plasma total protein concentration are presented in Fig. 30 and Appendix 45. There was no significant variation between groups of cows ($P > 0.05$) in TP concentration except for November 1997, May 1998, November 1998, January 1999 and March 1999. Plasma TP was high in the Ca supplemented cows in November 1997, November 1998 and January 1999 whereas TP was high in P supplemented and control cows in May 1998 and March 1999. Negative correlation was observed between TP and plasma Ca in the dry season in the control group only ($r = -0.35$, $P = 0.0249$).

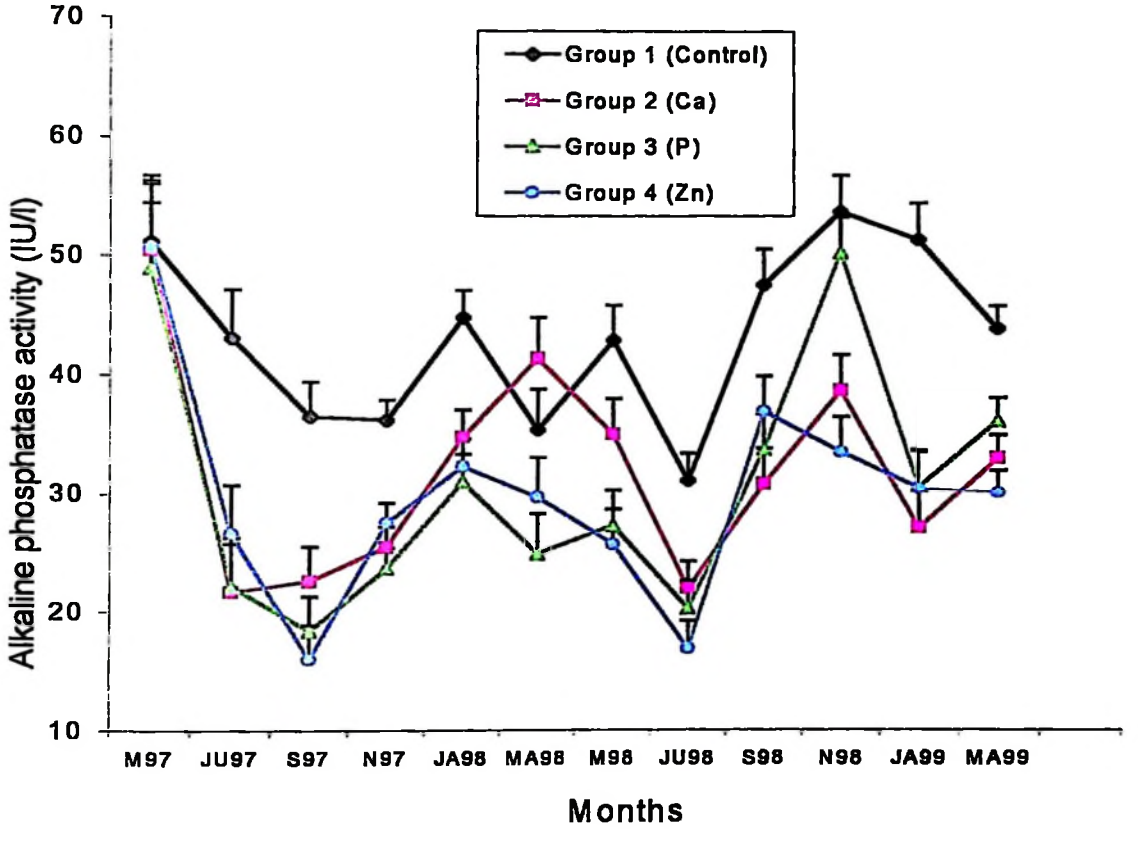


Figure 29 Plasma alkaline phosphatase activity (IU/l) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania.

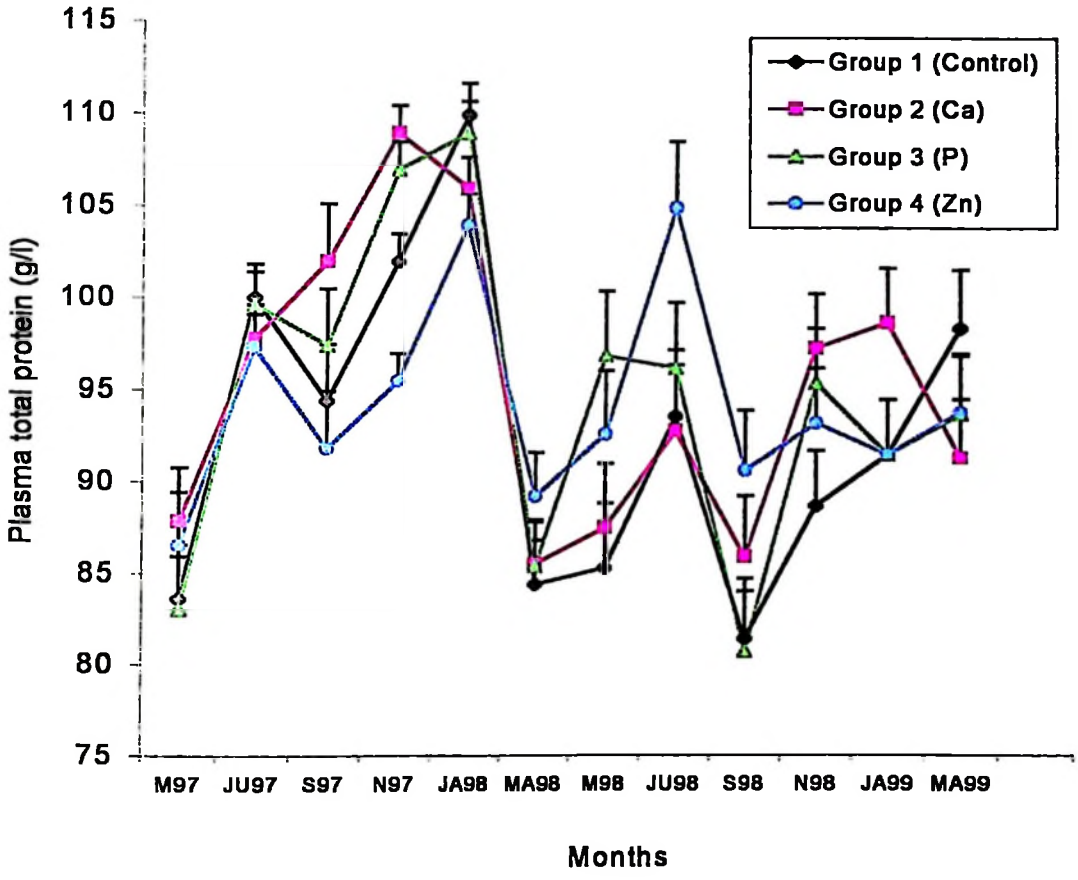


Figure 30 Plasma total protein concentration (g/l) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania.

Total protein was positively correlated to plasma Ca in both the control (r = 0.38, P = 0.0376), Ca (r = 0.80, P = 0.0001) and P supplemented cows (r = 0.56, P = 0.0012) in the rainy season. In addition negative correlation between TP and plasma Pi was observed in the rainy season in Ca (r = - 0.47, P = 0.0218) and P (r = - 0.43, P = 0.0164) supplemented cows. No correlation was observed between TP and plasma Ca, Pi or Zn in the Zn supplemented cows (Appendix.41, 42 and 43).

Plasma urea (Plu)

The means and ranges for plasma urea concentration are presented in Fig.31 and Appendix 46. Significant variation (P < 0.05) in Plu concentration was observed between groups. Cows supplemented with P tended to have high Plu compared to other groups. Plasma urea was negatively correlated to plasma Ca in the Ca (r = - 0.40, P = 0.0087), P (r = - 0.36, P = 0.0176) and Zn (r = - 0.48, P = 0.0013) supplemented cows during the dry season. Furthermore, plasma urea was positively correlated to plasma Pi in the control (r = 0.45, P = 0.0131) and Ca supplemented cows (r = 0.41, P = 0.0248) during the rainy season. No significant correlation (P> 0.05) was observed between Plu and plasma Zn in all groups (Appendix 41, 42 and 43).

Plasma glucose

The means and ranges for plasma glucose concentrations are presented in Fig. 32 and Appendix 47. A significant variation between the groups (P < 0.05) on plasma Glu concentration was observed at each sampling period except in May 1997,

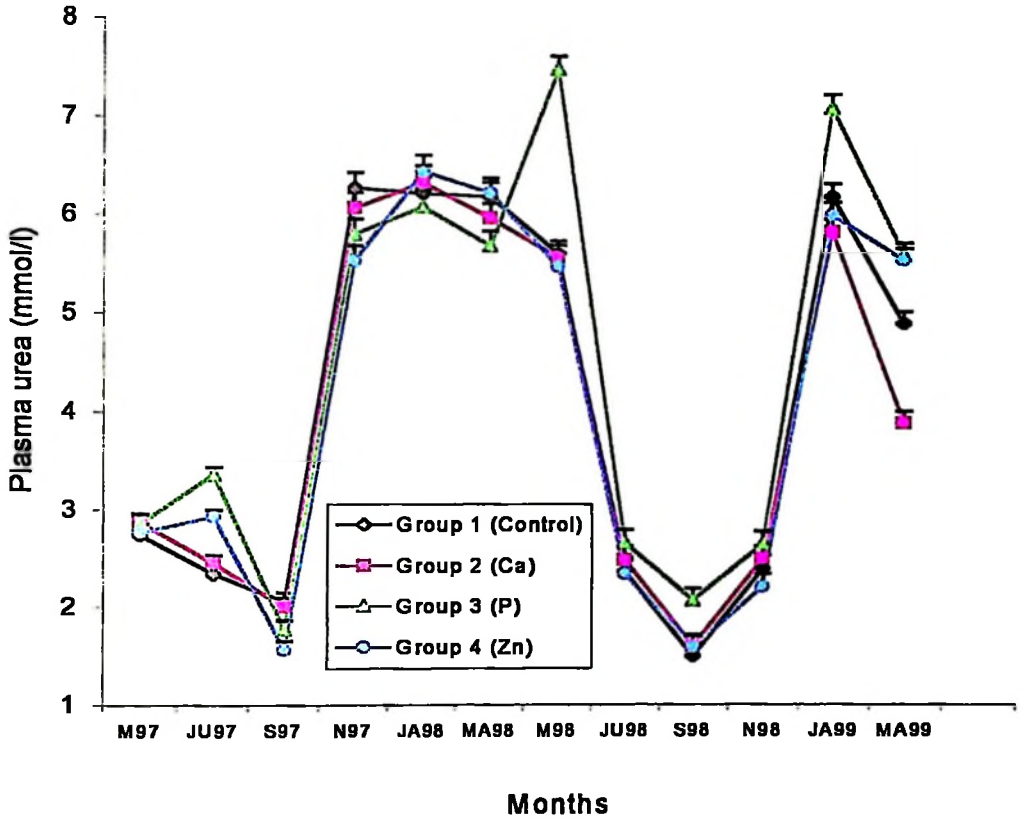


Figure 31 Plasma urea concentration (mmol/l) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania.

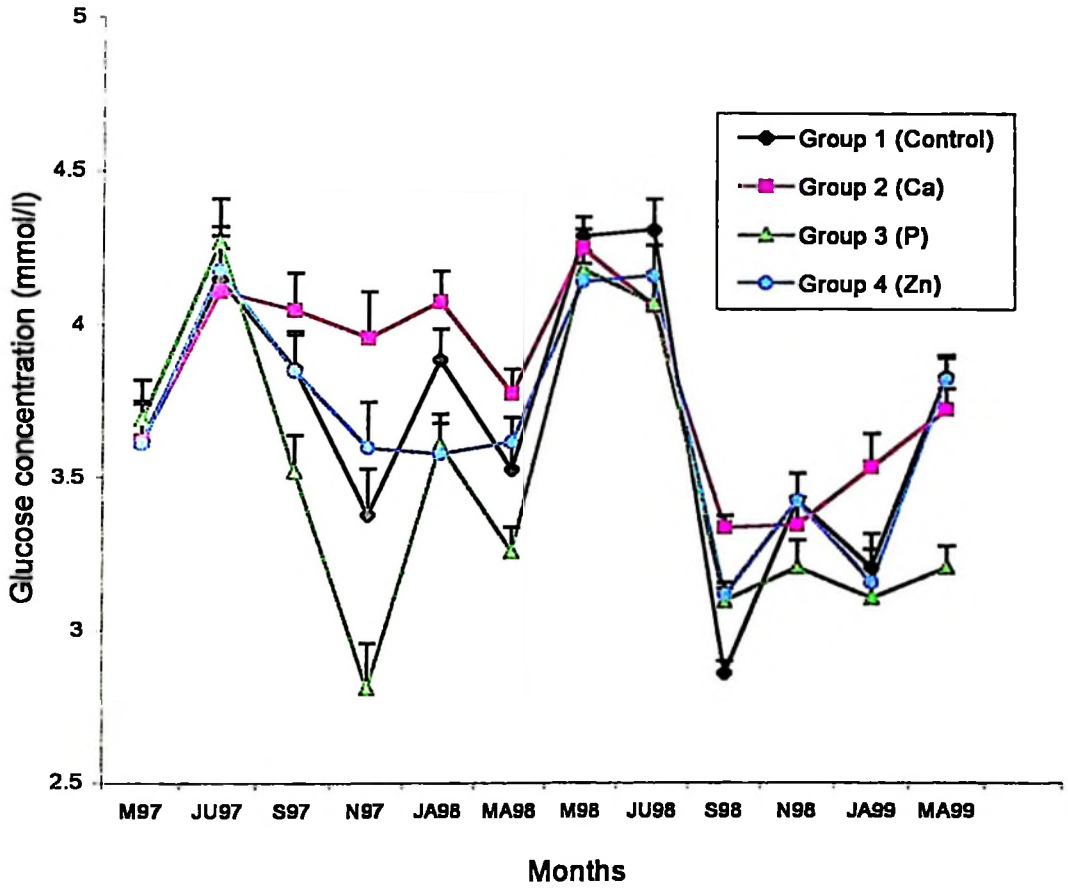


Figure 32 Plasma glucose concentration (mmol/l) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania.

July 1997 and November 1998. Cows receiving Ca had high Glu compared to other groups of cows in most of the sampling periods. Plasma glucose was negatively correlated to plasma Ca in the Ca ($r = -0.76$, $P = 0.0001$), P ($r = -0.70$, $P = 0.0001$) and Zn ($r = -0.82$, $P = 0.0001$) supplemented cows in the dry season but the relation was weak in the control cows ($r = -0.29$, $P = 0.0619$). Furthermore, plasma Glu was positively correlated to plasma Pi ($r = 0.44$, $P = 0.0146$) and plasma Zn ($r = 0.47$, $P = 0.0086$) in Ca supplemented cows during the rainy season whereas Glu was negatively correlated to plasma Zn in the P supplemented cows during the dry season ($r = -0.46$, $P = 0.0024$).

4.4 EFFECT OF CALCIUM, PHOSPHORUS AND ZINC SUPPLEMENTATION ON MILK PRODUCTION

4.4.1 Milk yield

Means milk yield per lactation in litres and lactation days are presented in Table 12 while mean and ranges of monthly milk yield are presented in Appendix 48. Significant variation ($P < 0.05$) on milk yield was observed between groups. High milk yield was recorded in the Ca and Zn supplemented cows compared to the control and P supplemented cows. There was no significant differences in milk yield per lactation in the first year 1997/98 but a significant variation ($P< 0.05$) in milk yield was observed in the second year 1998/99. Milk yield was negatively correlated to plasma Ca in the Ca ($r = -0.36$, $P = 0.0191$) and P ($r = -0.35$, $P = 0.0302$) supplemented cows only. No correlation was observed between milk yield and plasma Pi and Zn in all the groups.

Table 12 Milk yield per lactation and length of lactation for experimental cows at ASAS Dairy Farm, Iringa Tanzania. (Means with the same superscript letter within the same column and parameter do not differ significantly at $P > 0.05$, () = range).

Groups	n	Total milk yield per lactation			Lactation days		
		Year 1	Year 2	Mean	Year 1	Year 2	Mean
Group 1 (Co)	6	2555 ± 153^{a} (1978 – 3774)	2108 ± 290^{c} (1532 – 2880)	2332 ± 164^{b}	291 ± 15^{a} (235 – 378)	241 ± 28^{a} (223 – 322)	267 ± 16^{a}
Group 2 (Ca)	6	2589 ± 153^{a} (2122 – 3020)	3126 ± 290^{a} (2636 – 3429)	2857 ± 164^{a}	268 ± 15^{a} (219 – 302)	269 ± 28^{a} (250 – 308)	268 ± 16^{a}
Group 3 (P)	6	2390 ± 153^{a} (1792 – 3460)	2353 ± 290^{b} (2123 – 3273)	2372 ± 164^{b}	264 ± 15^{a} (189 – 346)	242 ± 28^{a} (189 – 319)	252 ± 16^{a}
Group 4 (Zn)	6	2766 ± 153^{a} (2172 – 3194)	2906 ± 290^{b} (2172 – 4052)	2836 ± 164^{a}	298 ± 15^{a} (273– 360)	298 ± 28^{a} (236– 526)	298 ± 16^{a}
Group 5 (Ca/P)	6	2660 ± 153^{a} (2218 - 3104)	2517 ± 290^{b} (1671 - 3876)	2589 ± 164^{b}	281 ± 15^{a} (265 - 303)	233 ± 28^{a} (159 - 359)	257 ± 16^{a}
Group 6 (Ca/Zn)	6	2632 ± 153^{a} (2180 – 2968)	2621 ± 290^{b} (1884 – 3580)	2627 ± 164^{b}	306 ± 15^{a} (292 – 324)	269 ± 28^{a} (189 – 375)	287 ± 16^{a}
Group 7 (P/Zn)	6	2618 ± 191^{a} (2318 – 2880)	2691 ± 290^{b} (2164 – 3321)	2654 ± 164^{b}	298 ± 15^{a} (257 – 337)	284 ± 28^{a} (248 – 326)	291 ± 16^{a}
Group 8 (Ca/P/Zn)	6	2534 ± 153^{a} (2210 – 2942)	2486 ± 290^{b} (1240 – 3404)	2511 ± 164^{b}	289 ± 15^{a} (223 – 332)	258 ± 28^{a} (102 – 327)	273 ± 16^{a}

4.4.2 Milk Fat (MF)

The means and ranges for percentage milk fat (MF) are presented in Table 13. Mean percentage milk fat between groups varied within sampling periods ($P < 0.05$) except for July 1997, July 1998, September 1998, November 1998 and March 1999. Calcium supplemented cows tended to have high MF compared to other groups of cows. Milk butterfat was positively correlated to plasma Ca in the rainy season in both the Ca supplemented cows ($r = 0.80$, $P = 0.0004$) and the control cows ($r = 0.65$, $P = 0.0048$). Furthermore, MF was positively correlated to plasma Zn in the control ($r = 0.36$, $P = 0.0376$) and P supplemented cows ($r = 0.56$, $P = 0.0230$) but negatively correlated in the Zn supplemented cows ($r = -0.66$, $P = 0.0056$) during the rainy season.

4.4.3 Milk Protein (MP)

The means and ranges for percentage milk protein (MP) are presented in Table 14. Mean percentage MP between the groups did not vary within sampling periods ($P > 0.05$) except for July 1997, September 1997, March 1998 and May 1998. Ca supplemented had high MP in July 1997 and September, 1997 when compared to the other groups of cows. Milk protein was positively correlated to plasma Ca in the rainy season in the control cows only ($r = 0.64$, $P = 0.0057$).

Table 13 Milk fat (%) in the milk from the experimental cows at ASAS Dairy Farm Iringa Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at $P > 0.05$, () = range).

Months	n	Group 1 (Control)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	P value
May 1997	6	4.80 ± 0.24^a (4.40 – 5.0)	4.82 ± 0.24^a (4.0 –5.50)	4.78 ± 0.24^a (3.60 – 5.50)	4.90 ± 0.24^a (4.40 –5.50)	0.8202
July 1997	6	5.56 ± 0.36^a (4.90 – 6.50)	5.52 ± 0.36^a (4.60 – 6.70)	5.14 ± 0.34^a (4.10 – 6.00)	4.37 ± 0.34^b (3.00 – 6.00)	0.0238
September 1997	5	5.42 ± 0.26^a (4.50 – 6.20)	5.10 ± 0.26^a (4.80 – 6.00)	5.18 ± 0.30^a (4.10 – 6.20)	4.92 ± 0.26^a (4.20 – 5.80)	0.0590
November 1997	3	5.05 ± 0.36^a (4.50 – 5.70)	4.90 ± 0.36^a (4.80 – 6.00)	4.18 ± 0.36^b (4.10 – 5.20)	4.92 ± 0.36^a (4.20 – 5.30)	0.0311
March 1998	6	3.60 ± 0.35^b (3.50 – 3.90)	4.15 ± 0.28^a (3.80 – 4.70)	3.85 ± 0.28^b (3.00 – 4.40)	4.22 ± 0.31^a (3.70 – 4.90)	0.0047
May 1998	6	3.48 ± 0.27^b (3.00 – 3.90)	4.02 ± 0.22^a (2.90 – 5.40)	3.78 ± 0.22^b (3.00 – 4.40)	4.26 ± 0.23^a (3.80 – 4.70)	0.0001
July 1998	6	4.58 ± 0.28^a (4.20 – 5.10)	4.43 ± 0.23^a (3.80 – 5.20)	4.72 ± 0.25^a (4.00 – 6.00)	4.22 ± 0.23^a (3.50 – 5.50)	0.2797
September 1998	6	5.08 ± 0.30^a (4.60 – 5.90)	4.63 ± 0.27^a (4.30 – 5.50)	4.88 ± 0.30^a (4.10 – 6.30)	4.40 ± 0.27^a (3.50 – 5.50)	0.2236
November 1998	6	4.54 ± 0.26^a (3.70 – 5.20)	5.17 ± 0.24^a (4.60 – 5.80)	4.60 ± 0.33^a (3.60 – 5.20)	4.43 ± 0.29^b (3.60 – 5.00)	0.0873
March 1999	6	5.00 ± 0.42^a (4.90 – 5.10)	4.30 ± 0.26^a (3.60 – 4.80)	4.54 ± 0.26^a (4.10 – 5.10)	4.90 ± 0.29^a (3.90 – 5.50)	0.1494

Table 14 Milk protein (%) in the milk from experimental cows at ASAS Dairy Farm Iringa, Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at P > 0.05, () = range).

Months	N	Group 1 (Con)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	P value
May 1997	6	3.61 ± 0.12^{a} (3.43 – 3.89)	3.59 ± 0.12^{a} (3.21 –3.88)	3.75 ± 0.12^{a} (3.35 – 3.96)	3.58 ± 0.12^{a} (3.17 –3.88)	0.9202
July 1997	6	3.50 ± 0.14^{b} (3.35 – 3.61)	3.87 ± 0.13^{a} (3.70 – 4.12)	3.73 ± 0.14^{a} (3.30 – 4.05)	3.48 ± 0.13^{b} (3.26 – 4.00)	0.0138
September 1997	5	3.41 ± 0.12^{a} (3.20 – 3.67)	3.74 ± 0.12^{a} (3.63 – 3.94)	3.18 ± 0.13^{b} (2.79 – 3.64)	3.49 ± 0.12^{b} (3.00 – 3.83)	0.0020
November 1997	3	3.69 ± 0.17^{a} (3.24 – 4.12)	3.19 ± 0.30^{a} (3.16 – 3.70)	3.37 ± 0.21^{a} (3.33 – 3.41)	3.43 ± 0.17^{a} (3.08 – 3.64)	0.6811
March 1998	6	2.65 ± 0.15^{c} (2.49 – 2.81)	3.12 ± 0.12^{a} (2.74 – 3.46)	3.15 ± 0.12^{a} (2.43 – 3.79)	2.99 ± 0.13^{b} (2.75 – 3.33)	0.0047
May 1998	6	2.54 ± 0.12^{c} (2.20 – 2.86)	3.06 ± 0.10^{b} (2.91 – 3.20)	3.33 ± 0.10^{a} (3.15 – 3.60)	2.81 ± 0.11^{c} (2.70 – 2.98)	0.0001
July 1998	6	3.27 ± 0.13^{a} (3.08 – 3.61)	3.27 ± 0.11^{a} (2.98 – 3.78)	3.16 ± 0.12^{a} (2.90 – 3.59)	3.28 ± 0.11^{a} (2.92 – 3.68)	0.2797
September 1998	6	3.05 ± 0.14^{a} (2.65 – 3.34)	3.44 ± 0.13^{a} (3.23 – 3.89)	3.20 ± 0.14^{a} (2.81 – 3.61)	3.37 ± 0.13^{a} (3.12 – 3.90)	0.2236
November 1998	6	3.37 ± 0.16^{a} (2.98 – 3.92)	3.69 ± 0.14^{a} (3.11 – 4.00)	3.26 ± 0.20^{a} (3.08 – 3.59)	3.11 ± 0.18^{a} (2.69 – 3.43)	0.0873
March 1999	6	3.30 ± 0.24^{a} (2.92 – 3.68)	3.52 ± 0.15^{a} (3.12 – 3.88)	3.31 ± 0.29^{a} (3.00 – 3.70)	3.52 ± 0.29^{a} (2.96 – 3.95)	0.1494

4.5 EFFECT OF CALCIUM, PHOSPHORUS AND ZINC SUPPLEMENTATION ON REPRODUCTION PERFORMANCE

Interval between calving and resumption of oestrous cycle (PRO), interval between calving and conception (PCO), calving intervals (CAI) and number of services per conception (SEC) are presented in Table 15. Cows supplemented with Ca or P had short PRO and PCO and CAI than control cows. Cows supplemented with Ca and P needed an average of 2 service before conception as compared to an average of 3 service in the other group of cows.

4.6 EFFECT OF CALCIUM, PHOSPHORUS AND ZINC SUPPLEMENTATION ON COPPER AND SELENIUM BALANCE

4.6.1 Ceruloplasmin activity (Cp)

The means and ranges of plasma ceruloplasmin activity are presented in Fig. 33 and Appendix 51. Significant variations ($P < 0.001$) on plasma Cp activity was observed between the groups except for May 1997 and September 1997. Cows supplemented with Ca had low plasma ceruloplasmin in January 1998 and March 1998 compared to other groups of cows whereas cows supplemented with P had low plasma ceruloplasmin activity in July1998 and March 1999. In the dry season plasma Cp was positively correlated to plasma Pi ($r = 0.41$, $P < 0.0068$) and plasma Zn ($r = 0.42$, $P < 0.0053$) in the Ca supplemented cows. Furthermore, Cp was negatively correlated to plasma Pi ($r = -0.47$, $P < 0.0090$) and plasma Zn ($r = -0.66$, $P < 0.0001$)

Table 15 Reproductive fertility indices in the experimental cows at ASAS Dairy Farm, Iringa, Tanzania. (Means with the same superscript letter within column do not differ significantly at $P > 0.05$).

Group	Calving intervals (Days)	Calving to first oestrus (Days)	Calving to conception (Days)	Number of service per conception
Group 1 (Control)	393 ± 21^{a}	69 ± 10^{a}	112 ± 19^{a}	3
Group 2 (Ca)	364 ± 21^{a}	30 ± 10^{b}	75 ± 19^{a}	2
Group 3 (P)	393 ± 21^{a}	45 ± 10^{b}	76 ± 19^{a}	2
Group 4 (Zn)	386 ± 21^{a}	53 ± 10^{a}	98 ± 19^{a}	3
Group 5 (Ca/P)	379 ± 21^{a}	43 ± 10^{b}	90 ± 19^{a}	3
Group 6 (Ca/Zn)	380 ± 21^{a}	43 ± 10^{b}	96 ± 19^{a}	3
Group 7 (P/Zn)	395 ± 21^{a}	48 ± 10^{a}	111 ± 19^{a}	3
Group 8 (Ca/P/Zn)	386 ± 21^{a}	43 ± 10^{b}	98 ± 19^{a}	3

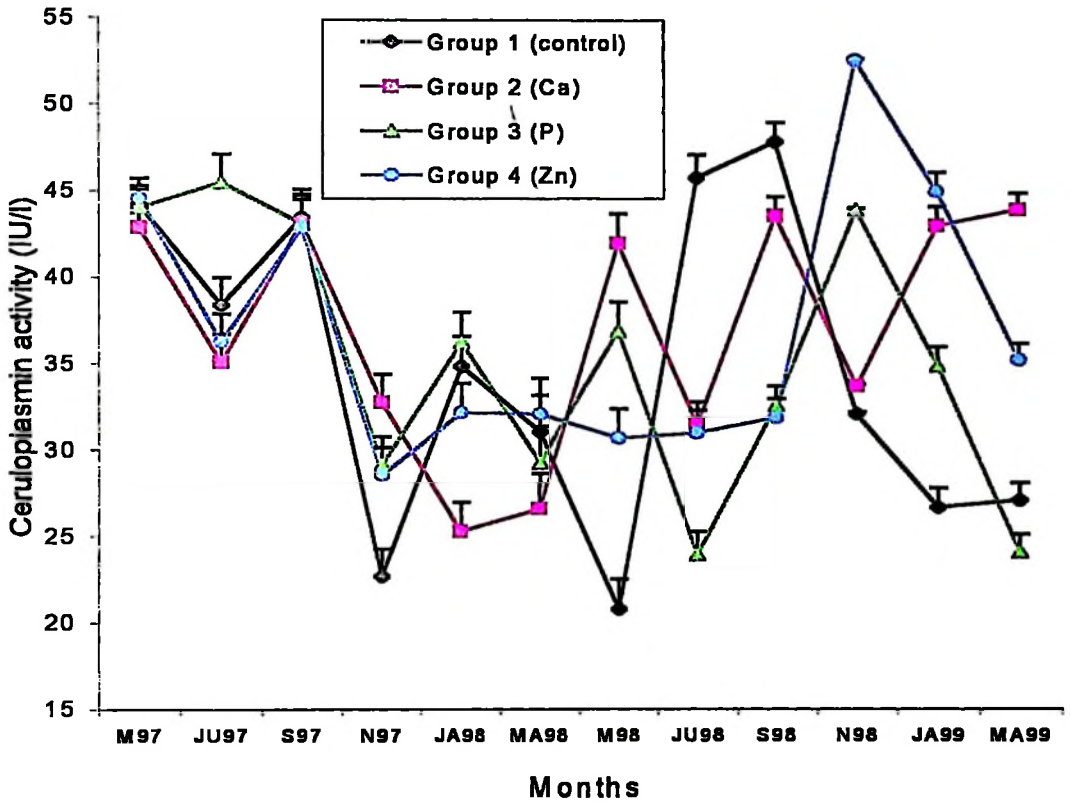


Figure 33 Ceruloplasmin activity (IU/l) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania.

in the same group during the rainy season. Plasma Cp was not correlated to plasma Ca or Pi or Zn in the control, P or Zn supplemented cows (Appendix 41, 42, 43).

4.62 Glutathione peroxidase activity (GSH.Px)

The means and ranges of whole blood GSH.PX activity are presented in Table 16. Significant variation ($P < 0.05$) on the mean GSH.Px activity was observed between the groups in January 1998. Calcium supplemented cows had lower GSH.Px activity (117 IU/l) compared to the control cows (164 IU/l), P (163 IU/l) and Zn (147 IU/l) supplemented cows.

4.7 CALCIUM, PHOSPHORUS AND ZINC INTERACTIONS

4.7.1 Effect of Ca, P and Zn interaction on plasma calcium, inorganic phosphate and zinc

Plasma total calcium

The means and ranges of plasma Ca concentration are presented in Figure 34 and Appendix 18. No significant variation ($P > 0.05$) on plasma Ca was observed between the groups during sampling periods. Season had effect on plasma Ca ($P < 0.001$). High levels of plasma Ca was recorded during the wet season however the last months of the dry season (September and November 1998) had also high plasma Ca. Plasma Ca was positively correlated to plasma Pi ($r = 0.66$, $P = 0.0001$) and Plasma Zn ($r = 0.81$, $P = 0.0001$) during the wet season in the P/Zn supplemented cows. A positive correlation of plasma Ca and Zn was also observed in Ca/P/Zn supplemented cows at the same period ($r = 0.77$, $P = 0.0001$).

Table 16 Whole blood glutathione peroxidase activity (IU/l) in the experimental cows at ASAS Dairy Farm, Iringa, Tanzania. (Means with the same superscript letter within column do not differ significantly at $P > 0.05$).

Group	1997 May	1997 September	1998 January
Group 1 (Control)	139 ± 8.8^{a} (102 – 163)	179 ± 8.6^{a} (141 – 193)	164 ± 10^{a} (107 – 237)
Group 2 (Ca)	132 ± 8.8^{a} (105 – 165)	169 ± 8.6^{a} (140 – 200)	117 ± 10^{c} (105 – 134)
Group 3 (P)	134 ± 8.8^{a} (115 – 173)	169 ± 8.6^{a} (148 – 195)	163 ± 10^{a} (126 – 189)
Group 4 (Zn)	140 ± 8.8^{a} (117 – 168)	174 ± 8.6^{a} (153 – 187)	147 ± 10^{b} (113 – 168)
Group 5 (Ca/P)	133 ± 8.8^{a} (102 – 169)	168 ± 8.6^{a} (151 – 185)	174 ± 10^{a} (157 – 189)
Group 6 (Ca/Zn)	135 ± 8.8^{a} (115 – 160)	166 ± 8.6^{a} (138 – 192)	165 ± 10^{a} (156 – 187)
Group 7 (P/Zn)	138 ± 8.8^{a} (110 – 170)	128 ± 8.6^{b} (100 – 158)	141 ± 10^{b} (104 – 186)
Group 8 (Ca/P/Zn)	138 ± 8.8^{a} (113 – 169)	159 ± 8.6^{a} (100 – 190)	160 ± 10^{a} (149 – 195)

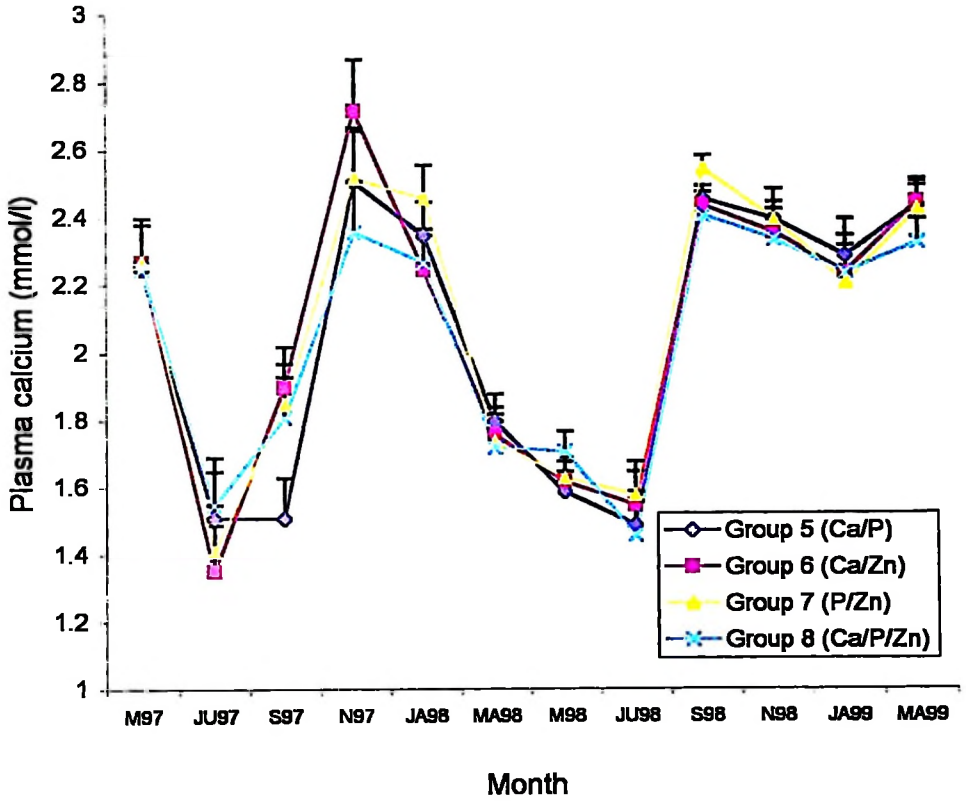


Figure 34 Plasma total calcium concentration (mmol/l) in grazing crossbred Zebu cows supplemented with different mineral combinations at ASAS Dairy Farm, Iringa, Tanzania.

Furthermore plasma Ca was positively correlated to plasma Zn in the dry season in Ca/P supplemented cows (r = 0.35, P =0.0207).

Plasma inorganic phosphate

The means and ranges of plasma Pi concentration are presented in Fig. 35 and Appendix 20. Mean monthly plasma Pi between the groups did not vary between groups within the sampling periods (P > 0.05) except for January 1998, March 1998, July 1998 and March 1999. Significant effect of season (P < 0.001), on Pi was observed. High plasma Pi was recorded during the dry season except for July 1997 and 1998. A positive correlation between plasma Pi and Zn was observed in the P/Zn supplemented cows (r = 0.56, P =0.0012) in the dry season. Plasma Pi was negatively correlated to plasma Zn during the dry season in the Ca/Zn supplemented cows (r = -0.38, P =0.0119).

Plasma zinc concentration

The means and ranges for plasma Zn concentration are presented in Fig. 36 and Appendix 21. Significant variations (P < 0.001) on plasma Zn concentration was observed between the groups except for January 1998, January 1999 and March 1999. Cows receiving a combination of Ca, P and Zn (Group 8) tended to have constant plasma Zn from May 1997 to January 1998 (12.1 – 12.6 μmol Zn /l) whereas Ca/Zn supplemented cows had a constant plasma Zn from November 1997 to May 1998 (12.1 – 12.5 μmol Zn /l). For the rest of the groups plasma Zn fluctuated between low and high values. Significant effect (P< 0.001) of season on plasma Zn was observed. High plasma Zn concentration was recorded in the dry

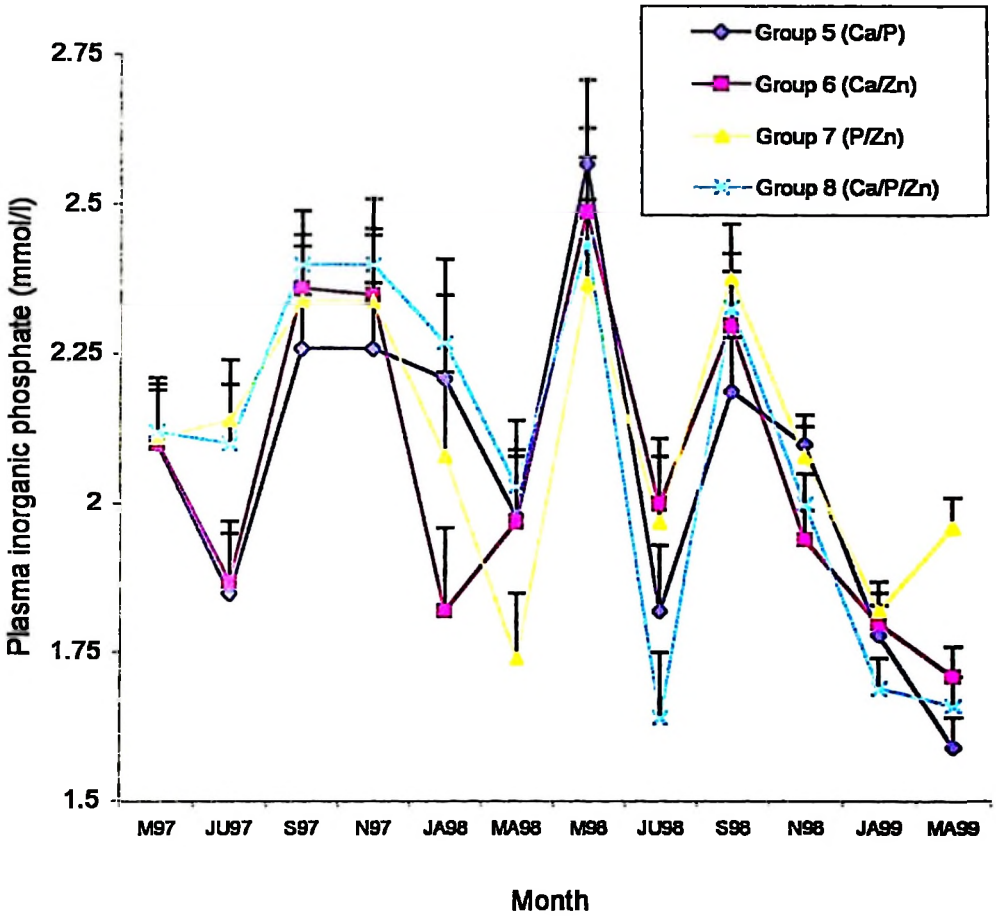


Figure 35 Plasma inorganic phosphate concentration (mmol/l) in grazing crossbred Zebu cows supplemented with different mineral combinations at ASAS Dairy Farm, Iringa, Tanzania.

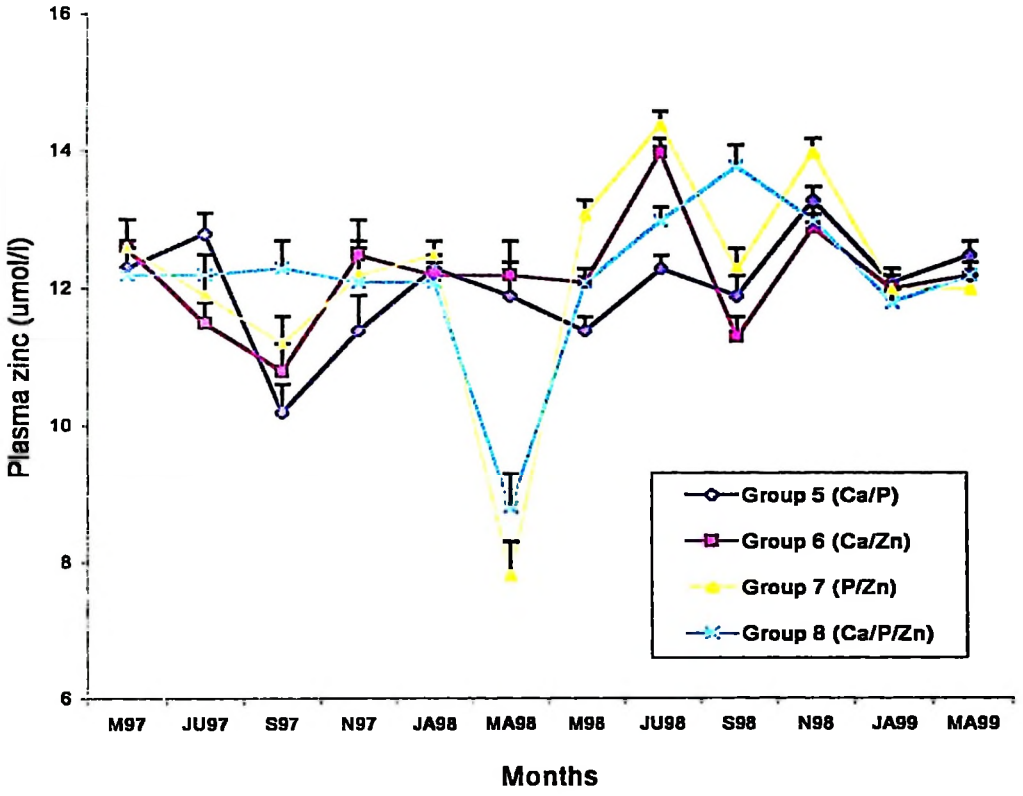


Figure 36 Plasma zinc concentration (mmol/l) in grazing crossbred Zebu cows supplemented with different mineral combinations at ASAS Dairy Farm, Iringa, Tanzania.

season. Plasma Zn was positively correlated to plasma Ca in the Ca/P supplemented cows only (r = 0.35, P =0.0207) during the dry season whereas plasma Zn was positively correlated to plasma Ca in the P/Zn supplemented cows (r = 0.81, P =0.0001) and in the Ca/P/Zn supplemented cows (r = 0.77, P =0.0001) during wet season.

4.7.2 Effect of Ca, P and Zn interaction on health and immune status

Disease conditions in experimental cows

Disease conditions for the study period are presented in Table 17 and Appendices 23 and 24. No differences were observed between occurrence of mastitis. However, cows supplemented with Ca/P and Ca/P/Zn suffered severe cases of suppurative granulomatous mastitis. Anaplasmosis incidences were high in the month of March 1998 and July 1998 (Appendix 24). One incidences of East Coast Fever was observed in one cow supplemented with Ca/P/Zn. In addition four cases of udder parakeratosis were observed in cows supplemented with P/Zn and Ca/P/Zn two case in each in January and February 1998. No cases of milk fever or clinical lameness were observed in all study groups. Furthermore, the number of cases of embryonic death, abortion, stillbirth, retained placenta pyometra and cystic ovaries observed during the study periods are presented in Appendix 25. The abortions were not time specific, however most of the stillbirth cases were recorded in December 1997 and January 1998.

Table 17. Number of cases for different disease conditions observed in cows supplemented with different mineral combinations at ASAS Dairy Farm Iringa, Tanzania.

	CONDITION	GROUP 5 (Ca/P)	GROUP 6 (Ca/Zn)	GROUP 7 (P/Zn)	GROUP 8 (Ca/P/Zn)
1.	Mastitis	15	13	14	14
2.	Anaplasmosis	3	5	2	4
3.	East coast fever	-	-	-	1
4.	Parakeratosis	3	-	2	2
5.	Others				
	Cytic ovaries	1	-	-	-
	Pyometra	1	-	-	-

Hoof health and lameness assessment

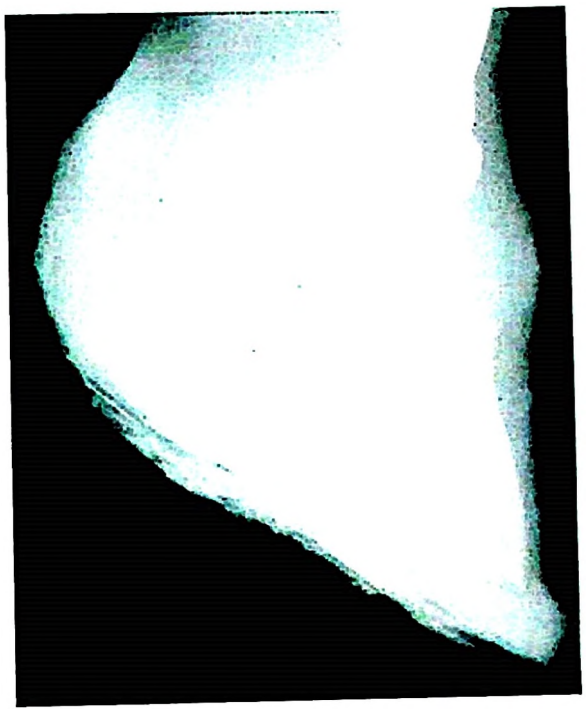
No fractures and abnormal hoof growth which, could lead to clinical lameness were observed during the whole experimental period. No claw abnormalities were observed between groups before and after 18 months of mineral supplementation as indicated in radiological pictures presented in Fig. 37 and 38.

Body weight and body condition scores

The mean and range for body weights are presented in Fig. 39 and Appendix 26. There was no significant statistical differences ($P > 0.05$) in live body weight between groups however there was a tendency towards high live body weight for Ca/P/Zn and P/Zn supplemented cows. No significant correlation was observed between BW and Plasma Ca or Pi or plasma Zn (Appendix 22 and 27).

The mean body condition scores are presented in Fig. 40 and Appendix 28. No significant variation ($P > 0.05$) in BCS was observed between sampling period except in November 1997, March 1998 and March 1999. Severe decrease in body condition score was observed in the Ca/P/Zn and P/Zn supplemented cows in March 1998. The period coincided with calving. Cows in these four groups had low BCS when compared to Ca supplemented cows. Body condition score was positively correlated to plasma Ca in the rainy season in P/Zn ($r = 0.43$, $P = 0.0181$) and Ca/P/Zn ($r = 0.50$, $P = 0.0051$). In addition BCS was positively correlated to plasma Zn in the rainy season in the same groups; P/Zn ($r = 0.59$, $P = 0.0007$) and Ca/P/Zn ($r = 0.54$, $P = 0.0019$) supplemented cows.

(a)



(b)

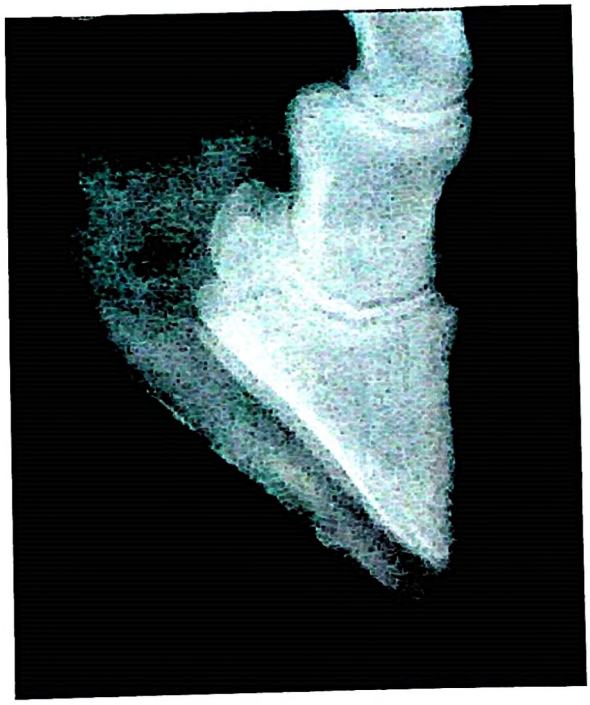


Figure 37 Lateromedial radiographic pictures of digital claws of cow supplemented with Ca/P. Top (before) bottom (after) 18 months of Ca/P supplementation. The bone contours are smooth, the basal surface is slightly concave.

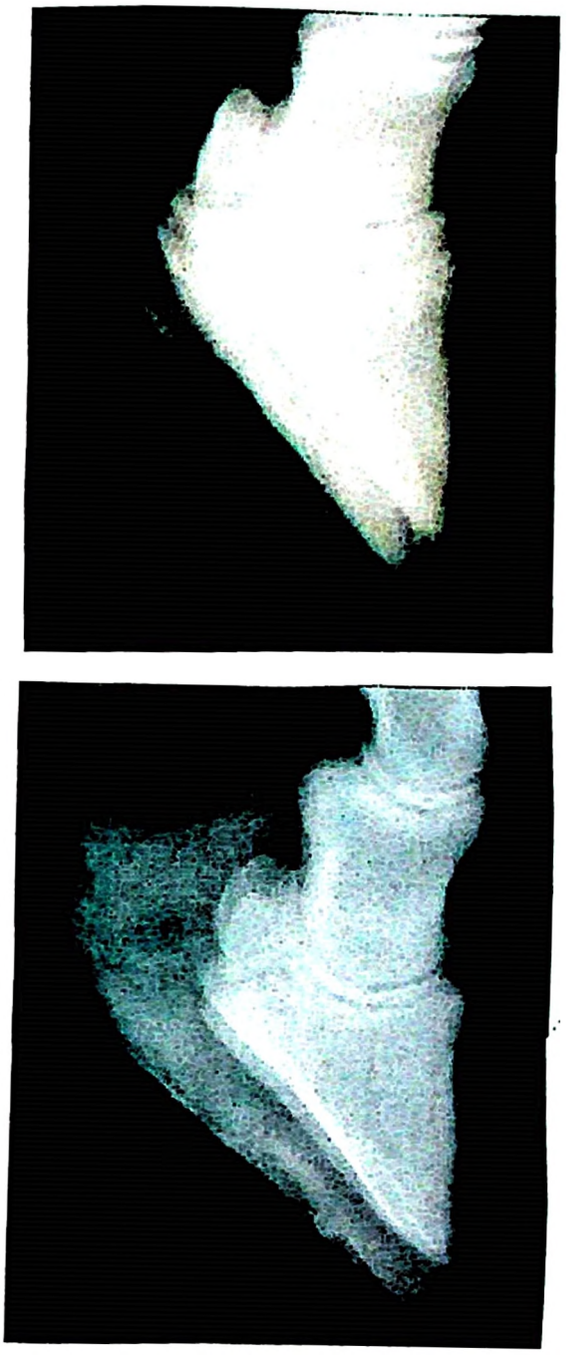


Figure 38 Lateromedial radiographic pictures of digital claws of cows supplemented with Ca/Zn (Top) and P/Zn (bottom) after 18 months of mineral supplementation. The bone contours are smooth, the basal surface is slightly concave.

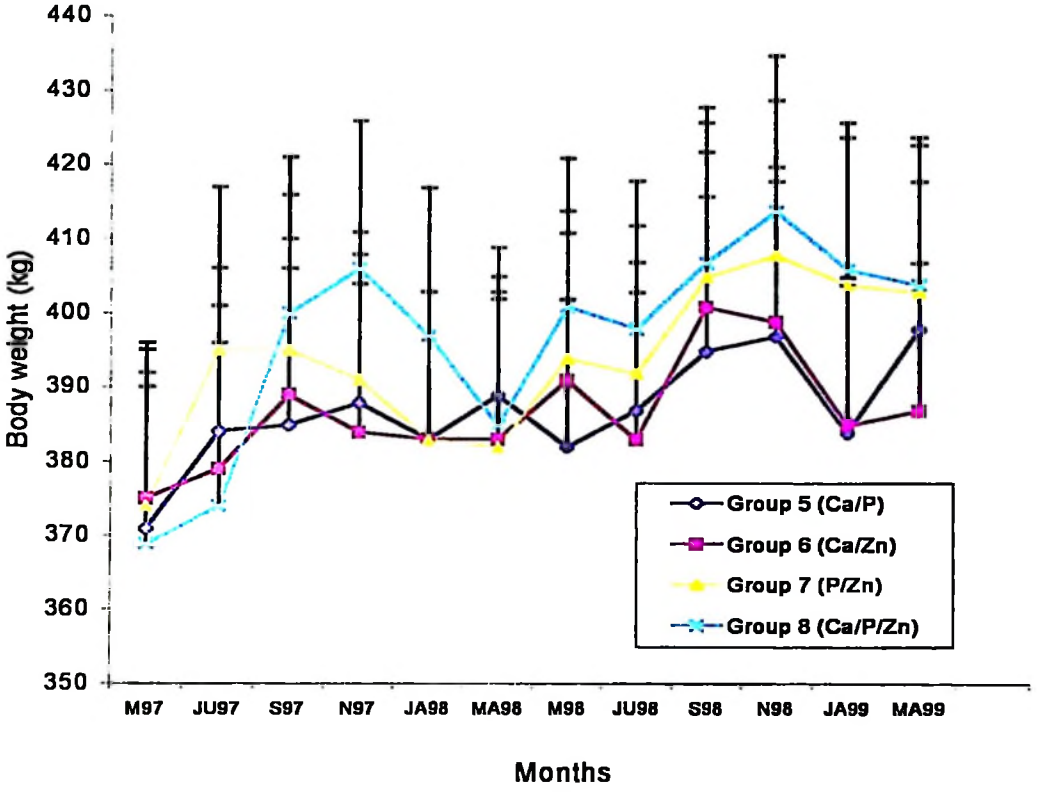


Figure 39 Live body weight (kg) in grazing crossbred Zebu cows supplemented with different mineral combinations at ASAS Dairy Farm, Iringa, Tanzania.

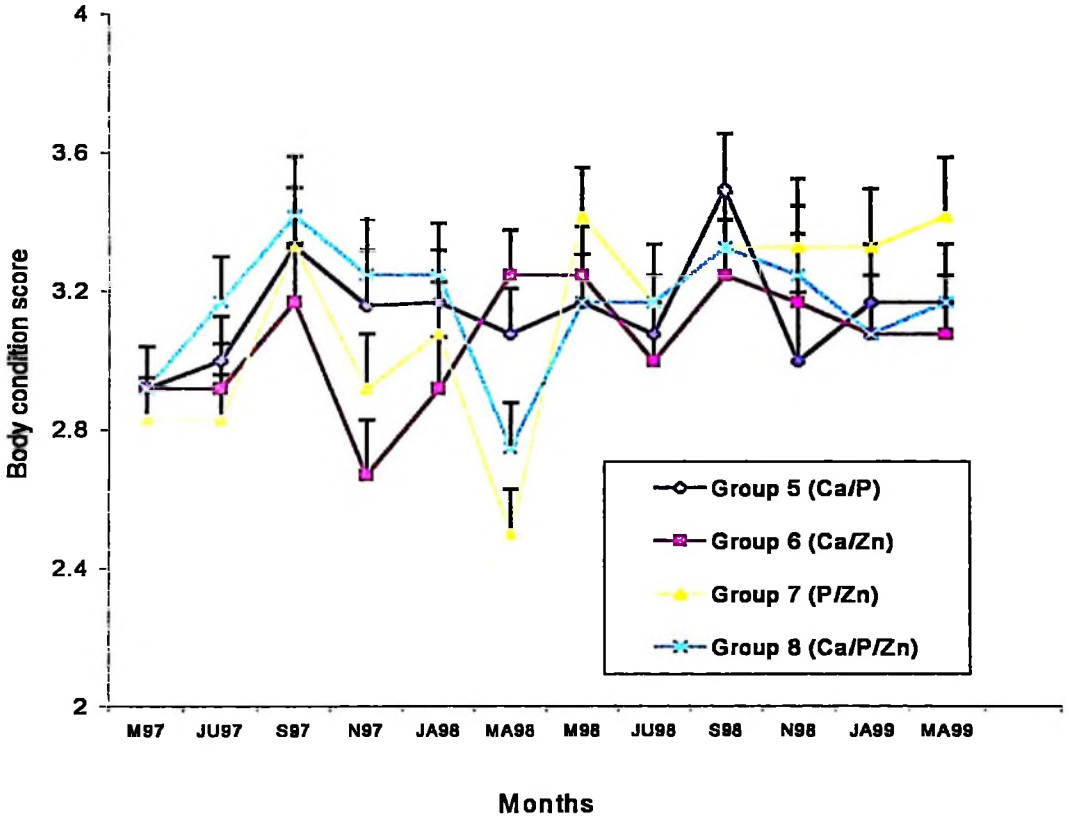


Figure 40 Body condition score in grazing crossbred Zebu cows supplemented with different mineral combinations at ASAS Dairy Farm, Iringa, Tanzania.

Packed cell volume (PCV%)

Means and ranges for packed cell volume percentage are presented in Fig. 41 and Appendix 29. Significant variation ($P < 0.05$) in the PCV% was observed between groups except for September 1997, January 1998, May 1998, November 1998 and March 1999. Cows supplemented with Ca/P tended to have low PCV compared to other groups of cows in most of the sampling periods. No significant correlation ($P > 0.05$) was observed between PCV and plasma Ca, Pi and Zn (Appendix 22 and 27).

Erythrocyte haemoglobin concentration

The means and ranges for haemoglobin concentration are presented in Fig. 42 and Appendix 30. No significant variation ($P > 0.05$) on haemoglobin concentration was observed between groups except in January1998, July 1998, September 1998 and January 1999. The high erythrocyte Hb was observed in cows receiving Ca/Zn whereas cows receiving P/Zn had the lowest Hb concentration in most of sampling periods. Haemoglobin was negatively correlated to Plasma Ca in the dry season in all the study groups; Ca/P ($r = -0.48$, $P = 0.0012$), Ca/Zn ($r = -0.52$, $P = 0.0004$), P/Zn ($r = -0.35$, $P = 0.0243$) and Ca/P/Zn ($r = -0.44$, $P = 0.0039$). Furthermore, Hb was negatively correlated to plasma Zn in the dry season in the P/Zn ($r = -0.44$, $P = 0.0034$) and Ca/P/Zn supplemented cows ($r = -0.32$, $P = 0.0508$).

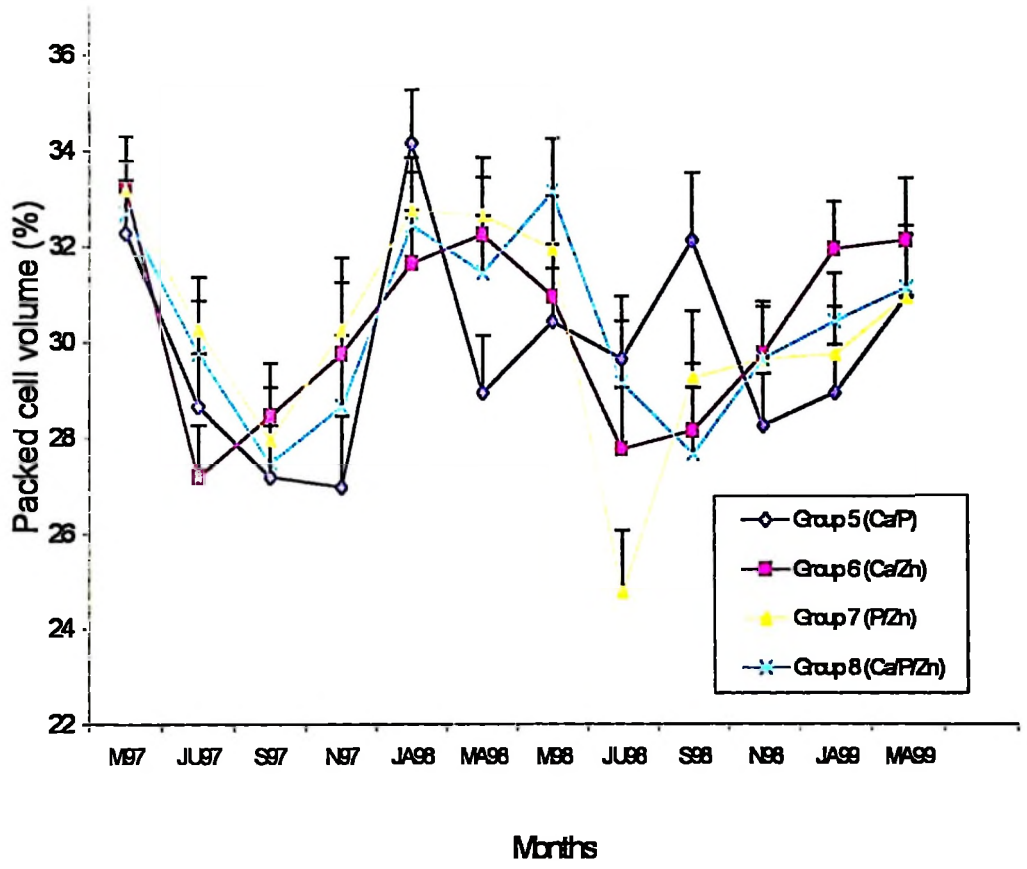


Figure 41 Packed cell volume percentage (%) of blood in grazing crossbred Zebu cows supplemented with different mineral combinations at ASAS Dairy Farm, Iringa, Tanzania.

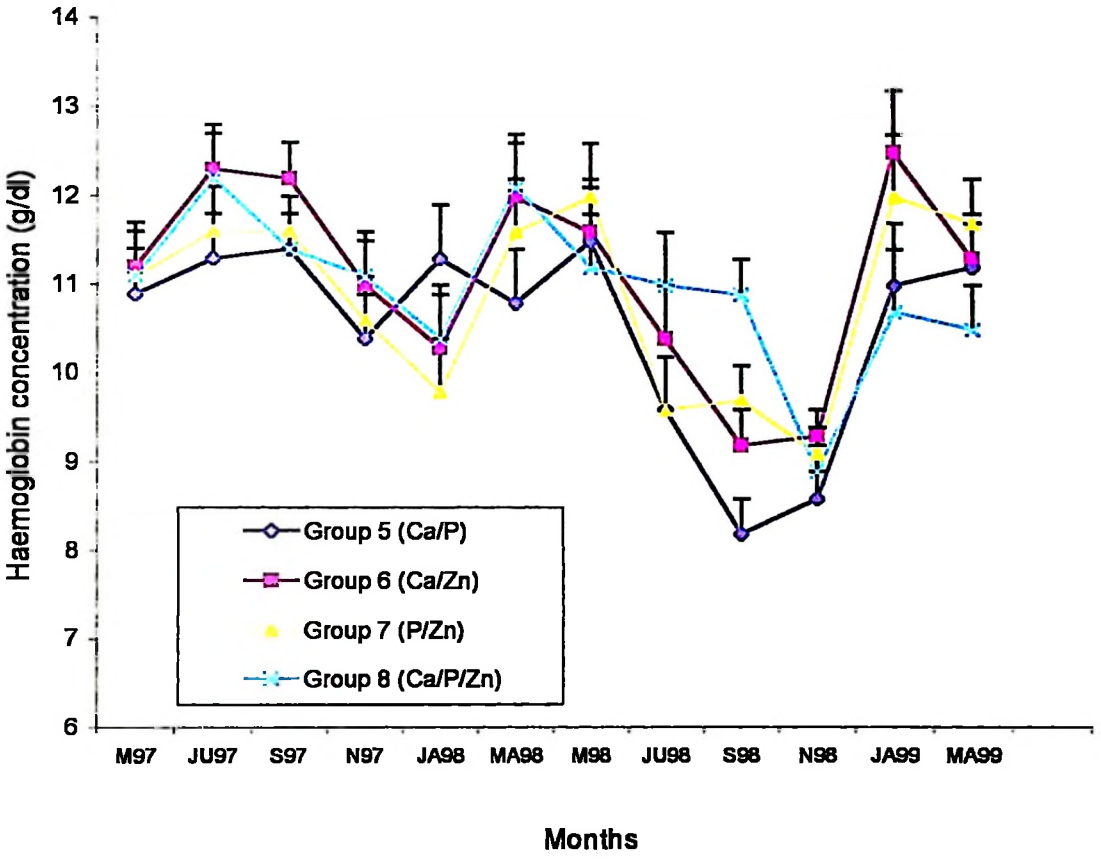


Figure 42 Haemoglobin concentration (g/dl) in grazing crossbred Zebu cows supplemented with different mineral combinations at ASAS Dairy Farm, Iringa, Tanzania.

Total white blood cell counts and differential WBC counts

The means and ranges of TWBC counts are presented in Fig. 43 and Appendix 31. There was no significant variation between the groups ($P > 0.05$) in TWBC at each sampling periods. However, there was a tendency of high TWBC in Ca/P/Zn supplemented cows in most of the sampling periods. Positive correlation between TWBC and plasma Ca was observed in the dry season in Ca/P ($r = 0.53$, $P = 0.0003$), Ca/Zn ($r = 0.44$, $P = 0.0037$) and P/Zn ($r = 0.55$, $P = 0.0002$) supplemented cows. Total white blood cell count was positively correlated to plasma Zn in the rainy season in Ca/P ($r = 0.51$, $P = 0.0043$), Ca/Zn ($r = 0.54$, $P = 0.0019$) and Ca/P/Zn ($r = 0.46$, $P = 0.0109$).

Lymphocyte counts (LC)

The means and ranges of lymphocyte counts are presented in Fig. 44 and Appendix 34. There was no significant variation between the groups ($P > 0.05$) in LC at each sampling period except for March 1998, May 1998 and July 1998. Cows supplemented with Ca/Zn had low LC in March 1998 and July 1997 whereas Ca/P had high LC in May 1998. Positive correlation between LC and plasma Ca was observed in the dry season in Ca/P ($r = 0.48$, $P = 0.0010$), Ca/Zn ($r = 0.54$, $P = 0.0002$) and P/Zn ($r = 0.46$, $P = 0.0020$) supplemented cows. In addition LC was positively correlated to plasma Ca ($r = 0.41$, $P = 0.0231$) and plasma Zn ($r = 0.45$, $P = 0.0119$) in the rainy season in Ca/Zn supplemented cows. A weak positive correlation between plasma Zn and LC was also observed in Ca/P/Zn ($r = 0.36$, $P = 0.0538$) during the same period.

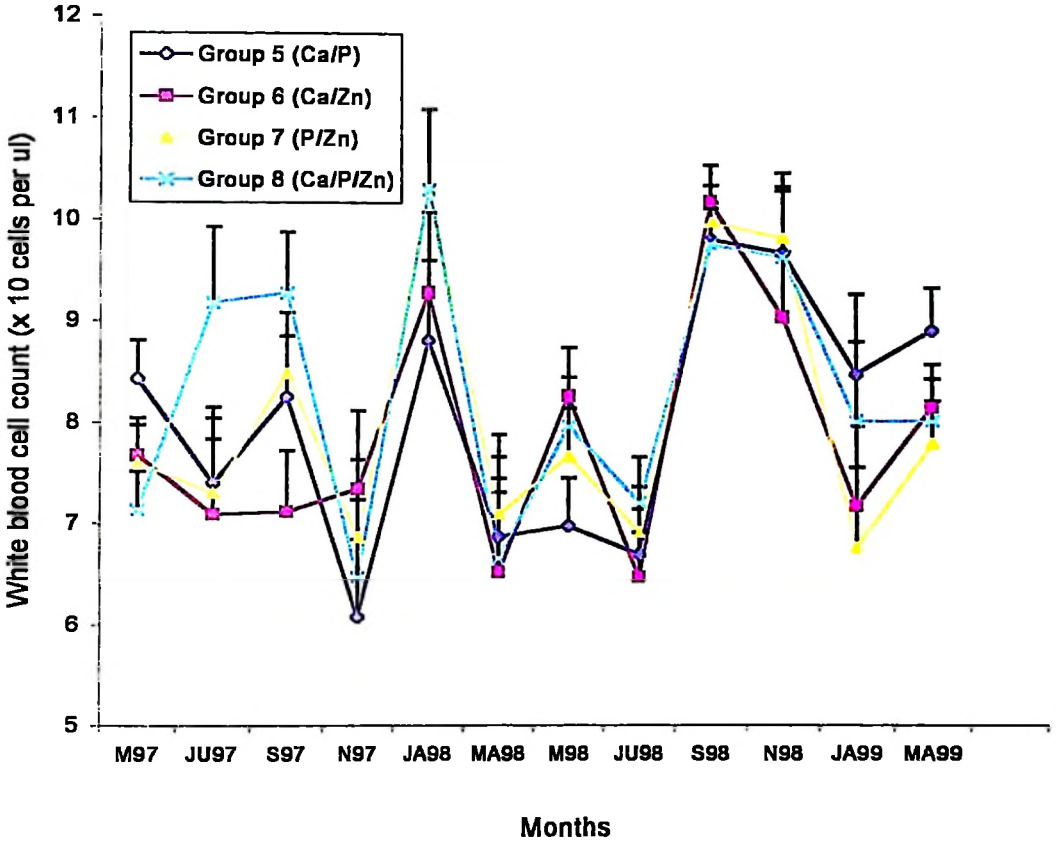


Figure 43 Total white blood cell count (x 10^3 cells per µl of blood) in grazing crossbred Zebu cows supplemented with different mineral combinations at ASAS Dairy Farm, Iringa, Tanzania.

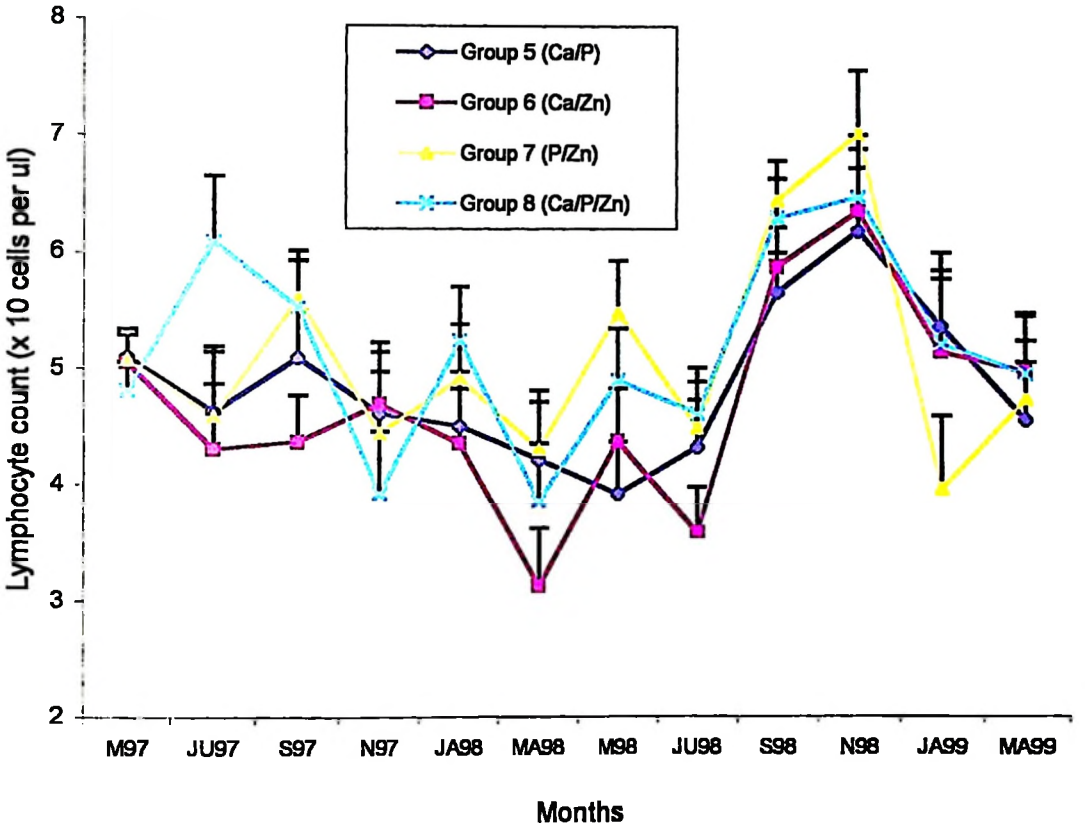


Figure 44 Lymphocyte count (x 10^3 cells per µl of blood) in grazing crossbred Zebu cows supplemented with different mineral combinations at ASAS Dairy Farm, Iringa, Tanzania.

Neutrophil counts (NC)

The means and ranges of neutrophil counts are presented in Fig. 45 and Appendix 36. There was no significant variation between groups ($P > 0.05$) in the NC at each sampling period except for November 1997, May 1998 and March 1999. Positive correlation between NC and plasma Zn was observed in the rainy season in Ca/P ($r = 0.55$, $P = 0.0016$), P/Zn ($r = 0.37$, $P = 0.0450$) and Ca/P/Zn ($r = 0.38$, $P = 0.0380$) supplemented cows. Neutrophil count was positively correlated to plasma Pi in the rainy season in P/Zn ($r = 0.33$, $P = 0.0772$) and Ca/P/Zn ($r = 0.46$, $P = 0.0104$) supplemented cows. Furthermore, NC was positively correlated to plasma Pi in the dry season in Ca/P ($r = 0.40$, $P = 0.0089$) and Ca/P/Zn ($r = 0.35$, $P = 0.0237$) supplemented cows.

Eosinophil counts (EC)

The means and ranges of eosinophil counts are presented in Fig. 46 and Appendix 37. There was no significant variation between groups ($P > 0.05$) in EC at each sampling period except for July 1998 and September 1998. During these periods Ca/Zn supplemented cows had high mean EC. Negative correlation between EC and plasma Zn in P/Zn ($r = -0.41$, $P = 0.0262$) and Ca/P/Zn ($r = -0.35$, $P < 0.0562$) supplemented cows were observed in the rainy season. Furthermore, plasma Pi was negatively correlated to EC in the dry season in Ca/P ($r = -0.37$, $P = 0.0413$), P/Zn ($r = -0.44$, $P = 0.0145$) and Ca/P/Zn ($r = -0.47$, $P = 0.0087$) supplemented cows. Eosinophil counts in P/Zn supplemented cows was further correlated to plasma Ca in the rainy season ($r = -0.45$, $P = 0.0126$) whereas in the dry season EC was negatively correlated to plasma Ca in Ca/P/Zn supplemented cows ($r = -0.31$, $P = 0.0438$).

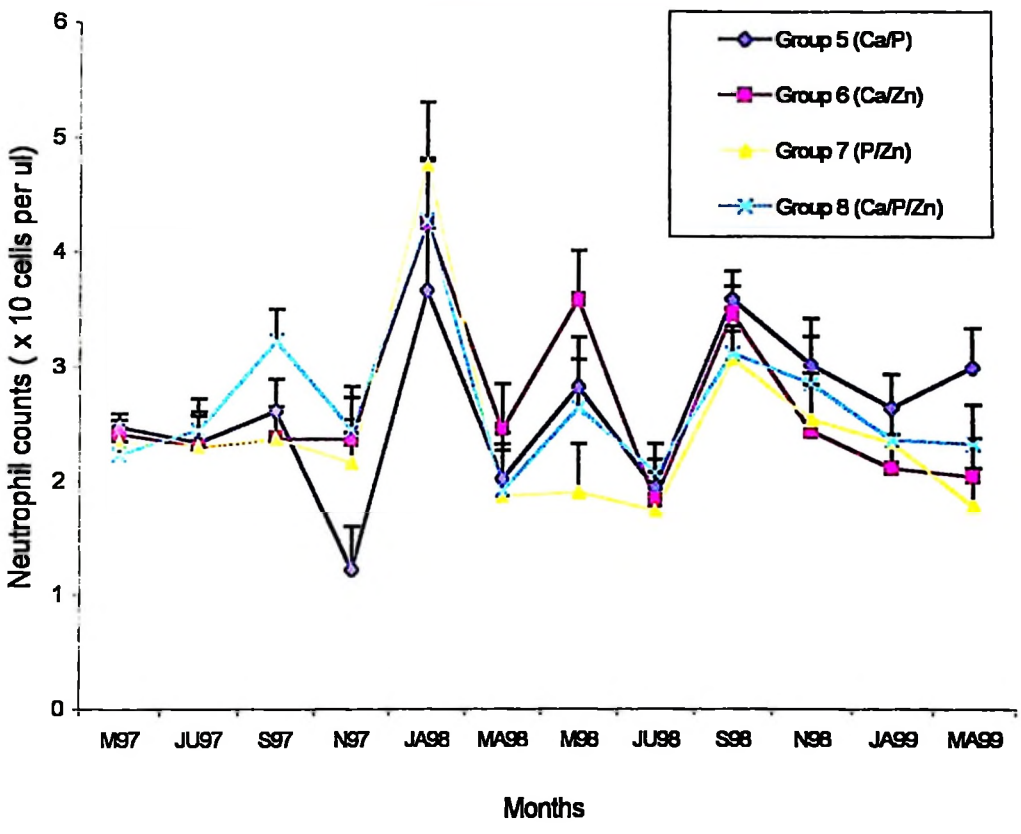


Figure 45 Neutrophil counts (x 10^3 cells per µl of blood) in grazing crossbred Zebu cows supplemented with different mineral combinations at ASAS Dairy Farm, Iringa, Tanzania.

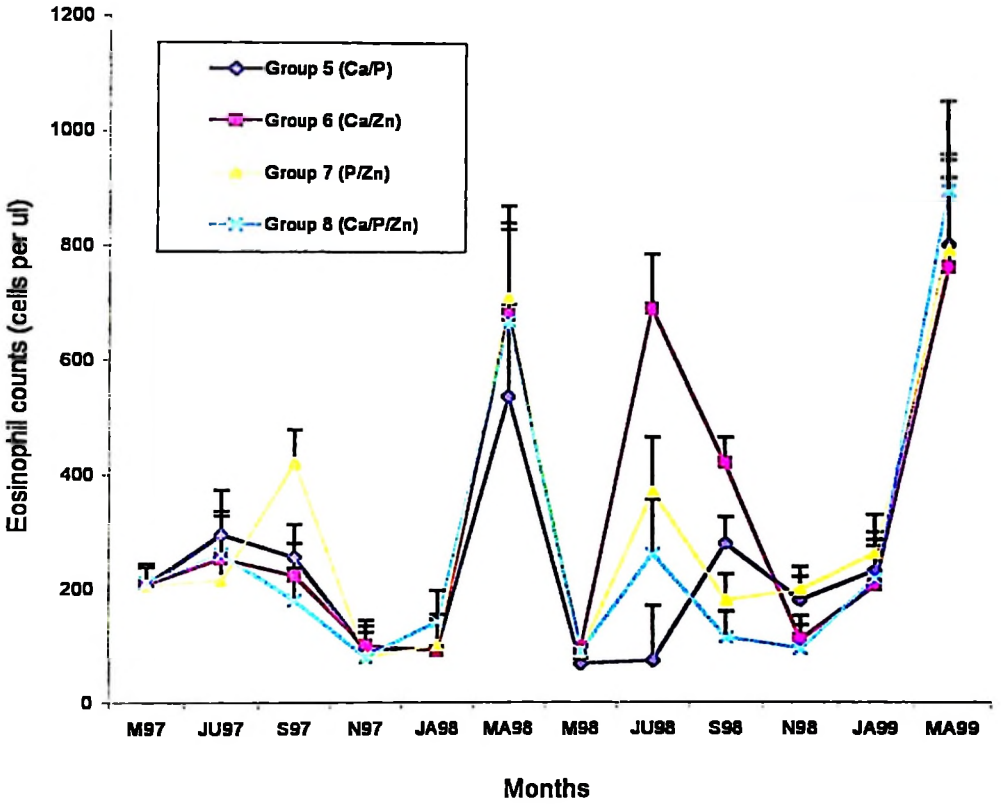


Figure 46 Eosinophil counts (cells per µl of blood) in grazing crossbred Zebu cows supplemented with different mineral combinations at ASAS Dairy Farm, Iringa, Tanzania.

Monocyte counts (MC)

The means and ranges for monocytes counts are presented in Fig. 47 and Appendix 38. There was no significant variation between groups ($P > 0.05$) in MC at each sampling period except for September 1997 and January 1998 in which cows supplemented with Ca/P and Ca/P/Zn tended to have high mean MC compared to other groups. Plasma Pi was negatively correlated to MC in the dry season in Ca/P ($r = -0.39$, $P = 0.0103$) supplemented cows. Furthermore, weak positive correlation was observed between MC and plasma Zn during the rainy season in Ca/P ($r = 0.34$, $P = 0.0641$) and Ca/P/Zn ($r = 0.33$, $P = 0.0740$) supplemented cows.

Serum immunoglobulins (SIM)

The means and ranges for serum immunoglobulins are presented in Fig. 48 and Appendix 39. There was significant variation between groups ($P < 0.05$) in SIM at each sampling period except for July 1997, November 1997, January 1998 and March 1999. Positive correlation was observed between SIM and plasma Ca in the dry season in Ca/Zn ($r = 0.38$, $P = 0.0131$) and Ca/P/Zn ($r = 0.46$, $P = 0.0021$) supplemented cows. Negative correlation between SIM and plasma Ca ($r = -0.54$, $P = 0.0020$) and plasma Zn ($r = -0.50$, $P = 0.0042$) in the rainy season was observed in the P/Zn supplemented cows. Furthermore, plasma Pi was negatively correlated to SIM in the rainy season in Ca/P ($r = -0.65$, $P = 0.0001$), P/Zn ($r = -0.59$, $P = 0.0005$) and Ca/P/Zn ($r = -0.67$, $P = 0.0001$) supplemented cows. Serum immunoglobulin was positively correlated to plasma Pi in the Ca/Zn supplemented cows in the rainy season ($r = -0.45$, $P = 0.0121$).

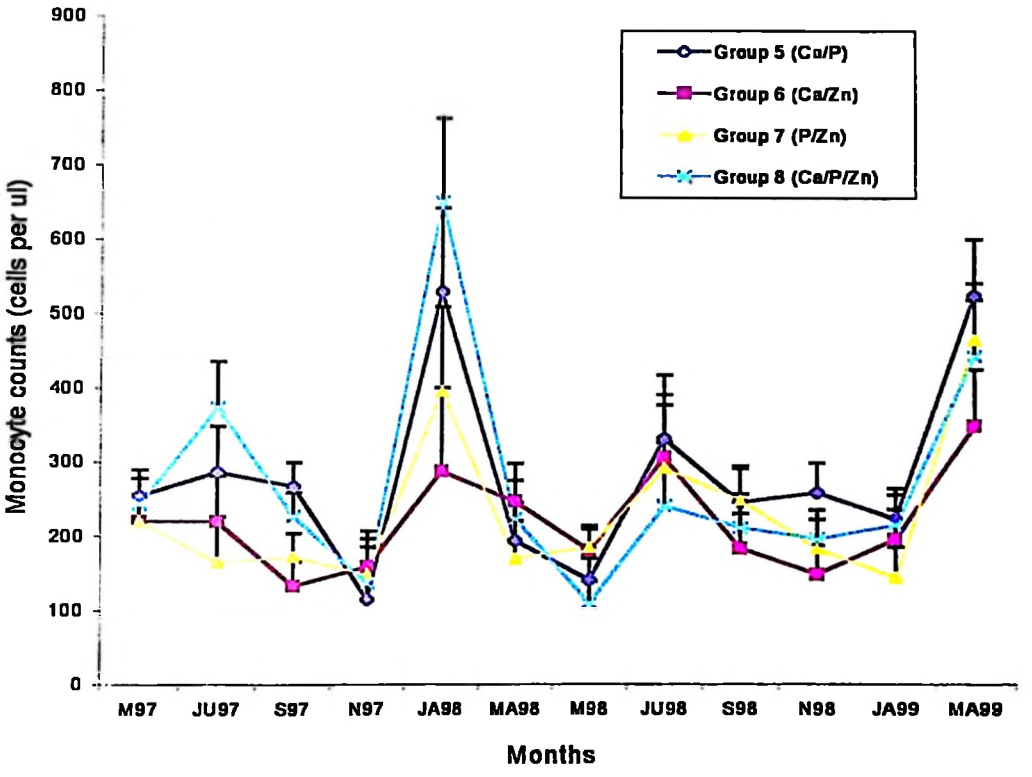


Figure 47 Monocyte counts (cells per µl of blood) in grazing crossbred Zebu cows supplemented with different mineral combinations at ASAS Dairy Farm, Iringa, Tanzania.

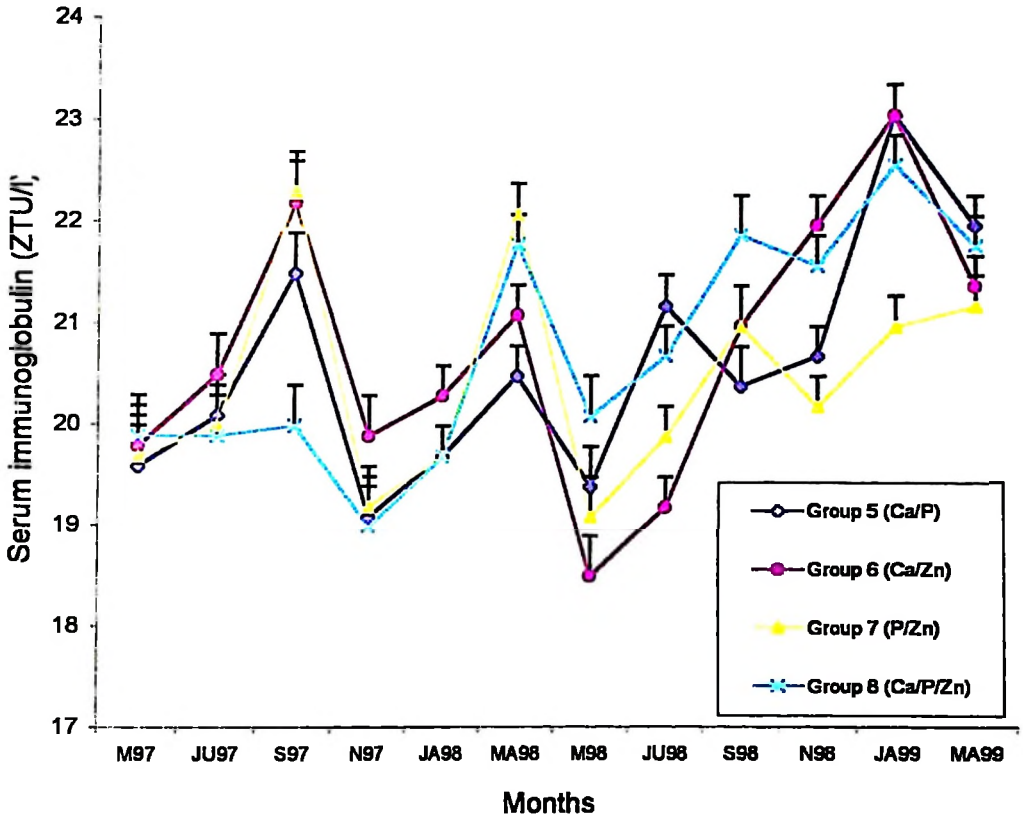


Figure 48 Serum immunoglobulin (ZTU/l) in grazing crossbred Zebu cows supplemented with different mineral combinations at ASAS Dairy Farm, Iringa, Tanzania.

Plasma aspartate aminotransferase (ASAT)

The means and ranges for plasma aspartate aminotransferase are presented in Fig. 49 and Appendix 40. Significant variation ($P < 0.001$) in plasma ASAT was observed between sampling periods. High plasma ASAT was recorded in the second year in the month of November 1998 (43.7 – 63.7 IU/l) whereas low ASAT was observed in September 1997 (24.0 – 33.5 IU/l). There was a significant variation between groups ($P < 0.05$) in plasma ASAT at each sampling period except in for January 1998, March 1998 and May 1998. Mean plasma ASAT tended to be low in the Ca/P/Zn supplemented cows and high in Ca/Zn supplemented cows. Positive correlation were observed between ASAT and plasma Ca in the dry season in Ca/Zn ($r = 0.47$, $P = 0.0014$) and Ca/P/Zn ($r = 0.33$, $P = 0.0353$) supplemented cows. The relation was non significant in Ca/Zn ($r = 0.28$, $P = 0.0688$) and P/Zn ($r = 0.28$, $P = 0.0670$) supplemented cows. Positive correlation between ASAT and plasma Zn ($r = 0.49$, $P = 0.0065$) and negative correlation between ASAT and plasma Pi ($r = - 0.52$, $P = 0.0030$) in the rainy season was observed in the P/Zn supplemented cows. Furthermore, plasma Zn was positively correlated to ASAT in the rainy season in the Ca/P ($r = 0.33$, $P = 0.0334$), Ca/Zn ($r = 0.31$, $P = 0.0419$) and P/Zn ($r = 0.49$, $P = 0.0009$) supplemented cows.

Plasma alkaline phosphatase (ALP)

The means and ranges for plasma ALP activity are presented in Fig. 50 and Appendix 44. Significant variation ($P < 0.05$) on ALP activity was observed between sampling periods. The highest plasma ALP activity (42.9 – 50.6 IU /l) was recorded at the start of the experiment i.e. May, 1997 whereas the lowest was observed in

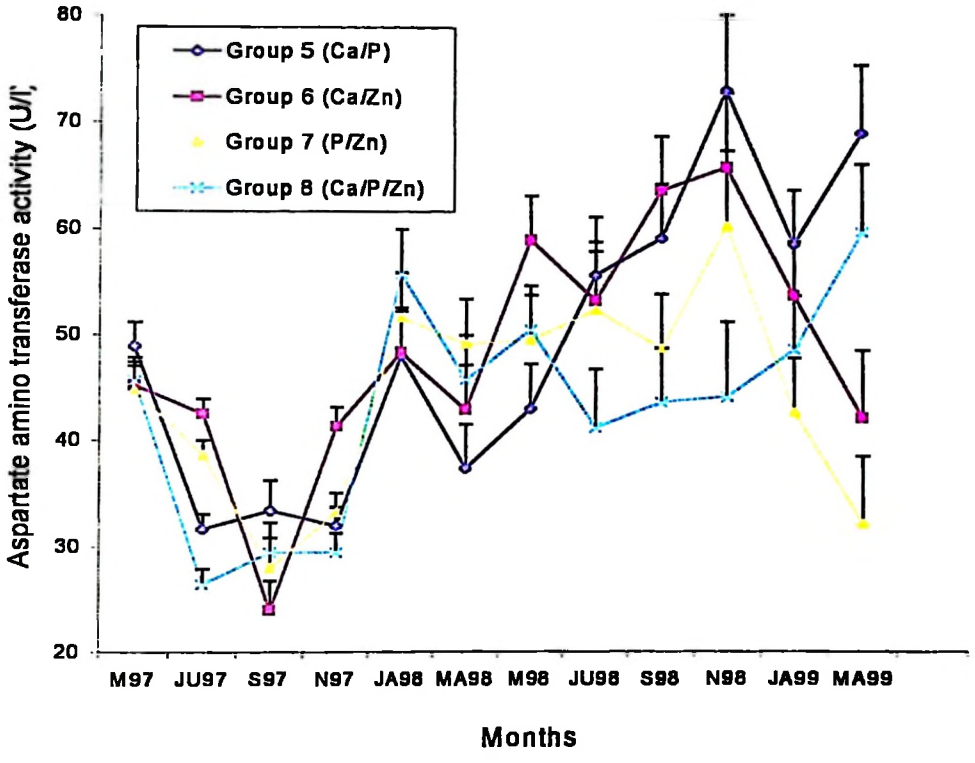


Figure 49 Plasma aspartate aminotransferase activity (IU/l) in grazing crossbred Zebu cows supplemented with different mineral combinations at ASAS Dairy Farm, Iringa, Tanzania.

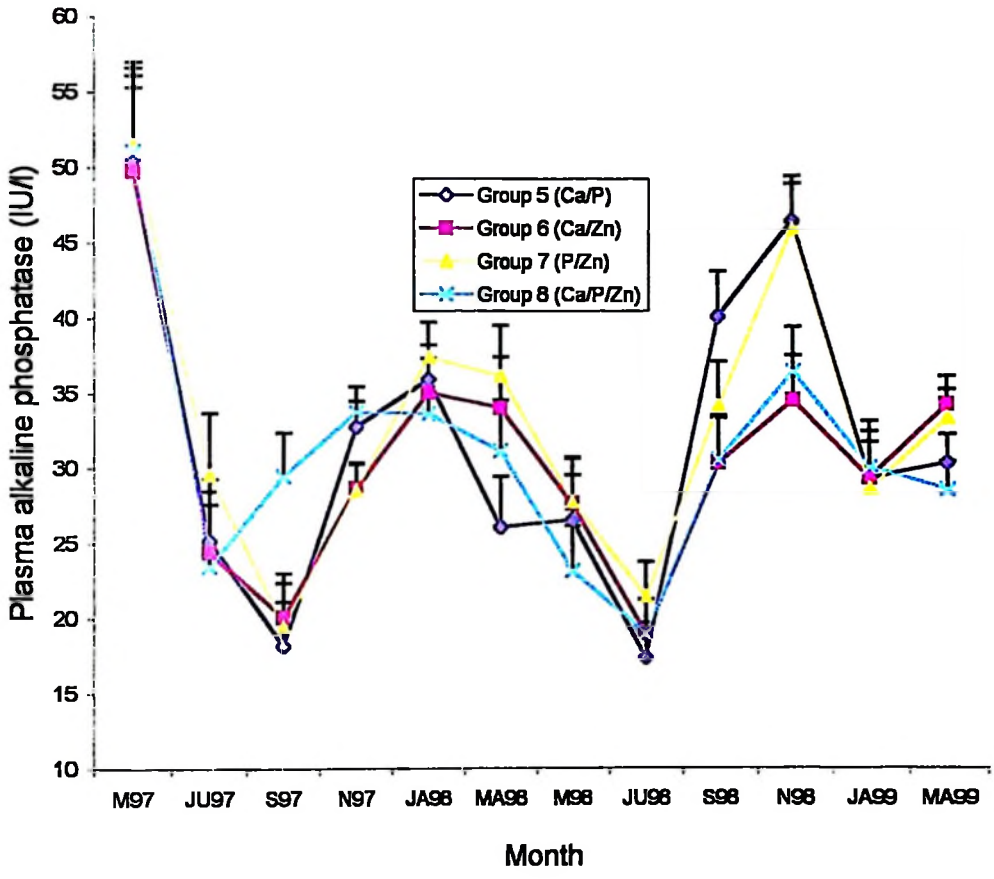


Figure 50 Plasma alkaline phosphatase activity (IU/l) in grazing crossbred Zebu cows supplemented with different mineral combinations at ASAS Dairy Farm, Iringa, Tanzania.

September 1997 (16.6 – 20.1 IU/l) and July 1998 (17.4 – 21.5 IU/l). Mean plasma ALP between groups varied within sampling periods ($P < 0.001$) except November 1997, March 1998, September 1998 and November 1998. Cows supplemented with Ca/Zn and Ca/P/Zn tended to have low plasma ALP when compared to Ca/P supplemented cows. Positive correlation was observed between ALP and plasma Ca in all groups of cows in the dry season; Ca/Zn ($r = 0.73$, $P = 0.0001$), Ca/Zn ($r = 0.47$, $P = 0.0019$), P/Zn ($r = 0.43$, $P = 0.0047$) and Ca/P/Zn ($r = 0.60$, $P = 0.0001$) supplemented cows. In the rainy season plasma ALP was only correlated to plasma Ca in the Ca/P supplemented cows only ($r = 0.37$, $P = 0.0418$). Furthermore, plasma Zn was positively correlated to ALP in the dry season in Ca/P supplemented cows ($r = 0.31$, $P = 0.0405$) but a negative correlation was observed in Ca/Zn supplemented cows ($r = -0.31$, $P = 0.0353$) in the rainy season. A weak positive correlation was observed between ALP and plasma Pi during the rainy season in Ca/P/Zn supplemented cows only ($r = 0.35$, $P = 0.0583$).

4.7.3 Effects on protein and energy metabolism

Plasma total proteins (TP)

The means and ranges for plasma total protein are presented in Fig 51 and Appendix 45. Significant variation ($P < 0.05$) in the TP was observed between sampling periods. High TP was recorded in the first year of the study 1997/1998 with highest record in January 1998 (105 - 114 g /l). Low TP was recorded in the second year (1998/1999) with the lowest record in September (81.6 - 85.8 g /l). There was no significant variation between groups ($P > 0.05$) in the TP concentration at each sampling period except for September 1997, November 1997, January 1998,

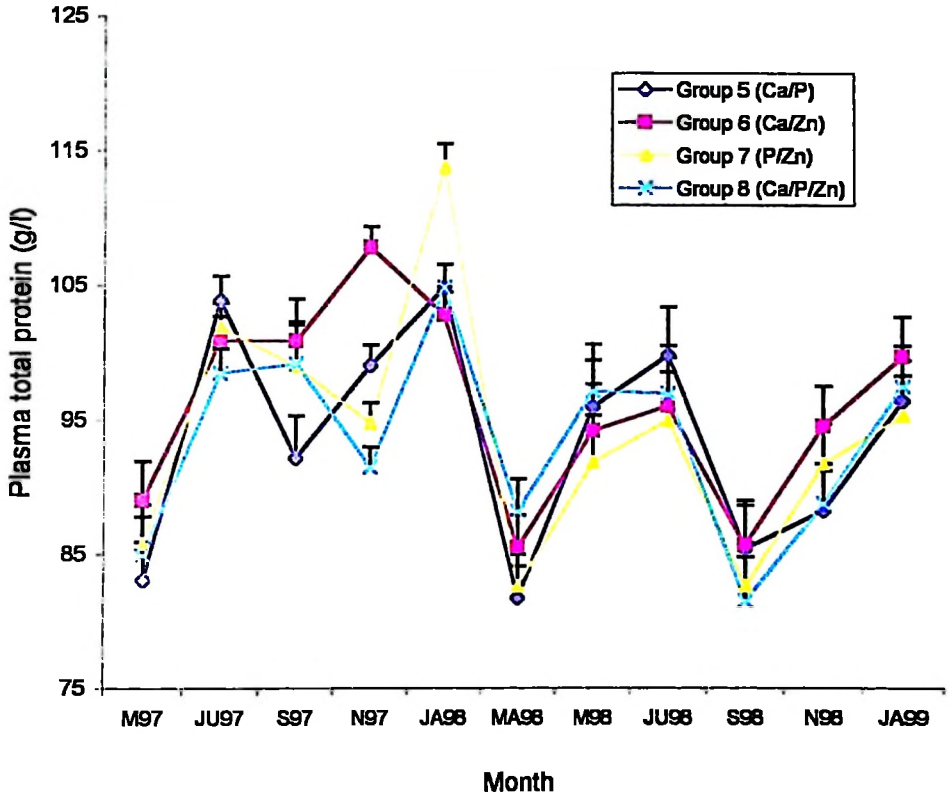


Figure 51 Plasma total protein concentration (g/l) in grazing crossbred Zebu cows supplemented with different mineral combinations at ASAS Dairy Farm, Iringa, Tanzania.

November, January 1999 and March 1999. High TP was recorded in Ca/Zn in most of these periods. Positive correlation was observed between TP and plasma Ca in all groups of cows in the rainy season i.e. Ca/Zn ($r = 0.62$, $P = 0.0003$), Ca/Zn ($r = 0.60$, $P = 0.0005$), P/Zn ($r = 0.43$, $P = 0.0182$) and Ca/P/Zn ($r = 0.34$, $P = 0.0661$). Furthermore, plasma TP was negatively correlated to Ca in the dry season in all groups of cows; Ca/Zn ($r = -0.38$, $P = 0.0119$), Ca/Zn ($r = -0.33$, $P = 0.0324$), P/Zn ($r = -0.42$, $P = 0.0054$) and Ca/P/Zn ($r = -0.66$, $P = 0.0001$). A positive correlation was observed between TP and plasma Pi during the rainy season in P/Zn supplemented cows only ($r = 0.38$, $P = 0.0365$).

Plasma urea (Plu)

The means and ranges of plasma urea are presented in Fig. 52 and Appendix 46. Significant variation ($P < 0.001$) in Plu was observed between sampling periods. The high Plu was recorded during the rainy season, with highest value measured in March 1998 (6.12 – 7.57 mmol /l). Low Plu was observed in the dry season (1.55 - 3.01 mmol/l). A significant variation between groups ($P < 0.001$) in Plu concentration was observed at each sampling period. There was a tendency towards low Plu in Ca/P/Zn supplemented cows. Negative correlation was observed between Plu and plasma Pi in the rainy season in Ca/P ($r = -0.44$, $P = 0.0147$) and Ca/P/Zn ($r = -0.43$, $P = 0.0183$) supplemented cows. However plasma Plu was positively correlated to Pi in the dry season in the same groups of cows; Ca/Zn ($r = 0.39$, $P = 0.0092$), and Ca/P/Zn ($r = 0.39$, $P = 0.0101$). Positive correlation were observed between Plu and plasma Ca in the rainy season; in the Ca/Zn ($r = 0.62$, $P = 0.0003$) and Ca/Zn ($r = 0.60$, $P = 0.0005$) supplemented cows.

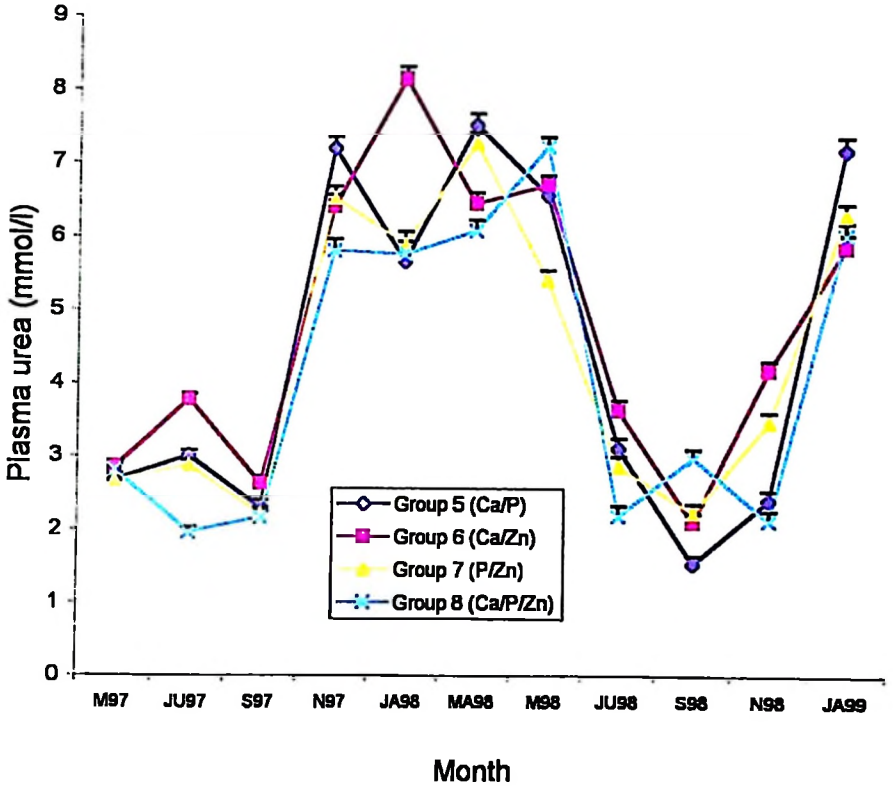


Figure 52 Plasma urea concentration (mmol/l) in grazing crossbred Zebu cows supplemented with different mineral combinations at ASAS Dairy Farm, Iringa, Tanzania.

and Ca/P/Zn (r = 0.34, P = 0.0661). Furthermore, plasma Plu was negatively correlated to Ca in the dry season in some groups of cows i.e. Ca/P (r = -0.27, P = 0.0820), Ca/Zn (r = -0.42, P = 0.0052) and P/Zn (r = -0.28, P = 0.0692).

Plasma glucose

The means and ranges for plasma glucose concentration are presented in Fig. 53 and Appendix 47. A significant variation between groups (P < 0.05) on plasma Glu concentration was observed at each sampling period except for July 1997, November 1997, July 1998, November 1998, January 1999 and March 1999. Negative correlation was observed between plasma Glu and plasma Ca in all groups of cows in the dry and the rainy seasons; Ca/Zn (r = -0.61, P = 0.0001; r = -0.50, P = 0.0050), Ca/Zn (r = -0.68, P = 0.0001; r = -0.40, P = 0.0270), P/Zn (r = -0.74, P = 0.0095; r = -0.47, P = 0.0095) and Ca/P/Zn (r = -0.65, P = 0.0001; r = -0.51, P = 0.0038) respectively. Furthermore, plasma Glu was negatively correlated to plasma Zn in Ca/P/Zn supplemented cows (r = -0.30, P = 0.0508) and plasma Pi in the P/Zn supplemented cows (r = -0.37, P = 0.0445) in the dry season. In addition negative correlation was observed between plasma Glu and plasma Zn in the rainy season in the P/Zn supplemented cows (r = -0.52, P = 0.0031) and Ca/P/Zn supplemented cows (r = -0.46, P = 0.0105).

4.7.4 Effect of Ca, P and Zn interaction on milk production

Milk yield

Means and ranges of milk yield are presented in Appendix 48. Mean milk yield per lactation and lactation days are presented in Table 12. There were no significant

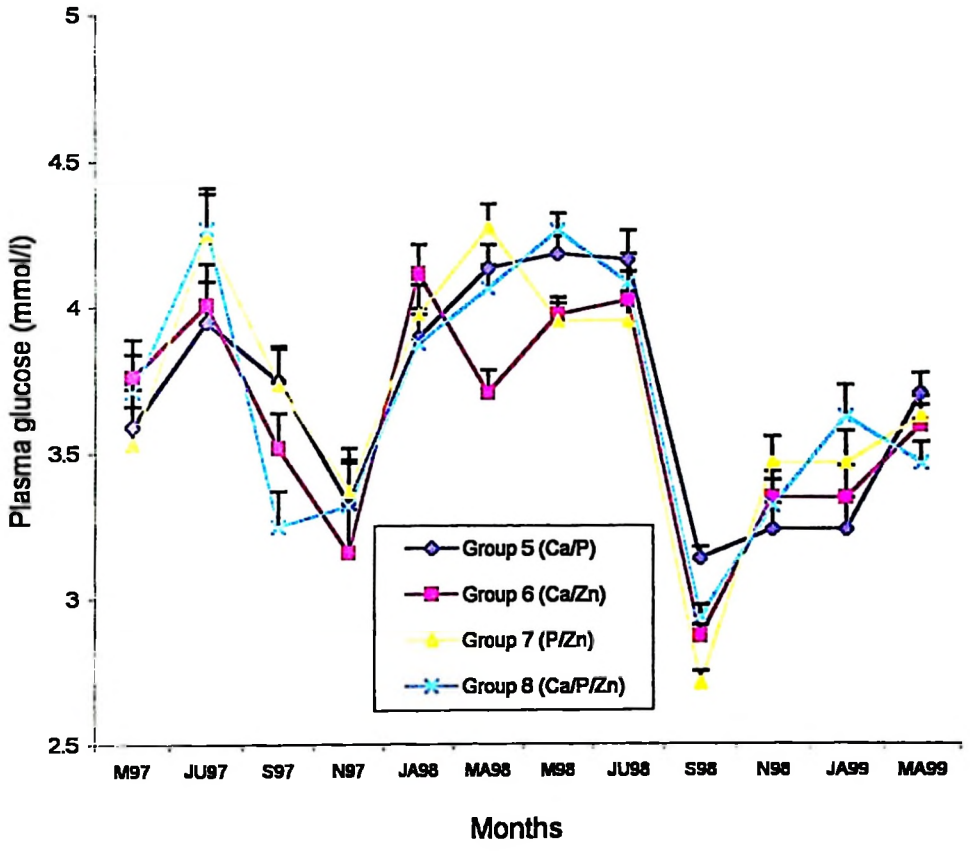


Figure 53 Plasma glucose concentration (mmol/l) in grazing crossbred Zebu cows supplemented with different mineral combinations at ASAS Dairy Farm, Iringa, Tanzania.

differences variation ($P > 0.05$) in milk yield per lactation and lactation days between groups. Significant variation ($P < 0.05$) on milk yield was observed between sampling periods except for July 1998, September 1998 and November 1998. Plasma Ca was negatively correlated to milk yield in the rainy season in Ca/Zn ($r = -0.40$, $P = 0.0533$) and P/Zn ($r = -0.54$, $P = 0.0078$).

Milk fat (MF)

The means and ranges of percentage milk fat (MF) are presented in Table 18. Mean percentage milk fat between groups varied within sampling periods ($P < 0.05$) except in September 1998. High mean MF was recorded in cows receiving Ca/Zn and the lowest MF was observed in cows receiving a combination of Ca, P and Zn. Mean MF was negatively correlated to plasma Zn ($r = -0.46$, $P = 0.0289$) and plasma Pi ($r = -0.45$, $P = 0.0309$) in the P/Zn supplemented cows in the rainy season. In addition negative correlation was observed between MF and plasma Zn in the dry season in Ca/Zn supplemented cows ($r = -0.39$, $P = 0.0226$). Plasma Pi was positively correlated to MF in the rainy season in Ca/P ($r = 0.73$, $P = 0.0006$) and Ca/Zn ($r = 0.45$, $P = 0.0451$) and plasma Pi was negatively correlated to milk yield in P/Zn supplemented cows only ($r = -0.45$, $P = 0.0309$).

Milk Protein (MP)

The means and ranges of percentage milk protein (MP) are presented in Table 19. Mean percentage MP between groups varied within sampling periods ($P < 0.05$) except for November 1997, September 1998 and March 1999. High mean MP was recorded in the cows supplemented with Ca/P/Zn. Mean MP was positively

Table 18 Milk fat (%) in the milk in cows supplemented with different mineral combinations at ASAS Dairy Farm Iringa, Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at P > 0.05, () = range).

Months	n	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	P value
May 1997	6	5.0 ± 0.24[a] (4.60 – 5.60)	5.0 ± 0.24[a] (4.80 – 5.30)	5.0 ± 0.24[a] (4.50 – 5.50)	4.72 ± 0.12[a] (4.20 – 5.50)	0.8202
July 1997	6	5.15 ± 0.34[a] (4.40 – 6.50)	5.07 ± 0.34[a] (4.50 – 5.30)	5.00 ± 0.34[a] (3.60 – 6.10)	4.93 ± 0.34[a] (4.00 – 6.80)	0.5238
September 1997	5	5.04 ± 0.26[a] (4.70 – 5.20)	5.12 ± 0.26[a] (4.80 – 5.50)	4.90 ± 0.30[a] (4.70 – 5.30)	4.44 ± 0.26[b] (3.70 – 5.20)	0.0510
November 1997	3	5.25 ± 0.36[a] (4.50 – 5.70)	4.98 ± 0.36[a] (4.80 – 6.00)	4.22 ± 0.36[b] (4.10 – 5.20)	4.97 ± 0.36[a] (4.20 – 5.30)	0.0411
March 1998	6	4.38 ± 0.31[b] (3.50 – 5.50)	4.85 ± 0.35[a] (4.60 – 5.10)	4.70 ± 0.31[a] (3.50 – 5.90)	4.28 ± 0.31[b] (3.50 – 5.80)	0.0047
May 1998	6	4.18 ± 0.23[a] (3.50 – 4.50)	4.38 ± 0.27[a] (4.00 – 4.80)	4.30 ± 0.24[a] (3.50 – 4.90)	4.30 ± 0.24[a] (3.60 – 5.40)	0.8346
July 1998	6	3.63 ± 0.32[b] (3.30 – 3.90)	4.50 ± 0.25[a] (4.10 – 5.20)	4.23 ± 0.23[a] (3.30 – 5.00)	4.62 ± 0.23[a] (4.40 – 4.90)	0.2797
September 1998	6	4.40 ± 0.38[a] (3.90 – 4.70)	4.84 ± 0.30[a] (4.40 – 5.10)	4.16 ± 0.30[a] (3.10 – 5.50)	4.38 ± 0.30[a] (3.90 – 4.80)	0.0636
November 1998	6	4.60 ± 0.29[a] (3.90 – 5.20)	4.55 ± 0.30[a] (4.00 – 5.30)	4.43 ± 0.30[a] (3.70 – 5.20)	4.43 ± 0.30[a] (3.70 – 5.20)	0.0873
March 1999	6	4.10 ± 0.24[a] (3.50 – 4.90)	4.00 ± 0.24[a] (3.50 – 4.50)	4.67 ± 0.26[a] (3.90 – 5.60)	4.38 ± 0.26[a] (3.60 – 5.60)	0.1494

Table 19 Milk protein (%) in the milk in cows supplemented with different mineral combinations at ASAS Dairy Farm Iringa Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at P > 0.05, () = range).

Months	N	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	P value
May 1997	6	3.71 ± 0.12^{a} (3.26 – 4.00)	3.60 ± 0.12^{a} (3.27 – 4.00)	3.71 ± 0.12^{a} (3.61 – 3.89)	3.73 ± 0.12^{a} (3.61 – 3.89)	0.9202
July 1997	6	3.70 ± 0.13^{b} (3.26 – 4.10)	4.16 ± 0.13^{a} (3.61 – 4.30)	3.86 ± 0.13^{b} (3.39 – 4.19)	3.90 ± 0.13^{b} (3.66 – 4.26)	0.0138
September 1997	5	3.57 ± 0.12^{a} (3.36 – 3.98)	3.07 ± 0.12^{c} (2.80 – 3.26)	3.15 ± 0.12^{b} (2.77 – 3.37)	3.16 ± 0.12^{b} (2.69 – 3.54)	0.0020
November 1997	3	3.53 ± 0.30^{a} (3.23 – 3.83)	3.32 ± 0.21^{a} (3.17 – 3.47)	3.34 ± 0.30^{a} (3.14 – 3.74)	3.17 ± 0.21^{a} (3.09 – 3.25)	0.6811
March 1998	6	3.19 ± 0.13^{a} (2.59 – 3.50)	2.60 ± 0.14^{c} (2.48 – 2.67)	2.49 ± 0.13^{c} (2.33 – 2.80)	2.89 ± 0.13^{b} (2.72 – 3.30)	0.0047
May 1998	6	3.35 ± 0.11^{b} (2.86 – 3.76)	3.25 ± 0.12^{b} (3.02 – 3.40)	3.43 ± 0.11^{a} (3.12 – 3.92)	3.36 ± 0.11^{b} (3.05 – 3.89)	0.0001
July 1998	6	3.15 ± 0.15^{a} (2.91 – 3.36)	3.20 ± 0.12^{a} (2.86 – 3.81)	2.98 ± 0.11^{a} (2.79 – 3.29)	3.41 ± 0.11^{a} (3.14 – 3.50)	0.2797
September 1998	6	3.45 ± 0.18^{a} (3.21 – 3.58)	3.39 ± 0.14^{a} (3.13 – 3.95)	3.10 ± 0.14^{a} (2.46 – 3.47)	2.41 ± 0.14^{a} (2.31 – 2.57)	0.2236
November 1998	6	3.28 ± 0.18^{b} (2.80 – 3.56)	3.01 ± 0.20^{c} (2.76 – 3.29)	3.47 ± 0.18^{b} (2.95 – 3.85)	3.63 ± 0.18^{a} (3.1 – 4.21)	0.0473
March 1999	6	3.15 ± 0.24^{a} (2.93 – 3.42)	3.18 ± 0.24^{a} (2.88 – 3.31)	3.45 ± 0.24^{a} (2.80 – 4.00)	3.37 ± 0.24^{a} (3.04 – 4.02)	0.1494

correlated to plasma Ca in the rainy season in the Ca/Zn (r = 0.50, P = 0.0246), P/Zn (r = 0.82, P = 0.0001) and Ca/ P/Zn (r = 0.68, P = 0.0014) supplemented cows. Milk protein was negatively correlated to plasma Pi in the dry season in Ca/Zn supplemented cows (r = -0.49, P = 0.0033) but it was positively correlated to Pi in the rainy season in Ca/P (r = 0.70, P = 0.0014) and P/Zn (r = 0.46, P = 0.0463) supplemented cows. In addition plasma Zn was positively correlated to MP in the rainy season in P/Zn (r = 0.74, P = 0.0002) and Ca/P/Zn (r = 0.53, P = 0.0198) supplemented cows only.

4.7.5 Effect of Ca, P and Zn interaction on reproduction performance

Interval between parturition to resumption of oestrous activity (PRO), interval between parturition to conception (PCO), calving intervals (CAI) and number of services per conception (SEC) are presented in Table 15. No variation was observed between groups in term of PRO, PCO, CAI and SEC.

4.7.6 Effect of Ca, P and Zn interactions on copper and selenium balance

Ceruloplasmin activity (Cp)

The mean and range of plasma ceruloplasmin activity are presented in Fig. 54 and Appendix 52. Significant variations (P < 0.05) on plasma Cp activity were observed between the groups. High plasma Cp was observed in May 1997 and September 1997 respectively. Low Cp was observed in November 1997. Cows supplemented with Ca/Zn and P/Zn tended to have high mean Cp in most sampling periods compared to other groups. Ceruloplasmin was positively correlated to plasma Ca in

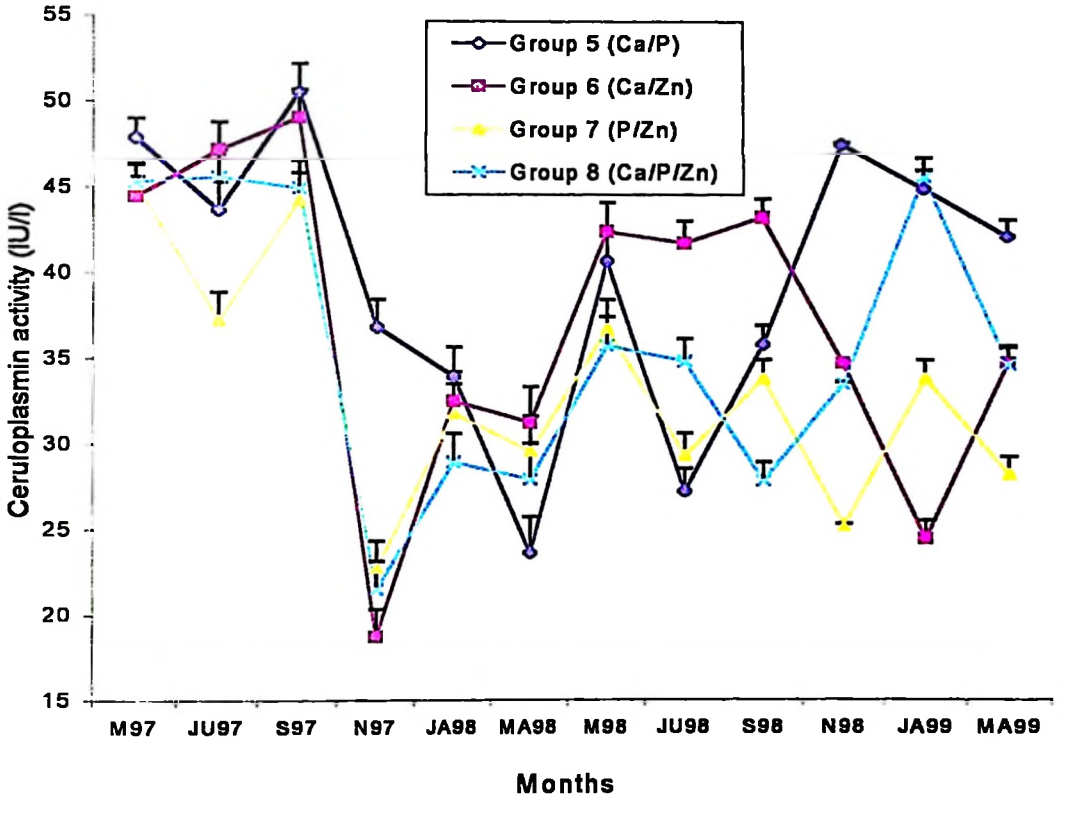


Figure 54 Plasma ceruloplasmin activity (IU/l) in grazing crossbred Zebu cows supplemented with different mineral combinations at ASAS Dairy Farm, Iringa, Tanzania.

the rainy season in the Ca/P supplemented cows only (r = 0.72, P = 0.0001). Ceruloplasmin was negatively correlated to plasma Ca in the dry season in the P/Zn (r = -0.32, P = 0.0360) and Ca/P/Zn (r = -0.35, P = 0.0212) supplemented cows. A negative correlation was also observed in the rainy season in the Ca/Zn supplemented cows (r = -0.36, P = 0.0485). Furthermore Cp was negatively correlated to Pi in the rainy season in the Ca/Zn (r = -0.36, P = 0.0536), P/Zn (r = -0.57, P = 0.0011), Ca/P/Zn (r = -0.65, P = 0.0001) and plasma Zn in the dry season in the Ca/Zn (r = -0.51, P = 0.0006), P/Zn (r = -0.62, P = 0.0001), P/Zn (r = -0.45, P = 0.0031) supplemented cows.

Glutathione peroxidase activity (GSH.Px)

The mean and range of whole blood glutathione peroxidase activity is presented in Table 16. Significant variation (P < 0.001) on GSH.Px activity was observed between groups in January 1998. Cows receiving P/Zn had low GSH.Px activity compared to other groups.

CHAPTER FIVE

DISCUSSIONS

5.1 PRELIMINARY EVALUATION ON MINERAL STATUS

5.1.1 Soil mineral concentration at ASAS Dairy Farm, Iringa.

Calcium concentration

The average Ca content in the soil at ASAS Dairy Farm, ranged from 4.46 – 5.67 me/100g. No variation on soil Ca content was observed between the dry season and the wet season. A mean concentration of 2.50 me/100 g Ca in soil has been suggested to be adequate for maintaining proper plant growth (Okalebo *et al*, 1992). If 2.50 me/100 g Ca is taken as the Ca requirements for plants then the soil at ASAS Dairy Farm had normal Ca contents and the results are in agreement with Underwood, (1981) who reported that Ca deficient soils are less common.

The amount of Ca content obtained in this study was in agreement with Ca content obtained by other workers in Tanzania like Mwakatundu, (1977) (4.20 – 6.2 me/100 g), Mtengeti, (1984) (3.20 – 5.4 me/100 g), Sendalo, (1986) (5.20 – 6.5 me/100 g), Muhikambele, (1990), (4.80 –6.4 me/100 g) and Pereka and Phiri (1998), (2.20 – 13.9 me/100 g). All these observations emphasise the view that the amounts of Ca found in the soil at ASAS Dairy Farm, are of the same magnitude as reported earlier by other workers in Tanzania.

Phosphorus concentration

The average P content in the soil ranged from 11.9 to 34.6 ppm (Table 6). These results are within the range which had been reported in other parts of Tanzania by

others such as Arusha, 8 – 70 ppm (Tengeru, Arusha) (Mwakatundu 1977), 10 – 150 ppm (Magadu, Morogoro) (Sendalo 1986) and 20 – 200 ppm (Magadu Morogoro) (Chauhan and Ndelingo, 1997). According to Okalebo *et al.*, (1992) soil with less than 15 ppm P are deficient. On this basis available P was inadequate in April 1997 and January 1998 and was high in September 1997, May 1998 and March 1999. General observation was that soil P was high during the dry season (28.1 – 31.8 ppm) and low in the wet season (11.9 – 14.8 ppm) except in March 1999 (34.6 ppm). In October 1998 the farmer applied farmyard manure in some plots hence a possible reason for having higher soil P in March 1999. Possible reason for less P during the rain season is loss of soil P by leaching. The loss of P by leaching may lead to deficiency of P in the soil as reported by (Landon, 1991). The tendency for cations to be leached from different soils depends on the Cation Exchange Capacity (CEC) of the soil. In acid mineral soils the effective (CEC) appears to be most closely correlated with the clay content (Okalebo *et a.*, 1992). Drainage water may wash large quantities of P from heavy clay soil (Landon, 1991). Part of ASAS Dairy Farm soil is characterised by acid soil and high clay content (Pereka and Phiri 1998). Farm yard manure apart from adding mineral to the soil may stabilise the soil and hence minimise the loss of P by leaching hence, high P in March 1998.

Zn concentration

The average Zn content in the soil ranged from 0.81 to 1.02 ppm. According to Landon (1991) soil with less than 1.0 ppm Zn are deficient. On this basis Zn in the soil was marginal in the dry season (0.99 – 1.02 ppm) and low in wet season (0.81 – 0.98 ppm) (Table 6). These observations are in agreement with Chauhan and

Nderingo, (1997) (0.30 – 1.20 ppm) who reported that Zn deficiency was wide spread in Tanzania.

Other minerals in the soil at ASAS Dairy Farm, Iringa.

Okalebo *et al* (1992) suggested soil levels of 0.17% Mg to be the lower safe level for cattle. In the present study all soil samples in both sampling periods were higher than this level indicating Mg adequacy. Potassium and Sodium were adequate as soil contained levels of K and Na above the critical levels of 0.13 and 0.07 me /100g respectively. Iron (75.8 – 101 ppm) and Copper (1.38 – 2.24 ppm) were also above the critical levels of 4.50 and 0.20 ppm, respectively.

5.1.2 Mineral concentration in forage and feed concentrates used at ASAS Dairy Farm

Calcium concentration in forage and feed concentrates

The average Ca contents in the pastures ranged from 0.31 – 0.39%. Calcium content in pasture tended to be high during the dry season (0.38 – 0.39%), than during the wet season (0.31 – 0.36%), although it was not statistically different at $P < 0.05$ (Table 8). These observations were in agreement with the general view that Ca content does not vary much or it may rise with maturity (McDowell, 1992), which has been the case in this study. The Ca levels obtained in this study were in agreement with those obtained by other workers like Mwakatundu, (1977) (0.26 – 0.74%) and Pereka and Phiri (1998) (0.38 – 0.64%). However, forage Ca contents obtained in this study are lower as compared to levels of Ca obtained by Sendalo,

(1986) (0.45 – 0.78%) at Magadu Farm, SUA Morogoro. It is possible that low soil Ca was reflected on pasture Ca concentrations.

A mean concentration of 0.30% Ca in pasture grass has been suggested to be adequate for maintaining ruminants (NRC, 1989). If 0.30% Ca is taken as the Ca maintenance requirements for ruminants then the pastures at this area had marginal Ca content. Calcium contents in alfalfa were high Ca i.e 1.4 % (Table 2) and the level was within the normal range (1.33 – 1.96 %) given by McDowell, (1992) but on the lower side. In view of these results, it would be advisable to supplements Ca to the dairy cows on this farm.

A positive correlation observed between calcium in the soil and percent Ca in the pastures ($r = 0.394$, $P = 0.0018$) was in contrast to the findings by Mwakatundu (1977) who reported a non significant correlation at ($P > 0.05$). It is known that absorption of ions from soil by plants follow different mechanisms and are dependent on many environmental factors such as temperature, light, oxygen, pH, ion concentrations and interactions with other minerals (McDowell, 1992), hence a possible reason for the difference in correlation between Ca in the soil and pastures.

Phosphorus concentration in forage and feed concentrate

The average P content of 0.25% P in the pasture was considered sufficient for ruminants (McDowell, 1983). Underwood (1981) suggested a P level of 0.17 % to be marginal for grazing ruminants. In the present study P in pasture (Table 8) grass hay, and concentrate (Table 3) exceeded these values indicating that P was adequate

at this farm. These results on forage P were similar to those reported by Mtengeti (1984) (0.30% - 0.45%), Sendalo (1986) (0.27 – 0.54), Chauhan and Ndelingo, (1997) (0.35 – 0.60%) at Magadu Farm SUA Morogoro. However, P deficiency has been reported in other parts of Tanzania and East Africa e.g. Mbulu and Tengeru, Tanzania (Rodger, 1975; Mwakatundu, 1977), Kenya (Dougall and Bogdan, 1958; Howard, 1962), Uganda (Long *et al.*, 1970, 1972) and Ethiopia (Kabaija and Little, 1988). It was therefore clear that there was a marked variation in forage P content in the different agro -ecological zones, within and outside Tanzania.

Zinc concentration in forage and feed concentrates

Mean Zn concentration in the pasture grass was 21.2 – 27 ppm. These were inadequate when compared to normal levels (< 40 ppm) given by McDowell, *et al.*, (1983) (Table 8). The findings in this study were similar to those given in the previous report by Phiri (1995) at the same farm, which were 19.2 to 23.8 ppm. Animals depending on forages only were likely to suffer from Zn deficiency. However, concentrates had high Zn concentration (48 – 68 ppm) (Table 1 and 3), thus if fed to the animals would reduce the severity of the deficiency.

Other minerals concentration in the feed and forages

McDowell *et al.*, (1983) suggested a dietary level of 0.20% Mg in forage to be the lower safe level for cattle. In the present study all pasture grasses in both sampling periods were below this level indicating Mg deficiency. However, concentrate mixture had significant amount of Mg (0.24 – 0.36 %) (Table 1 and 3). Potassium and Sodium were adequate as all feedstuff contained K and Na above the critical levels of

0.8 % and 0.07 %, respectively. This was in contrast to observations from other parts of Tropical Africa, where Na deficiency was a common feature for instance Kenya Chamberlain, 1955; Howard *et al.* 1962), Uganda (Long *et al.*, 1970, 1972), Zambia (Walker, 1957), Malawi (Mtimuni, 1982) and Ethiopia (Khalili *et al.*, 1993).

5.1.3 Plasma calcium, inorganic phosphate and zinc concentration in animals at ASAS Dairy Farm, Iringa.

Calcium concentration

A reference interval for total plasma calcium of 2.17 – 2.84 mmol /l was suggested by Rosol *et al.* (1995) in dairy cattle. Plasma Ca concentration in the present study was within the normal suggested range (Table 10) though on the lower side in the lactating cows (2.20 mmol/l). Non lactating pregnant cows (2.14 mmol/l), pregnant heifers (2.14 mmol /l) and steers (1.90 mmol/l) had low plasma Ca. Theoretically, plasma Ca concentration should have been higher in steers than in lactating cows because it is known that the demand for Ca are maximal during lactation (McDowell, 1992). It is possible that the low levels of plasma Ca in steers was due to being fed pasture only without any mineral or concentrates supplementation in contrast to lactating cows, which were given mineral supplements and concentrates at every milking. Low levels of Ca may contribute to fragile bones, which are prone to fractures (McDowell, 1992). Bone fractures were frequently observed in calves and steers at this farm probably because of low levels of Ca in the diet. In the present study plasma Ca levels ranged from (2.14 - 2.20 mmol/l) in the dairy cattle. This range was low compared to what Mwakatundu (1977) reported in different parts of

Tanzania during the rainy season. Mwakatundu (1977) reported high levels of plasma Ca in lactating cows at Iwambi Mbeya (2.34 mmol/l), Tanga (2.73 mmol/l), Tengeru (2.78 mmol/l), Malya (2.67mmol/l), West Kilimanjaro (2.58 mmol/l), Mpwapwa (2.58 mmol/l) and Morogoro (2.55 mmol/l). The differences between the present study and result reported by Mwakatundu (1977) could have been due to seasonal, physiological state of the animals, breed of the animals, time of sampling and feeding regime. Sampling in the present study was done towards the end of the rainy season and most of the animals were in their second, third and fourth parities and in their mid lactation. It has been reported that plasma Ca decreases as a function of age and lactation number (Bendixen *et al.* 1987, Oetzel, 1991, Kusumanti *et al.* 1993).

Plasma inorganic phosphate concentration

Plasma Pi levels of 1.80 to 2.90 mmol/l are considered to be normal in cattle while levels below 1.25 mmol Pi /l are considered to be low (Ternouth, 1990; Underwood and Suttle, 1999). McDowell *et al.*, (1984) suggested a higher critical level of > 1.45 mmol Pi /l for zebu cows and their crosses. In the present study animals were deficient in Pi except the steers, which had normal levels, but at the lower end (1.80 mmol/l) (Table 1). High levels of P in steers as compared to other groups indicated physiological differences in the demand for P. Maternal demand for P is greater during pregnancy and lactation as a result of the additional requirements for the foetus and for milk synthesis (McDonald, *et al.*, 1995) compared to dry and non pregnant cows. The levels in the lactating cows in the present study were lower than the levels reported by Mwakatundu (1977) during the rainy season at Malya, Mwanza

(1.89 mmo/l), Iwambi, Mbeya (1.81 mmol/l), Tengeru, Arusha (1.80 mmol/l), West Kilimanjaro, (1.76 mmol/l) and Mpwapwa, Dodoma (1.63 mmol/l). The present and the observation by Mwakatundu, (1977) were in agreement that there is phosphorus deficiency in some parts of Tanzania.

Plasma zinc concentration

Normal plasma Zn concentration lie within the range of 12.0 to 18.5 µmol Zn /l (Underwood and Suttle, 1999). In the present study the lactating cows had marginal Zn concentration (12.2. µmol Zn /l). The rest of the animals i.e. steers (8.8 µmol Zn /l), pregnant cows (11.4. µmol Zn /l) and heifers (11.8. µmol Zn /l) had low plasma Zn concentration. As for the phosphorus it was possible that the low levels of plasma Zn in steers was due to the animals being fed pasture only without any mineral or concentrates supplementation in contrast to the lactating cows, which were supplemented with minerals and concentrates which contained high amount of Zn (48 ppm). The reported low levels of Zn in the present study were comparable to the findings reported by Chauhan and Nderingo (1997) (< 10µmol Zn /l) in dairy cattle at SUA, Morogoro. In addition a high negative correlation between pasture Ca and plasma Zn concentration ($r = -0.975$, $P = 0.0051$) was observed in this study. It has been reported that high dietary Ca decreases the absorption of Zn (Clark, *et al.*, 1995), therefore could be a possible reason for low Zn in plasma when Ca levels were high in pastures.

5.2 EFFECTS OF CALCIUM, PHOSPHORUS AND ZINC SUPPLEMENTATION ON PLASMA MINERAL BALANCE IN THE GRAZING CROSSBRED ZEBU COWS

5.2.1 Effects of Ca, P and Zn supplementation on plasma calcium

Plasma total Ca was between 1.35 and 2.88 mmol/l during the experimental periods. Most researchers investigating subclinical hypocalcaemia have defined the lowest subclinical level of Ca between 1.90 and 2.20 mmol/l (Hove, 1986; Daniel *et al.*, 1990; Jonsson *et al.*, 1999). In the present study, the mean plasma Ca was below the suggested lower critical levels in all groups during the dry season i.e July to September 1997 (1.35 - 1.77 mmol/l), May 1998 to July 1998 (1.44 – 1.83 mmol/l) and slightly higher but still lower than recommended levels during the calving period i.e. March 1998 (1.70 – 2.09 mmol/l) (Fig. 11 and Appendix 18). During the other periods of the year plasma Ca was within the suggested normal range. It is known that seasonal fluctuations in forage availability do cause feed shortage in the dry season either because of scarcity in pasture available for grazing (quantitative factor) or by a reduction in nutrients including minerals (qualitative factor) McDowell, (1992). During the rainy season, plants and plant parts of high nutritive value in term of minerals are preferably selected and chances for selection diminish as the dry season progresses. When the dry season intensifies animals get less feed. It is possible that the low levels of plasma Ca was due to this reason.

However, when cows were given 4 kg of concentrate, hay and fresh forage, plasma

Ca was increased in all groups and it remained high during the first two months of the rainy season. The raised plasma Ca was probably due to additional Ca from concentrates fresh forage and stored hay (Table 3).

High levels of plasma Ca was observed in Ca supplemented cows (Group 2) in November 1997 and 1998 and January 1998 and January 1999 was probably due to additional Ca given to these animals. The low plasma Ca levels observed in March 1998 and 1999, which coincided with the calving period were slightly lower compared to those observed by other researchers (Hove, 1986; Daniel *et al.*, 1990; Jonsson *et al.*, 1999).

Mineral supplemented cows had low plasma Ca between March, 1998 and May 1998 compared to the control cows (Fig. 11). Adequate calcium and phosphorus nutrition depends not only on a sufficient supply but also upon their mutual ratio in the diet and on the presence of other compounds or ions in the diet (Bondi 1987). Excess of either P or Ca interferes with the absorption of the other leading to lower plasma concentration (Cunha, 1973). Plasma calcium/phosphorus ratio of 2:1 was considered optimal for performance of dairy cows (McDowell, 1992). Calcium/phosphorus ratio of below 1:1 as observed in the months of July 1997 and 1998, March 1998, May 1998 resulted in lower plasma Ca. High plasma Ca was observed when Ca/P ratio was above or equal to 1:1 in the rest of sampling period (Appendix 53). It is possible that additional P supplementation from concentrates during the dry season worsened the condition that was already existing. The ratio was raised during the dry season of September 1998 and November 1998 and during

the rainy season when additional Ca from pasture was available and the ratio of maize bran to sunflower seedcake was increased from 3: 1 to 4: 1.

A positive correlation between plasma Ca and Pi was observed in the control cows ($r = 0.42$, $P = 0.0194$), Ca ($r = 0.38$, $P = 0.0393$) and Zn ($r = 0.48$, $P = 0.0076$) during the rainy season. During the rainy season plasma Pi was low when compared to dry season. In addition plasma Ca was positively correlated to plasma Zn in the dry season in all groups of cows except Zn supplemented cows. Calcium supplemented cows had a stronger correlation ($r = 0.46$, $P = 0.0023$) compared to the P supplemented cows ($r = 0.39$, $P = 0.0110$) and the control cows ($r = 0.30$, $P = 0.0518$). These observations indicate an indirect effect of P or Zn supplementation on Ca balance either through Zn metabolism as it has been reported that high amount of Zn or P interferes absorption of Ca (Underwood and Suttle, 1999). Furthermore, it is possible that the low plasma Ca in P supplemented cows during the rainy season was contributed to the high plasma Zn and it is supported by a negative correlation between plasma Ca and plasma Zn ($r = -0.42$, $P = 0.0201$) in the rainy season.

5.2.2 Effects of Ca, P and Zn supplementation on plasma inorganic phosphate

Plasma Pi levels of 1.80 – 2.90 mmol/l were considered normal for cattle while below 1.25 mmol Pi /l were considered low (Ternouth, 1990; Underwood and Suttle, 1999). For Zebu cows and their crosses McDowell *et al.*, (1984) suggested a higher critical level of > 1.45 mmol P /l. In the present study plasma Pi ranged in all groups between 1.57 and 2.43 mmol/l, which was above the suggested critical levels. There

was a tendency towards high Pi in Ca supplemented cows when compared to the control, P and Zn supplemented cows. The control cows receiving no mineral supplementation had most of time the lowest plasma Pi (Fig. 12). It has been suggested that plasma Pi is a good indicator of P status in ruminants only if stress factors like diseases are not present (McDowell, 1992). It is possible that the fluctuation of Pi between low and high in the groups of cows was a result of the presence of diseases like mastitis and anaplasmosis, feeding management, physiological state of the cows and season. Calcium and Zn supplemented cows had few cases of mastitis and anaplasmosis compared to the control and P supplemented cows (Table 11).

High levels of plasma Pi (Fig. 12 and Appendix 20) were recorded during the dry season compared to the rainy season possibly because during the dry season animals received high amount of P in the concentrates and stored hay but during the rainy season the amount of concentrates was reduced. Low P contents of the soils and seasonality of pasture production and quality, has been stated to be the cause of cattle grazing in the tropical semi arid pasture to frequently experience dietary deficiencies of P during both the dry and the rainy seasons (Lamerle *et al.*, 1980; Elliot and McMenimen 1987; Mclean *et al.*, 1990; Kerridge, 1990). The difference between these results and others may be attributed to the concentrates fed during the dry season which, had high amount of P (0.71%) in this study. Another reason might be the physiological state of cows as they calved during the rainy season in which demand for energy, protein and minerals are increased due to pregnancy and lactation.

In the second year levels of plasma Pi were reduced in all groups probably because of changing the concentrate mixture from one containing high amount of P (0.71%) to one containing low amount of P (0.43%) (Table 3). The present study suggests that better feeding management can raise or keep Pi levels above or within the marginal range.

No direct correlation was observed between plasma Pi and Ca in all groups of cows during the dry season. However, during the rainy season Pi was negatively correlated to plasma Zn in the control and the P supplemented cows. However, the P supplemented cows had a stronger correlation ($r = -0.40$, $P = 0.0285$) than the control cows ($r = -0.37$, $P = 0.0467$). It has been demonstrated that large intake of Zn interferes with the absorption of phosphorus by forming insoluble phosphates hence reducing the amount of plasma Pi (Bondi, 1987). It is possible that an increase in Zn from pasture during the wet season as indicated by a rise in plasma Zn (Fig. 13) was the cause of low Pi.

5.2.3 Effects of Ca, P and Zn supplementation on plasma zinc

Plasma Zn concentration lie within the range of 12.0 to 18.5 µmol Zn /l (Underwood and Suttle, 1999). In the present study plasma Zn ranged between 9.70 and 14.3 µmol Zn /l. Cows supplemented with Ca had high plasma Zn in most of the study period (Fig. 13 and Appendix 21). Plasma Ca was positively correlated to plasma Zn in the dry season in the control, Ca and P supplemented cows. Ca supplemented cows had a stronger relationship ($r = 0.42$, $P = 0.0194$) than P supplemented cows ($r = 0.39$, P

=0.0110) and the control cows (r = 0.30, P = 0.0518). Furthermore, plasma Zn was positively correlated to plasma Pi (r = 0.44, P = 0041) in the Ca supplemented cows during the dry season whereas plasma Zn was negatively correlated to Pi (r = -0.37, P = 0467) in the wet season. Hence a possible reason for having high plasma Zn in the Ca supplemented cows in both seasons. In the dry season cows receiving Ca had high plasma Pi concentration compared to the control cows whereas in the rainy season plasma Pi was low in the Ca supplemented cows. These observations suggest an interaction between Ca, P and Zn.

In ruminant adequately fed Zn, there is no direct evidence that Ca, phytic acids or other factors in the plant proteins decrease Zn absorption (Mills 1987; Miller, 1970). However in monogastric animals, orthophosphates (Cabell and Earle, 1965) as quoted by Zemel and Bidari, (1983), has been reported to decrease Zn absorption and utilisation in animals. The effects have been reported to be exacerbated by high levels Ca intake which increases faecal excretion of Zn (Suttle *et al* 1970; Zemel and Bidari, 1983). It may be speculated that Ca levels in Group 2 cows were not high enough to prevent Zn absorption in the dry season. Furthermore, it is possible that $CaCO_3$ apart from supplying Ca increased the absorption or availability of Zn by several suggested mechanisms. Calcium or carbonate may compete with antinutritive factor including Fe or Mn, which may be present in pasture or concentrate. Iron concentrations was high in soil (76.4 – 88.9 me/ 100 g), concentrates (364 ppm) and pasture (144 –167 ppm) (Table 3. 7 and 9). Iron and Mn have been reported to decrease Zn absorption and utilisation (Underwood, 1981).

Furthermore, calcium carbonate may buffer rumen and gastrointestinal tract pH thereby retarding the release of iron from food ligands (Prather and Miller, 1992). Iron absorption especially non heme has been shown to be absorbed better at low pH (McDowell, 1992). Another mechanism is formation of insoluble iron salts with the anion from the calcium salt (O' Neil- Cutting and Crosby 1986). A third possible mechanism is that calcium ions may compete with iron for ligands present in the gastrointestinal lumen. This could inhibit iron absorption, if iron is bound to soluble low molecular weight ligands, its displacement by calcium could result in polymerisation or precipitation of the iron thus decreasing availability (Prather and Miller 1992). Moreover, calcium may inhibit Fe absorption by competing with Fe for entry into the mucosal cell or by affecting the rate of transfer of iron from the mucosal cell into the circulation (Barton *et al.*, 1983). It is possible that one or all of these suggested mechanisms could have been in operation hence favouring Zn absorption in Ca supplemented cows. The same mechanism may have been operating in case of P absorption resulting in high plasma Pi in this group.

Plasma Zn concentration in the Ca supplemented cows was lower in July 1997 and 1998 as compared to the other periods (Fig. 13). It is known that many factors other than dietary zinc affect plasma Zn concentrations (King, 1987). Plasma Zn can rapidly drop as a result of a variety of stress such as infectious and environmental insults (Wegner *et al.*, 1973; Corrigall *et al.*, 1976; Dufty *et al.*, 1977, King, 1987). Marked decreases in plasma Zn concentrations have been reported to occur in cattle after experimental intravenous and intramammary administration of endotoxin (Verheijden *et al.*, 1983). Esrkine and Barlet (1993) reported a mean reduction of

28% serum Zn concentrations of prechallenge concentration in experimental induced *Escherichia coli* mastitis. The higher the dose of endotoxin, the lower was plasma Zn concentration. It is possible that the lowered plasma Zn concentration in Ca supplemented cows in July 1997 and 1998 was due to mastitis (Fig. 13). Mastitis cases were high during this period in the Ca supplemented cows.

Plasma Zn was also observed to decline towards the end of the gestation and the first month of lactation in all groups (Fig. 13). A number of the physiological states and hormonal signals such as high level of cortisol or other steroid hormones can trigger metallothionein synthesis and hence reduce plasma or serum Zn concentration in humans and animals (Gibson *et al.*, 1989; Goff and Stabel, (1990); Ruz *et al.*, 1991) therefore a possible reason for a decrease in plasma Zn at this period. It has been stated that cows with hypocalcaemia are likely to be more stressed than normal calcaemic cows and studies indicate that hypocalcaemic cows have higher plasma cortisol concentrations (Goff and Stabel, 1990). In the present study cows that had adequate store of Ca like the Ca supplemented cows did not show a severe decline in plasma Zn concentration. It is possible that cows in the Ca and Zn supplemented groups were less stresses compared to the control and P supplemented cows (Fig. 13).

5.2.4 Supportive indicator of Ca, P and Zn adequacy

Alkaline phosphatase (ALP) activity is used as a supportive measure in experiment designed to determine Ca, P and Zn adequacy in animals, (Boyd *et al.* 1983; Gibson,

1994). In the present study a positive correlation was observed between ALP and plasma Ca in the dry season in Ca ($r = 0.47$, $P = 0.0018$), P ($r = 0.68$, $P = 0.0001$) and Zn ($r = 0.58$, $P = 0.0001$) supplemented cows. However, ALP was negatively correlated to plasma Ca in the rainy seasons ($r = -0.54$, $P = 0.0019$) in the Ca supplemented cows. In the first four months of the dry season Ca levels were low when compared to the rainy season. These observations are in agreement with the findings by other researchers (Boyd *et al.* 1983) who reported a positive correlation between serum ALP and Ca. The negative correlation observed during the rainy season indicates that possibly plasma Ca was low. Hurwitz and Griminger (1961) demonstrated, using chicks fed graded levels of Ca, that elevated plasma ALP activity was indicative of insufficient calcification and that ALP activity appeared to decline in a linear manner until the Ca requirements was met. Positive correlation between ALP and plasma Zn was observed during the rainy season in both the control cows ($r = 0.38$, $P = 0.0388$) and Ca supplemented cows ($r = 0.42$, $P = 0.0206$), during this period levels of plasma Zn were high in the Ca supplemented cows (Fig. 13) hence ALP. Zinc is an intergral part of the enzyme ALP (Nackels, 1994). No significant correlation was observed between plasma Zn and ALP in the Zn supplemented cows. It is known that ALP is influenced by many factors other than Zn such as type of protein, magnesium or manganese deficiency (McDowell, 1992; Chae *et al.*, 2000), hence a possible reason for weak correlation. Non significant correlation was observed between plasma Pi and ALP in all groups of cows indicating that at high levels of Pi there is no correlation. Plasma Pi was within the suggested normal levels throughout the sampling periods.

5.3 EFFECTS OF CALCIUM, PHOSPHORUS AND ZINC SUPPLEMENTATION ON HEALTH AND IMMUNE STATUS IN THE GRAZING CROSSBRED ZEBU COWS

5.3.1 Effect of Ca, P and Zn supplementation on disease conditions

Mastitis and anaplasmosis were the major clinical diseases observed in the lactating cows at this farm (Table 11). Mastitis is one of the disease conditions of the mammary gland affecting milk producing animals (Jain, 1979). Complex interactions between pathogens, nutrition, immunity, milk yield, environment, management and genetic factors have been suggested to be involved in the disease (Bartlett *et al.*, 1992; Sender *et al.*, 1992; Lam *et al.*, 1997). Several alteration in the udder defence mechanisms including decreased level of neutrophils, phagocytic activity and increased cortisol have been proposed to be involved in development of mastitis (Kehrli *et al.*, 1989; Cai *et al.*, 1994).

In the present study fewer cases of mastitis were observed in cows supplemented with Ca and Zn compared to the P supplemented and control cows. High cases of mastitis occurred in the month of July 1997, September 1998 and March 1998 in all groups. These periods coincided with very low plasma Ca (< 1.80 mmol Ca/l) in all groups of cows. However, cows supplemented with Ca had high plasma Ca compared to other groups of cows in other sampling periods, hence less incidences of mastitis. It has been reported that cows with hypocalceamia have a greater chance of having mastitis than if hypocalcaemia is absent (Curtis *et al.*, 1983). Most cows

with mastitis had less than 1.50 mmol Ca/l as compared to non mastitic cows indicating the importance of this mineral in body defence against diseases.

Calcium is an essential nutrient that influences resistance to diseases (McDowell, 1992; Hogan *et al.*, 1996). It is involved in antioxidant systems that maintain the integrity of phagocytic cells and lymphoid tissues (Hogan *et al.*, 1996). Impairments of the antioxidant system can result in a higher incidence and more severe clinical signs of disease (Miller *et al.*, 1996).

Parturient paresis or milk fever is a metabolic disorder of cattle associated with parturition and initiation of lactation. It has been reported that older dairy cows 5 —10 years of age are particularly prone to the disorder (Horst *et al.*, 1990; Underwood and Suttle, 1999). Cows used in the present study were in their first, second and third parity at the beginning of the study, thus, a possible reason for failure to observe any case of milk fever in experimental cows, even in cows which produced 10 litres of colostrum per day. Aged cows are at greater risk of developing milk fever than young cows because both basal and hormonally stimulated intestinal Ca absorption and bone resorption decline with age (Goff *et al.*, 1991). In addition milk production is greater in older cows than in young cows (Littledike and Goff, 1987).

Zinc is among the list of essential nutrients that affect the resistance to diseases (McDowell, 1992; Hogan *et al.*, 1996) in animals. Zinc is important in antibody production and stabilisation of membrane against bacterial endotoxins, (Nackels, 1994). It is involved in antioxidant systems that maintain the integrity of phagocyte

cells and lymphoid tissues (Miller *et al.*, 1996). Impairments of the antioxidant system can result in a higher incidence and more severe clinical signs of disease. Cows supplemented with Ca and Zn had fewer cases of mastitis and anaplasmosis compared to the control and P supplemented cows (Table 11 and Appendices 22 and 23) possibly they had adequate Zn as indicated by high plasma Zn concentration in most of the sampling periods (Fig. 13). Examination of some cows with mastitis revealed low concentration of plasma Zn (< 12.0 µmol/ l) compared to non mastitic cows (>12,0 µmol/ l). Zinc play an important role in the prevention of diseases by stabilising epithelial cells (Moynaham, 1981). Zinc deficiency has been reported to reduce incorporation of amino acids into the skin proteins resulting in parakeratosis with lesions commonly occurring at the teat skin (Miller, 1979). Teat canal keratin, which is composed of desquamified epidermal cells, is the primary barrier to intrammamary infections (Kincaid *et al.*, 1984). Zinc deficiency may alter keratin composition and render the mammary gland more susceptible to infections (Kincaid *et al.*, 1984; Aquilar *et al.*, 1988). It is possible that the low plasma Zn concentration noted especially in the months of November 1997 to March 1998 (Fig. 13) in the control and P supplemented cows could have resulted into disrupting the teat canal keratin rendering the cows vulnerable to intramammary infection and parakeratosis.

Aspartate aminotransferase activity is used in detecting hepatic or muscular injury in domestic animals including cattle (Kaneko, 1989). Among factors affecting the activity of this enzyme include nutritional factors such as different and unstable composition of ration (Kaneko, 1989). In this study P supplemented cows had high ASAT activity and high cases of diseases like mastitis and anaplasmosis possibly

indicating detrimental effect of P supplementation. No correlation was observed between ASAT and Pi in the P supplemented cows but a positive correlation between ASAT and plasma Ca ($r = 0.51$, $P = 0.0005$) and plasma Zn ($r = 0.51$, $P = 0.0001$) was observed in the P supplemented cows in the dry season possibly indicating an indirect effect of P supplementation on Zn and Ca metabolism. This indicates that at low plasma Ca and Zn the activity of ASAT is reduced and *vice versa*. The role of Ca and Zn in the activity of this enzyme is not known. However it is known that Zn and Ca are important minerals involved in a number of enzymatic reactions in the animal body (Kaneko, 1989).

5.3.2 Effect of Ca, P and Zn supplementation on lameness

Dietary Ca, P and Zn level have been shown to affect bone development and associated chemical and physical properties (McDowell, 1992). Chronic inadequacy in dietary Ca, P or Zn supply may be reflected by reduction in mineralisation of bones leading to fractures (Williams *et al.*, 1991a; McDowell, 1992; Underwood and Suttle, 1999). Failure to observe bone fractures possibly indicates that the level of dietary Ca, P and Zn were adequate for bone structure. Baggort *et al.*, (1988) found that high Ca, P and Zn were related to hardness of the claw keratin, whereas low levels were associated with lameness. Poor mineralisation of bone matrix can be reflected in radiological pictures (Williams *et al.*, 1991a). In the present study the claw size, contour and density were normal (Fig.14, 15, 16 and 17) indicating absent of disturbance in mineralisation and osteoid formation. Plasma Ca, Pi and Zn concentrations were above critical levels (Fig.11, 12 and 13) in most of the sampling

period hence ruling out chronic deficiency of Ca, P and Zn. It is possible, that failure to observe any radiological difference was contributed to this factor.

In addition resorption of minerals does not take place equally from different parts of the adult skeleton. The spongy bones, ribs, vertebrae, sternum and cancellous ends of the long bones, which are lowest in ash, are the first to be affected, while the compact shafts of the long bones and small bones of the extremities are the last to be affected (Underwood and Suttle, 1999). This could be another possible reason for failure to observe any radiological change in digital bones.

5.3.3 Effect of Ca, P and Zn supplementation on body weight (BW) and body condition (BCS)

Body condition scoring of cows during the production cycle provides an opportunity to monitor and manage dairy cows by observing body reserves relative to changes in health, reproductive performance and productivity (Ruegg and Milton, 1995). Body condition information has a qualitative as well as quantitative significance. The qualitative information express the body reserve participation in the energy balance (mobilisation or deposition) of the lactating cow (Randel, 1990). The quantitative information expresses the actual reserves at any given time (Ruegg and Milton, 1995). Changes in BCS are related to both liveweight and change in body composition in animals (Wright and Russel 1984; Mertens, 1985). Persistent significant decline in BW and BCS can be considered as an indicator of disease or failure of metabolic processes e.g. poor feed absorption or utilisation (Maltz *et al.*,

1997). A significant reduction in DM intake may be expressed by a decline in BW (Maltz *et al.*, 1992; Spahr *et al.*, 1993). In the present study cows supplemented with Ca and Zn maintained better body weight and body condition in most of the study period compared to the control and P supplemented cows (Fig. 18 and 19). It is possible that cows in the other groups were in negative energy balance and hence scored less and had high cases of diseases. Diseases may have resulted in reduced DM intake, poor absorption and or utilisation of nutrients.

Calcium carbonate apart from supplying Ca has an effect on pH, which has been suggested to improve apparent total starch digestibility and feed utilisation (Wheeler and Noller, 1976, 1977; Nocek *et al.*, 1983; Evans *et al.*, 1984; Wohlt *et al.*, 1986; Clark *et al.*, 1989). It has been suggested by Wheeler and Noller (1977) that $CaCO_3$ supplementation might increase ruminal fluid, cecal and colonic pH thereby increasing microbial activity and starch breakdown by action of α-amylase (Gray, 1992; Harmon, 1993). It is possible that the observed body condition and body weight in the Ca supplemented cows was due to the above mentioned advantage.

5.3.4 Effect of Ca, P and Zn supplementation on percentage packed cell volume (PCV%) and haemoglobin concentration

The normal Hb and PCV% in the literature are highly variable and range from 8.7 to 16.5 g Hb /dl and 32 to 35 PCV% (Schalm *et al.*, 1975; Gentry, 1984). Mean PCV% and Hb were within this range in most of the sampling period (Fig. 20, 21 and Appendices 29 and 30). Mean PCV% and Hb were significant different ($P < 0.05$)

between control and Ca supplemented cows. Control cows had high PCV compared to cows supplemented with Ca, P and Zn in most of the sampling periods (Fig. 20) indicating that Ca, P and Zn supplements have an effect on PCV%. Packed cell volume was negatively correlated to plasma Ca ($r = -0.37$, at $P = 0.0416$) and Pi ($r = -0.34$, at $P = 0.0287$) in the Ca supplemented cows during the rainy and the dry season, respectively. In the dry season Pi was high in Ca supplemented cows (Fig. 12) hence low PCV in this group. In the wet season Pi was low resulting in high PCV%. In addition Hb concentration was positively correlated to plasma Ca ($r = 0.37$, $P < 0.0162$) in the Ca supplemented cows in the dry season but was negatively correlated during the rainy season ($r = -0.35$, $P < 0.0501$). This implies that at low levels of plasma Ca, Hb was low and *vice versa*. Furthermore Hb concentration was negatively correlated to Zn in control group ($r = -0.38$, $P = 0.0136$) during the dry season but was positively correlated to plasma Zn in the Ca supplemented cows during the wet season ($r= 0.46$, $P = 0.0111$). During the dry season control cows had low plasma Zn hence high Hb whereas in Ca supplemented cows plasma Zn was high in the wet season hence raised Hb. This again indicates that Ca, P and Zn supplements have an effect on Hb.

Iron, copper and cobalt are principle minerals required for red blood cell production. Iron is an integral part of haemoglobin and copper is required in haem synthesis whereas cobalt is essential for vitamin B_{12} synthesis in the rumen (Kaneko, 1989). It has been reported that Ca and Zn may reduce Cu and Fe availability (McDowell, 1992) hence a possible reason for low PCV% and Hb in the Ca and Zn supplemented cows compared to the control cows. Haemoglobin and ceruloplasmin

a Cu containing enzyme were negatively correlated to plasma Ca in the control cows in the dry season ($r = -0.38$, $P = 0.0146$), hence a possible reason for a decrease in Hb when plasma Ca was elevated and *vice versa* in this group. Mean haemoglobin concentration in all the groups was reduced in the second year (Fig. 21), when alfalfa was fed to cows from March 1998 to March 1999. Alfalfa contained 1.4 % Ca, which may have reduced the absorption or availability of Cu or Fe resulting in low haemoglobin and erythrocyte synthesis. In addition concentrates mixture composition of maize bran /sunflower seedcake was changed in July 1998 from a ratio of 3:1 to 4:1 due to scarcity of sunflower seedcake. Changing of the ratio worsened the situation by reducing the amount of Fe, Cu and probably Co in the diet. Concentrates in a ratio of 3:1 contained more Fe and Cu than in a ratio of 4:1 (364 ppm Fe, 12.3ppm Cu) vs (262 ppm Fe, 8.0 ppm Cu), respectively.

No significant ($P > 0.05$) correlation was observed between PCV% or Hb and plasma Pi in both the control and P supplemented cows. This implies that P supplementation did not have a direct influence on the level of PCV% or Hb, but possibly it may have an indirect effect through interacting with mineral or nutrients required for erythrocyte and Hb formation resulting in low level of PCV% or Hb. Therefore more research is needed to quantify the low PCV% and Hb in the P supplemented cows.

5.3.5 Effect of Ca, P and Zn supplementation on total white blood cell (TWBC) and differential counts (DWBC)

Normal mean total white blood cell counts in cattle is 8.0 x 10^3 per µl of blood with a range of 4.0 x 10^3 – 12.0 x 10^3 cells per µl of blood (Schalm *et al.*, 1975). Total white blood cell counts were within this range during most of the sampling period (Fig. 22 and Appendix 31). However, for November 1997 and July 1998 TWBC was at the lower end of the normal levels (less than 6.90 x 10^3 cells per µl of blood). Total white blood cell counts is a reflection of the balance between supply and the need for leukocyte functions in the various body tissues (Schalm *et al* ., 1975). Changes in leukocyte counts during stress or diseases are a reflection of systemic stress and participation of various leukocyte types responding to the underlying problem (Tizard, 1982). In the present study cell counts below 7.0 x 10^9 per µl of blood and above 10.0 x 10^3 cell per µl of blood were associated with health problems regardless of supplementation package. Total WBC was drastically reduced in November 1997 and July 1998 probably because of mastitis and anaplasmosis and change of climate. November was the beginning of rainy season whereas July was in the mid of the dry season and it was during these periods when more cases of mastitis were reported (Appendix 31).

Calcium supplemented cows tended to have high TWBC compared to other groups of cows. Total white blood cell counts was positively correlated to plasma Ca in the dry season in both groups of cows, however cows supplemented with Ca had stronger correlation (r = 0.53, P = 0.0003) than P (r = 0.48, P = 0.0013), Zn

(r = 0.44, P = 0.0040) supplemented cows and the control cows (r = 0.38, P = 0.0123). Furthermore, TWBC was positively correlated to plasma Zn in the dry season in Ca supplemented cows only (r = 0.46, P = 0.0023) whereas TWBC was positively correlated to plasma Ca in the rainy season in the control cows (r = 0.37, P = 0.0439). These relations contributed to variations of TWBC between the control and Ca supplemented cows and indicated that Ca is important in WBC production. At low level of Ca as indicated by the plasma levels (Fig. 11) TWBC was reduced and was increased when the plasma Ca level was increased.

No direct correlation was observed between TWBC and plasma Pi in both the control and P supplemented cows. However, TWBC was positively correlated to plasma Ca in the dry season in both group of cows, but cows supplemented with P had stronger relation (r = 0.48, P = 0.0013) than the control cows (r = 0.38, P = 0.0123). Furthermore, TWBC was negatively correlated to plasma Ca (r = -0.43, P = 0.0185) and positively correlated to plasma Zn in the rainy season in P supplemented cows only (r = 0.37, P = 0.0424) whereas TWBC was positively correlated to plasma Ca in the rainy season in the control cows (r = 0.37, P = 0.0439). These correlations contributed to variations of TWBC between the control and P supplemented cows and possibly suggesting an indirect effect of P supplementation on TWBC.

The proportion of lymphocytes among the blood leukocytes in cattle ranges from 2.5 – 7.5 x 10^3 cells per µl of blood with a mean of 4.5 x 10^3 cells per µl of blood (Schalm, *et al.*, 1975). In the present study the lymphocyte numbers were between

1.24 – 9.05 x 10^3 cells per µl of blood. Number exceeding 7.0 x 10^3 per µl of blood and below 3.0 x 10^3 cells per µl of blood were accompanied with mastitis. Lymphocyte counts were positively correlated to plasma Ca in the dry season in cows supplemented with Ca only (r = 0.52, P = 0.0004). However, plasma Pi was positively correlated to lymphocyte count in the control cows (r = 0.40, P = 0.0082) during the same period. The levels of plasma Ca and Pi fluctuated between periods and groups hence contributing to the difference in lymphocyte counts (Fig. 23). No correlation was observed between LC and plasma Ca or Pi or Zn in the P supplemented cows (Appendix 31, 32 and 33) possibly indicating that at high level of P the correlation was diminished.

Low plasma Zn concentration had been reported to be associated with reduced lymphocyte proliferation, decreased antibody production as well as impairment of phagocytic functions i.e ingestion and chemotactic migration (Spears and Kegley, 1991; Nackels, 1994). The reduction in T cell proliferation in Zn deficient animals is attributed to interference of DNA synthesis for leukocytes formation in lymphoid tissues (Kruse –Jares, 1989). In the present study a strongly positive correlation was observed between lymphocyte and plasma Zn in the Ca (r = 0.52, P = 0.0004) and Zn supplemented cows (r = 0.48, P = 0.0067) when compared to the control cows (r = 0.36, P = 0.0477). Calcium and Zn supplemented cows had high plasma Zn in most of the sampling period. This implies that a decrease of plasma Zn may result in a decrease of lymphocytes. These observations are in agreement with those of other researchers (Miller *et al.*, 1996; Hogan *et al.*, 1996) that Zn is very important for the integrity of phagocytic cells.

Neutrophils constitute one of the essential body defence mechanisms against invading microorganisms. The proportion of neutrophils among the blood leukocytes in cattle ranges from 0.6 – 4.0 x 10^3 per µl of blood with a mean of 2.0 x 10^3 cells per µl of blood (Schalm, *et al.*, 1975). In the present study neutrophil counts (NC) numbers were between 0.12 – 7.57 x 10^3 cells per µl of blood. Number exceeding 4.0 x 10^3 cells per µl of blood and below 1.0 x 10^3 cells per µl of blood were accompanied with mastitis. In most sampling period Ca supplemented cows tended to have high NC when compared to the control cows. Neutrophil counts were positively correlated to plasma Ca in the dry season in cows supplemented with Ca only ($r = 0.30$, $P = 0.0494$) but the relation was weak and non significant in control cows ($r = 0.27$, $P = 0.0789$). Plasma Pi was negatively correlated to NC in control cows ($r = -0.32$, $P = 0.0353$) during the same period. Furthermore, NC was positively correlated to plasma Zn in dry season in Ca supplemented cows only ($r = 0.43$,$P = 0.0039$). Calcium supplemented cows had low plasma Ca (Fig. 11), high levels of Pi (Figure 12) and Zn (Fig. 13) during the dry season compared to control cows. It has been reported that adequate Zn may result in high neutrophils and lymphocytes cells production in the blood (Droke *et al.*, 1993) hence, the reason for high neutrophils cell counts in Ca supplemented cows.

No correlation was observed between Pi and NC counts in the P supplemented cows however plasma Pi was negatively correlated to NC in the control cows ($r = -0.32$, $P = 0.0353$) during the rainy season possibly indicating that at low P supply, the level of NC decreases. Hence a possible reason for low NC in the control cows during this period. Neutrophil counts were positively correlated to plasma Ca in the

dry season in cows supplemented with P only (r = 0.32, P = 0.0424). Furthermore, NC was positively correlated to plasma Zn in the dry season in P supplemented cows (r = 0.33, P = 0.0325) indicating that P supply affected plasma levels of Ca and Zn. which are important in production of neutrophils (Droke *et al.*, 1993). Hence a possible reason for NC fluctuation between periods when plasma Ca and Zn changed (Fig. 24).

There are comparatively few eosinophils in the circulation in healthy animals, yet numbers are sufficient to reflect significant changes in the pathophysiology under certain conditions (Schalm, *et al.*, 1975). The proportion of eosinophils among the blood leukocytes in cattle varies with the parasite or stress burden of an animal but ranges from 0 – 2.4 x 10^3 per µl of blood with a mean of 0.7 x 10^3 per µl of blood (Schalm, *et al.*, 1975). In the present study the eosinophil counts were between 0.05 – 1.5 x 10^3 cells per µl of blood (Fig. 24 and Appendix 36). Calcium supplemented cows tended to have a low EC. Negative correlation between EC and plasma Ca (r = - 0.66, P = 0.0001) and plasma Pi (r = -0.53, P< 0.0024) in the rainy season were observed in Ca supplemented cows. During these periods plasma Ca and plasma Pi were high when compared to the control cows. The role of Ca supplementation in this process is not known. However fewer diseases were observed in this group and no cases of parakeratosis were observed.

No significant difference on EC was observed between the control and P supplemented cows however, P supplemented cows tended to have high EC (Fig. 23 and Appendix 36). Positive correlation between EC and plasma Zn (r = 0.44,

P = 0.0146) was observed in the rainy season in the P supplemented cows, possibly indicating the effect of P supplementation on zinc nutrition on the animal. During this period an increase in plasma Zn resulted in an increase in the number of EC. The mechanism involved is not known but probably P supplementation increased absorption of Zn in the cows resulting in an increase in EC counts.

No significant variation was observed in eosinophil count between the control and Zn supplemented cows. However, Zn supplemented cows tended to have low EC. A negative correlation between EC and plasma Zn was obseved (r = -0.49, P = 0.0060) and Pi (r = -0.47, P = 0.0084) in the dry season in Zn supplemented cows. During this period decrease in plasma Zn resulted in high number of EC while an increase in plasma Zn resulted in low EC. Furthermore, cows in the Zn supplemented group were in positive energy balance as they scored high in BCS (Fig. 19) compared to the control cows, hence an additional possible reason for low incidences of mastitis and anaplasmosis compared to the control animals.

The proportion of monocytes among the blood leukocytes in cattle ranges from 0 025– 0.84 x 10^3 per µl of blood with a mean of 0.4 x 10^3 per µl of blood (Schalm *et al*, 1975). In the present study the monocyte numbers were between 0.03 – 1.5 x 10^3 per µl of blood. Monocytosis generally reflects chronicity of disease conditions (Kaneko, 1989), for instance it is a characteristic feature of chronic suppurative and granulomatous diseases (Schalm, *et al.*, 1975). A small increase in MC was observed in Ca supplemented cows in July 1998 and September 1998 due to mastitis (Appendix 23). A temporary small increase in MC was observed in P supplemented

cows in July 1997, November 1997 and January 1998 due to mastitis (Appendix 23). No cases of chronic monocytosis were observed in the Zn supplemented cows, indicating absent of chronic diseases in this group.

In general regardless of the supplementation package the mean neutrophil and monocyte counts were reduced in all groups of cows, with a marked increase in neutrophils and monocyte before parturition (January 1998 and 1999) and reduced neutrophils after parturition (March 1998). However, the number of lymphocyte and eosinophils were low before parturition but high after parturition. In a study by Moreira da Silva *et al.* (1998) in which 10 cows, from one week prior to calving to eight weeks after parturition were used; a decrease in total leukocyte before parturition and after three weeks after calving was observed. Lymphocytes and neutrophils showed an analogous trend to that of the total leukocytes but monocytes and eosinophils did not change during the entire period. In the present study total and differential WBC cell counts were taken at seven to fourteen days before calving and after calving hence a possible reason for lack of agreement with the findings by Moreira da Silva *et al.*, (1998).

Stress has been shown to influence the total leukocyte count (Schalm, *et al.*, 1975; Tizard, 1982; Kaneko, 1989). Increased susceptibility to intramammary and other infections is known to occur more frequently in the peripartum period in contrast to any other period (McDonald and Anderson, 1981; Saad *et al*; 1989, Burvenich *et al.*, 1995). Physiological stress due to lactation (Paape *et al*, 1974) and a decrease in the number of circulating neutrophils capable of phagocytosis and bacteria elimination

(Newbold, 1976; Guidry *et al.*, 1976) are main factors leading to increased diseases susceptibility. It is known that many of the hormonal and metabolic changes that prepare the mammary gland for lactation take place three weeks preceding parturition (Kaneko, 1989). During this critical period, the animal's body metabolism shifts from demands for pregnancy to those for lactation with increased body demands for energy and protein. The negative energy and protein balances that exist during early lactation may also contribute to the impaired lymphocyte and neutrophil function and thus account for some of the periparturient immunosuppression (Kaneko, 1989).

5.3.6 Effect of Ca, P and Zn supplementation on serum immunoglobulin (SIM)

The term immunoglobulin is used to describe all proteins with antibody activity as well as some proteins that have the characteristic immunoglobulin structure but do not have antibody activity (Tizard, 1982). Immunoglobulin classes and sub-classes are important in mediating protection of the body against infectious diseases (Kaneko, 1989). The deficiencies of immunoglobulin will make an animal succumb to various infections (Tizard, 1982). In the present study Ca supplemented cows had high immunoglobulin concentrations (Fig. 27) compared to other groups of cows, hence fewer cases of diseases. Positive correlation were observed between SIM and plasma Ca in the dry season ($r = 0.36$, $P = 0.0209$) and plasma Zn in the rainy seasons in the control cows ($r = 0.45$, $P = 0.0124$). Negative correlation between SIM and plasma Ca ($r = -0.43$, $P = 0.0177$) and plasma Zn ($r = -0.40$, $P = 0.0284$) in the rainy

season were observed in Ca supplemented cows. This possibly indicates that Ca and Zn are important in immunoglobulin metabolism.

Calcium apart from being involved in production of a number of binding proteins such as superoxide oxidases, it is important for activation of neutrophils (Sohnle *et al.*, 1991, 1996; Srivastava *et al.*, 1994; Sandholm *et al.*, 1995). Activated neutrophils have the capability to secrete interleukin –1 an important regulatory cytokine involved in proliferation and differentiation of T and B lymphocytes, (Canning and Neill, 1989; Hamblin, 1988; Dinarello, 1987). Lymphocytes of B type are important for antibody production, whereas lymphocyte of T cells have antigen receptors, which share some characteristics with immunoglobulin receptors present on B cell membranes (Kaneko, 1989) hence a possible reason for high SIM in Ca supplemented cows.

Negative correlation was observed between SIM and plasma Pi in P supplemented cows during the dry season ($r = -0.43$, $P = 0.0177$). Furthermore, plasma Pi was negatively correlated to plasma Zn in the P supplemented cows during the rainy seasons ($r = -0.64$, $P = 0.0124$). All these correlations possibly indicates that P and Zn are important in determining the level of immunoglobulin. In the present study, P supplemented cows had high immunoglobulin concentrations (Fig. 27) compared to the control cows.

5.3.7 Effect of Ca, P and Zn supplementation on proteins and energy metabolism in cows

Normal plasma protein concentration in cattle is between 68 – 85 g /l (Schalm, *et al.*, 1975). In the present study mean plasma protein ranged between 80 - 114 g /l. Total plasma protein tended to be high in Ca supplemented cows as compared to the control cows in most of the sampling periods (Fig 28). Calcium is important in a number of enzymes involved in protein metabolism or utilisation (Underwood and Suttle, 1999) hence a possible reason for high TP. Furthermore, it is supported by a negative correlation between TP and plasma Ca in the dry season in the control cows receiving no mineral supplementation ($r = -0.35$, $P = 0.0249$). In the control cows TP was low as compared to Ca supplemented cows. However, TP was positively correlated to plasma Ca in both groups of cows in the rainy seasons with a stronger correlation in Ca supplemented cows ($r = 0.80$, $P = 0.0001$) than in the control cows ($r = 0.38$, $P = 0.0376$) indicating the importance of Ca in TP. In addition, a positive correlation between TP and plasma Pi ($r = 0.47$, $P = 0.0218$) was observed in the rainy season in Ca supplemented cows only. Calcium supplemented cows had high plasma Pi when compared to the control cows (Fig.12) possibly indicating interactions between Ca and P.

No direct correlation was observed between plasma Pi and plasma protein in P supplemented cows during the dry season, however Pi was positively correlated to TP ($r = 0.43$, $P = 0.0164$) during the rainy season. Possibly indicating that at low levels of Pi there is a decrease in TP but when the level of Pi are increased the correlation

disappears, hence a possible reason for high TP during dry season when Pi was high. Furthermore, plasma total protein was negatively correlated to plasma Ca in the dry season in both the control (r = -0.34, P = 0.0249) and the P supplemented cows with a stronger correlation in P supplemented cows (r = -0.53, P = 0.0003) (Appendix 40). However, during the rainy season a positive relation was observed between plasma Ca and TP with P supplemented cows (r = 0.56, P = 0.0012) having a stronger correlation than the control cows (r = 0.38, P = 0.0376). It is possible that P supplementation indirectly affected plasma Ca balance which resulted in change of TP. Phosphorus is important in formation and utilisation of energy and protein (Ternouth and Sevilla 1990b). Concentration of protein in the plasma at any given time is a function of the hormonal balance, nutritional status, water balance, and other factors affecting the state of health of the animal (Schalm *et al.*, 1975), it is possible that these factors modified the relationships that were observed.

Zinc is critical for the metabolism of many nutrients including proteins and carbohydrates (Spears, 1994). Zinc as a component of RNA and DNA polymerase is involved in protein biosynthesis (Miller and Miller, 1962). Plasma total protein in the present study was not correlated to plasma Zn in the Zn supplemented cows. Plasma Zn is mostly used as an index of Zinc status in animals however, it has several limitations (Gibson, 1994). The levels of Zn in plasma may be affected by other conditions unrelated to Zn nutrition sucha as diseases like mastitis caused by bacteria endotoxins and physiological state of the animal such as lactation (King, 1987). Therefore failure to see any correlation in this group.

Serum or plasma proteins constitute a portion of the amino acid pool of the body (Doornenbal *et al.*, 1988). The body tends strongly to conserve its proteins (Kaneko, 1989). Any excess of amino acids over that necessary to maintain the circulating pool is quickly converted to carbohydrate or fat and utilised for energy supply (Kaur and Arora, 1995). In the present study, in most occasions the level of total plasma protein was above the normal range (Fig. 30). One possibility for high plasma protein might be that either the animals were given excess digestible proteins in the first year, when the ratio of maize bran /sunflower seedcake in concentrates was 3:1. When the ratio was changed to 4. 1 in the second year plasma proteins decreased to normal levels in all groups (Fig. 30).

Plasma urea (Plu) provides information on nitrogen losses following absorption from the gut particularly the rumen (Annison and Bryden, 1999). Normal range of urea varies from 3.3 – 6.0 mmol /l for dairy cows (Carlsson, 1994;Whitaker 1998). If these values are considered applicable to the cows in this study then all groups of cows had low Plu in the dry season and high levels of Plu during the rainy season (Fig. 31). Ammonia in the blood is used in formation of urea in the liver and it comes mainly from the rumen and lower gut and in part from the breakdown of amino acids stored in the body reserves (Hungington, 1989; Van der Walt, 1993; Hirvonen *et al.*, 1999). Part of the ammonia may also originate from urea returned to the alimentary tract via blood and saliva (Reynolds, 1995). The amount of ammonia produced in the rumen is dependent on the amount of rumen degradable protein (RDP) in the diet. This ruminal ammonia can be converted into microbial protein provided that there is sufficient readily fermentable energy (FME) to the microbes

(Meijer *et al.*, 1990). Although there was no significant difference between the control and the Ca, P and Zn supplemented cows in Plu there was a tendency towards low Plu in the control and Ca supplemented cows. Plasma urea was negatively correlated to plasma Ca ($r = -0.40$, $P = 0.0087$) in the Ca supplemented cows during the dry season. Furthermore, plasma urea was positively correlated to plasma Pi in both the control ($r = 0.45$, $P = 0.0131$) and Ca supplemented cows ($r = 0.41$, $P = 0.0248$) during the rainy season. A weak positive correlation between Plu and plasma Zn in the rainy season was observed in the Ca supplemented group ($r = 0.35$, $P < 0.0589$). The significance of these observation is not clear. However, it may be suggested that low urea in the Ca supplemented cows could be due to better utilisation of protein with resulting ammonia being shifted to microbial protein rather than urea production.

Ammonia, which is not used by the microbes, will be absorbed from the rumen, transformed into urea in the liver and passed into the blood (Perhason, 1996). A positive correlation between the concentration of ammonia in the ruminal fluid and the concentration of urea in blood has been observed by Gustafsson and Palmquist (1993). Excess of RDP and adequate FME or adequate RDP and shortage of FME in the diet gives rise to a high concentration of urea in blood and milk (Oltner and Wiktorsson, 1983; Refsdal *et al.*, 1985; Ferguson *et al.*, 1988). A low blood urea can come about because the diet is short of RDP or because the cow did not eat as much of the crude protein component of the ration (Whitaker, 1998). Therefore, the concentration of urea in blood and milk is affected not only by dietary intake of digestible crude protein but also by the balance between the quantities of energy and

protein in the diet (Carlsson, 1994; Whitaker, 1998). Therefore, it is possible that the low blood urea observed in the dry season in all groups of cows was due to low crude protein in the diet caused by reduced availability of feed. The period coincided with the dry period where pasture availability was scarce. It is also possible that the high level of urea during rain season was due to excess of RDP or shortage of FME. It is known that cows grazing lush pasture of high protein and non protein N content often exhibit raised NH_3 levels, which result in high rates of urea production (Lobley *at al.*, 1996). Hence a possible reason for high level of urea in November 1997 as the period coincided with the beginning of the rainy season.

Temporary increase in urea in moderately and severely affected mastitic cows has previously been reported (Hirvonen *et al.*, 1999). It has been suggested that endotoxin shock may decrease glomerular filtration rate and cause prerenal azotemia (ureamia) thus increasing serum urea, (Coles, 1986). It is possible that mastitis and contributed to high levels of urea in P supplemented cows (Fig. 31) which had high cases of mastitis (Table 11).

No correlation was observed between plasma Pi and Plu in P supplemented cows in both the dry and the rainy season. However, plasma urea was negatively correlated to plasma Ca ($r = - 0.36$, $P = 0.0176$) in P supplemented cows during the dry season. During the dry season plasma Ca levels were low in the P supplemented cows. The significance of these observations is not clear, it is suggested that P supplementation may affect Ca balance resulting in change in Plu.

Glucose concentrations in whole blood or plasma from cows have been used extensively as an indicator of energy status, (Rowlands, 1970). Normal plasma glucose concentration in cows range between 2.5 and 4.16 mmol/l (Kaneko, 1989). In the present study plasma glucose ranged between 2.86 and 4.08 mmol/l in the control and Ca supplemented cows. Cows receiving Ca had high Glu compared to the other groups of cows in most of the sampling periods (Fig. 32).

Calcium is important in energy metabolism, hypocalcaemia may induce negative energy balance (Daniel, 1983). Direct relationship between plasma glucose and Ca concentration has been reported (Jonsson, 1999). Littledike *et al.* (1970) demonstrated a significant and steeply negative, linear relationship between plasma Ca concentration and glucose concentration at low levels of plasma Ca. However, in the same study the relationship was not evident at normal levels of plasma Ca concentration. A strong negative correlation observed in the Ca ($r = -0.76$, $P = 0.0001$), P ($r = -0.70$, $P = 0.0001$) and Zn ($r = -0.82$, $P = 0.0001$) supplemented cows during the dry season in the present study supports the observations by Littledike *et al* (1970). No correlation was observed during the rainy season when plasma Ca levels were high in the Ca and Zn supplemented cows. Furthermore plasma Glu was positively correlated to plasma Pi ($r = 0.44$, $P = 0.0146$) and plasma Zn ($r = 0.47$, $P = 0.0086$) in the Ca supplemented cows during the rainy season. Cows supplemented with Ca had high plasma Pi and plasma Zn compared to the control cows hence a possible reason for having high plasma glucose. In addition, plasma glucose was negatively correlated to plasma Ca in the rainy season ($r = -0.53$, $P = 0.0023$) in the P supplemented cows. Furthermore

plasma Glu was negatively correlated to plasma Zn ($r = -0.46$, $P = 0.0024$) in the P supplemented cows during the rainy season. Hence a possible reason for having low plasma glucose when plasma Ca and Zn was reduced.

Furthermore, hypocalcemia has been reported to be associated with reductions in plasma insulin release resulting in hyperglycaemia (Littledike *et al.*, 1970; Blum *et al.*, 1972; Wollheim and Sharpe, 1981). It is possible that high Glu levels in some period was due to reduction in insulin. It is not clear whether hyperglycaemia was due to alterations in insulin secretion or insulin binding (Jonsson, 1999). The role of Ca in insulin secretion needs more research as emphasised by the relationship of Ca to glucose metabolism.

In addition, it has been reported that on average 5 – 20% of starch consumed by cattle is digested post ruminally (Huntington, 1997). The assimilation of starch as glucose is energetically more efficient than fermentation to short chain fatty acids in the rumen or lower gut (Preston and Leng, 1987). In a review of production data from cattle fed on maize or sorghum based diets, Owen *et al.* (1986) concluded that starch digested in the small intestine provided 42% more energy than starch digested in the rumen. It is possible that the high glucose content in cows during the study period especially the dry season was due to additional concentrate in the diets. Concentrate was made by a mixture of maize bran and sunflower seedcake at a ratio of 3: 1.

Plasma glucose was low in March 1998 in all groups and this period coincided with the calving period and early lactation. In early lactation, energy derived from the

intake of feed is less than what is required for maintenance and milk yield. Utilisation of body reserves resulting in a loss of condition and body weight is necessary to meet energy and protein requirement of peak milk production (Haresign, 1981). Peak milk yield is reached four to seven weeks after parturition whereas the highest food intake is not reached until eight to ten weeks after calving (Foster, 1988). Due to the negative energy balance the blood concentration of glucose falls and the body reserves of fat and protein are mobilised (Bergman 1983, Blum *et al.*, 1983).

In the present study, the level of plasma glucose was high during the first year except for P supplemented cows compared to the second year (Fig. 32). Phosphorus supplemented cows had low plasma glucose as in the first year. One possibility for high plasma glucose might be that either the animals were given excess energy in the first year, when the ratio of maize bran /sunflower seedcake in concentrates was 3:1. When the ratio was changed to 4. 1 in the second year plasma glucose decreased in all groups (Fig. 32).

5.4 EFFECTS OF CALCIUM, PHOSPHORUS AND ZINC SUPPLEMENTATION ON MILK PRODUCTION IN THE GRAZING CROSSBRED ZEBU COWS

5.4.1 Effect of Ca, P and Zn supplementation on milk yield

Milk yield is not only influenced by general and healthy status of the mammary gland but also by nutrients supplied to the animal particularly energy and protein,

vitamins and minerals (McDowell, 1992). There is approximately 1 g of calcium in every 1 kg of milk. (NAS, 1988). Calcium input must be continuous to meet the needs of the cow for high milk production. Feeding a diet low in Ca to lactating cows may cause a depletion of Ca from bones, and reduced milk yield (McDowell, 1992). In the present study cows supplemented with Ca in the form of $CaCO_3$ had significant ($P< 0.05$) high milk yield (2831 l) compared to the control cows (2332 l). Increase in milk yield when cows were fed 0.9% coarse $CaCO_3$ has also been reported by Wohlt *et al.* (1986). The mechanism behind is not yet known, however it has been suggested that feeding $CaCO_3$ improve apparent total tract starch digestibility and feed utilisation (Wheeler and Noller, 1976 and 1977; Clark *et al.*, 1989).

It has long been known that milk formation depends on glucose supply, which is the precursor for lactose formation (Linzell and Peaker, 1971; Pereka, 1991). Lactose accounts for 60% - 85% of milk components (Bergman, 1983; Blum *et al.*, 1983). It is possible that the high milk observed in cows supplemented with Ca was due to high glucose, which was high in this group compared to other groups. It is supported by a reduction in milk yield when plasma Ca was low as indicated by a negative correlation between milk yield and plasma Ca in the Ca ($r = - 0.36$, $P = 0.0191$) and P ($r = - 0.35$, $P = 0.0302$) supplemented cows

The average lactation length was short for all groups of cows i.e. the control (267 days), Ca (268), P (252) and the Zn (298) supplemented cows. Disease and feeding management may have modified the lactation length. It is known that high energy

intake can result into excessive weight gain resulting in reduction of milk production and later complete cessation of milk secretion (Julien *et al.*, 1977). Cows in Ca supplemented group gained more weight as indicated by body condition score of 4.0 in a five scale point in their third lactation stage and stopped milk production. This period coincided with the dry season where concentrate amount was increased and hay was fed to the animals. However, in the control cows mastitis (15 cases) was a big problem, a significant reduction in milk yield was observed when mastitis occurred. The reduction on milk yield depended on stage of lactation, number of teats involved and severity of the infection. Cows supplemented with Ca and Zn had few mastitis cases (7 cases) each compared with the control cows (15 cases) and P supplemented cows (16) hence, more milk in the latter group.

Body condition of the dairy cow must be optimal during each stage of lactation cycle for maximal return. The average lactation length and milk yield was short for the control and the P supplemented cows in the second year probably because of mastitis and poor body condition (Fig. 19).

The effect of P on milk production has been disputed (Karn, 2001). No conclusive evidence was reported by Cohen (1975) to indicate that P supplementation increased milk production in cows. In another study by Davison *et al.*, (1986) P supplementation of cows grazing tropical grass legume pastures increased milk production, but in later research Walker *et al.*, (1997) found supplementing P to cows grazing P fertilized pastures had no effect on milk yield. The inconsistent responses were either due to low protein or energy, not P, being the first limiting

nutrient, P availability among forages, interactions with other minerals such as Ca or Zn (Karn, 2001). It is possible that in the present study low milk production was contributed by mineral interactions as indicated by low Ca/P ratio (Appendix 54).

Furthermore, there was no direct correlation between milk yield and glucose in the P supplemented cows however, a negative correlation was observed between plasma Zn and plasma glucose (r = -0.53, P= 0.0023). Zinc is important for glucose metabolism, as it has been shown that Zn deficiency may cause impairment of glucose metabolism (Kaneko, 1989). The impairment in glucose metabolism is reported to be secondary to reduced insulin release. It is possible that P supplementation affected Zn metabolism resulting into low glucose, hence milk yield.

5.4.2 Effect of Ca, P and Zn supplementation on milk proteins and fat

Milk protein accounts for about 25% of total milk solids and is now a more valuable component of milk than fat (Rosen *et al.*, 1996). *De novo synthesis* of proteins and fatty acids in the mammary gland depends on the presence of insulin, glucocorticoids and prolactin (Speake *et al.*, 1976; Pereka, 1991; Rosen *et al.* 1996). Synthesis is concomitantly decreased in cows in negative energy balance (DePeters and Cant, 1992; Graml, 1993; Palmquist *et al.*, 1993; Franzen, 1994; Sandholm *et al.*, 1995). It is possible that the high milk protein and fat observed in Ca supplemented cows was contributed by positive energy and protein balance during most period of the study. It was only in the Ca supplemented cows where plasma

protein was positively correlated to milk fat ($r = 0.54$, $P < 0.001$) and milk proteins ($r = 0.38$, $P < 0.01$). Furthermore milk butterfat was positively correlated to plasma Ca in the rainy season in the Ca supplemented cows ($r = 0.80$, $P < 0.0004$) and control cows ($r = 0.65$, $P < 0.0048$) and it was in these groups where MF was high as compared to Zn and P supplemented cows.

There was no positive correlation between plasma Zn and milk fat or protein, however a negative correlation was observed between plasma Zn and milk protein ($r = -0.36$, $P = 0.0394$) in the P supplemented cows. It is possible that P supplementation affected Zn metabolism resulting into low milk protein. It has been reported that synthesis of milk protein and fat decreases in cows in negative energy balance and Zn deficiency (DePeters and Cant, 1992; Graml, 1993; Palmquist *et al.*, 1993; Franzen, 1994). It is possible that the low milk protein and fat observed in P supplemented cows was contributed by negative energy and low plasma Zn during most periods of the study in P supplemented cows (Fig. 13, 20, 21). This is supported by a positive correlation between plasma Zn and milk fat ($r = 0.56$, $P = 0.0230$) and a negative correlation between milk proteins and plasma Pi ($r = -0.37$, $P = 0.0313$) and Zn ($r = -0.56$, $P = 0.0394$) during the rainy season where plasma Pi and Zn were low. High dietary Zn may lead to reduction in milk fat as indicated by negative correlation between plasma Zn and milk fat in the Zn supplemented cows ($r = -0.66$, $P = 0.0056$) during the rainy season.

5.5 EFFECTS OF CALCIUM, PHOSPHORUS AND ZINC SUPPLEMENTATION ON REPRODUCTIVE PERFORMANCE IN THE GRAZING CROSSBRED ZEBU COWS

Minerals are an important factor, which influence the reproductive performance of cattle (Call *et al.*, 1986; McDowell, 1992; Underwood and Suttle, 1999). Hypocalcaemia episodes have been reported to be associated with poor reproductive performance (Scharp, 1980; Mckay, 1994). It was observed by Scharp (1980) that cows with an average serum Ca concentrations of 2.27 mmol Ca /l, required only one service per pregnancy as compared to cows which had serum concentration of 2.17 mmol Ca /l requiring more services. Wards *et al.* (1971) found that cows fed more Ca ovulated earlier compared to inadequate Ca fed cows (14 Vs 22 days post partum) and the interval from calving to conception was also shorter by 37 days in cows supplemented with vitamin D. Jonsson *et al* (1999) measured Ca in 161 cows over 35 to 60 days after calving and observed that, cows in which plasma Ca concentration fell below 2.00 mmol /l on at least one occasion outside the first three days after calving were likely to ovulate later than cows not experiencing subclinical hypocalcaemia. There was no significant variation ($P > 0.05$) in reproductive performance between the control and mineral supplemented cows. However, the Ca supplemented cows tended to have short calving intervals and returned to oestrus earlier (364 days, 30 days) when compared to the control cows (393 days, 69 days), P (393 days, 45 days) and Zn (386 days, 53 days) supplemented cows. This was possibly due to better body condition, fewer cases of diseases such as mastitis and

anaplasmosis and positive energy balance as compared to the another groups of cows.

There are several mechanisms suggested by which hypocalcaemia might be expected to impair reproductive performance. These include poor uterine contractility, effects on energy balance, reduced blood flow to hypothalamus and ovary and interference with normal endocrine signal transmission at cellular level (Jonsson, 1999). The ability of hypocalcaemia to reduce ovarian blood flow has been demonstrated by Jonsson and Daniel (1997). Kamgarpour (1996) recently examined the follicular dynamics of hypocalcaemia and normal calcaemic cows and found that hypocalcaemic cows had significant ($P< 0.05$) lower mean numbers of ovulatory size follicles during the first 30 days post partum than normal cows. Plasma progesterone concentration as a function of corpus luteum during dieoestrous was also reduced. Therefore, it is possible that supplemented calcium contributed to the high reproductive performance observed in the supplemented cows.

Whitman (1975) found that cows calving at body condition scores of 7 to 9 in a nine scale (1 emaciated and 9 obese) were capable of returning to oestrus within 60 days after calving regardless of pre and postpartum change in body weight. In the present study, cows which had a pre partum body condition score of 3.5 or 4 which corresponded to score 6 and 7 of Whitman (1975) returned to oestrous in less than 35 days and most of them were in the Ca supplemented groups. Dziuk and Bellow (1983) and Richard *et al.* (1986) suggested a minimum body condition score of ≥ 5 (in a nine scale) which correspond to ≥ 3 in a five point scale with half points at

calving. These authors indicated that this minimum body condition score would ensure that body stores of nutrients are adequate for postpartum reproductive performance. The present study confirms these findings in that cows which returned to oestrous in less than 35 days had body condition score of ≥ 3 before calving. It is possible that poor body condition and negative energy balance were responsible for poor reproductive performance in the control cows.

Poor reproduction has been a regular feature of grazing cattle, goats and sheep confined to P deficient pasture (Underwood and Suttle, 1999). The subnormal fertility is associated with wide range of conditions including low ovarian activity and low conception rates (Read *et al.*, 1986a; Call *et al.*, 1986). The present results indicates that the P supply given to these cows was adequate.

5.6 EFFECT OF CALCIUM, PHOSPHORUS AND ZINC SUPPLEMENTATION ON COPPER AND SELENIUM BALANCE IN THE GRAZING CROSSBRED ZEBU COWS.

5.6.1 Copper

Ceruloplasmin is an extracellular antioxidant and free radical scavenger, (Yang *et al.*, 1996) and it accounts to 75 – 90 % of plasma Cu (Blakely and Hamilton, 1985). Activities of ceruloplasmin in plasma associated with Cu deficiency are below 40 IU/l of plasma and when the activity reach below 5 IU /l of plasma it indicates severe deficiency of Cu (Paynter, 1987). In the first month of the experiment, initial

mean ceruloplasmin activity was above 40 IU /l (Fig. 33). This was an indication that cows were in a positive Cu balance. Following supplementation with minerals, the activity of ceruloplasmin was maintained in the P supplemented cows but was reduced in the control, Ca and Zn supplemented cows. Levels were reduced in all groups in November 1997. Only cows supplemented with Zn maintained a stable ceruloplasmin activity from November 1997 to September 1998 indicating protective effect of Zn supplementation on changing ceruloplasmin activity. However the activity, was below the normal suggested range (40 IU /l) (Fig. 33). In the dry season plasma Cp was positively correlated to plasma Pi ($r = 0.41$, $P < 0.0068$) and plasma Zn ($r = 0.42$, $P < 0.0053$) in Ca supplemented cows. Possibly indicating that at high levels of P and Zn as indicated by plasma Pi and Zn the activity of Cp in the Ca supplemented cows was increased either due to P and Zn inhibiting Ca absorption. It has been reported that Cp activity may be reduced due high Ca, which may impair Cu absorption (Paynter 1987; McDowell, 1992). However, in the rainy season Cp was negatively correlated to plasma Pi ($r = -0.47$, $P < 0.0090$) and plasma Zn ($r = -0.66$, $P < 0.0001$) resulting in lower plasma Cp activity. No correlation between plasma Ca, Pi, Zn and ceruloplasmin was observed in the control and P supplemented cows in both the dry season or rain season. However, a positive correlation ($r = 0.31$, $P < 0.0447$) was observed between plasma Ca and Cp in the Zn supplemented cows during the dry season. All these relations may explain the fluctuation of ceruloplasmin in different groups. This possibly indicates that Cp activity is not affected only by minerals but also by other unknown factors.

High Cp in plasma of mastitic cows has been reported by Conner *et al.* (1986). It is possible that the high Cp observed in September 1997 in some cows was contributed by mastitis. The mechanism involved in release of Cp in mastitic cows is not clear. Chassagne *et al.* (1998) suggested that higher Cp activity in mastitic cows could reflect the activation of a Cp dependent mechanism of defence against oxidative stress in the subsequently mastitic cows. Increased Cp activity will eventually compensate the decreased efficiency of other antioxidant systems due to inadequate dietary supplies.

5.6.2 Selenium balance

Selenium exerts important biological functions in mammals via the enzyme glutathione peroxidase GSH.Px (Levander, 1987). Glutathione peroxidase is the only selenoenzyme well characterised in higher animals (Kaneko, 1989). It has been firmly established that the blood Se concentration and the activity of the GSH.Px in whole blood or erythrocytes is highly correlated in many species including cattle (Levander, 1987). The normal range in cattle is above 130 IU / gHb according to RANSEL kit manufacturers (RANSOD, 1994). In the present study the initial levels (132 - 138 IU / gHb) implies that all cows were in their normal GSH.Px (Table 16), which corresponded to selenium sufficiency. The present initial levels are lower compared to previous reported levels in heifers (254 IU /gHb) at the same farm (Phiri, 1995). It has been reported that certain protein sources, notably linseed oil meal, exert some protective action against selenosis by inhibiting Se uptake (McDowell, 1992). It is possible that sunflower seed oil may have the same effect, hence possible reasons for low GSH.Px activity in cows. Heifers at the previous

study were given a concentrate mixture of cotton seedcake twice per week compared to cows in this study in which they were fed 1-2 kg per day of concentrate mixture made by mixing maize bran and sunflower seedcake at a ratio of 4:1.

In general following supplementation with minerals the levels of GSH.Px was increased in all groups. Plants, which accumulate Se, usually remain green during the dry season when other forages are not available (McDowell, 1992). It is possible that the increased GSH.Px in cows in September 1997 was probably due to consumption of these plants. Identification of Se accumulator plants was not carried out.

In January 1998 GSH.Px were reduced to 117 IU/ gHb in Ca and Zn (147 IU/ gHb) supplemented cows (Table 16), the period coincided with the rainy season. This indicates that different mineral combinations have an effect in plasma GSH.Px. High amount of Ca and Zn may inhibit Se absorption (House and Welch, 1989; Hogan *et al.*, 1996). It possible that the reduction of GSH.Px in these groups was contributed by the amount of Ca and Zn, which was high in pastures during the rainy season.

5.7 EFFECT OF CALCIUM, PHOSPHORUS AND ZINC INTERACTIONS IN CROSSBRED GRAZING ZEBU COWS

There was no observed improvement in plasma Ca or Pi or Zn in all the groups of cows fed different combinations of Ca, P and Zn when compared to the Ca supplemented cows (Fig. 34, 35 and 36). A large excess of either Ca or P or Zn

interferes with the absorption of the other leading to lower plasma concentration (De boer *et al.*, 1981; McDowell, 1992; Underwood and Suttle, 1999). There was no observed change in the plasma Ca/P ratio when compared to single element supplementation (Appendix 54). However a positive correlation between plasma Ca and plasma Pi was observed only in the P/Zn supplemented cows ($r = 0.66$, $P = 0.0001$) during the wet season. Possibly indicating that the ratio of Ca to P in P/Zn supplemented cows was adequate and for other groups (Ca/Zn, Ca/P) probably the ratio was not adequate either one element was exceeding the other.

Plasma Ca or Pi or Zn are good indicators for Ca, P and Zn status of ruminants when stress factors like diseases are not present (Gindler and King, 1972; Belonje, 1978; McDowell, 1992). It is possible that mastitis and / or anaplasmosis irrespective of supplementation package may have modified the relationships. Plasma Zn concentration was maintained in a stable concentration in Ca/P/Zn supplemented cows from May 1997 to January 1998, similarly stable plasma Zn concentration was observed in Ca/Zn supplemented cows from November 1997 to May 1998, possibly indicating a protective mechanism of Ca and or P on fluctuating plasma Zn. In the rest of the groups plasma Zn was fluctuating between high levels and low levels because of diseases like mastitis and anaplasmosis or other unknown factors.

No significant difference was observed in incidences of mastitis between groups, when compared to the Ca or Zn supplemented cows. Some cows supplemented with Ca/P and Ca/P/Zn suffered severe cases of suppurative granulomatous mastitis in January and March 1998. During these periods plasma Ca and Zn was low when

compared to normal levels of > 2.17 mmol/l Ca and > 12.0 µmol/l Zn) possibly indicating an interaction between the elements resulting in reduced plasma Zn or Ca. Calcium and Zn are essential nutrients for the resistance to diseases (McDowell, 1992; Hogan *et al.*, 1996), the impairment of the antioxidant system can result in a higher incidence and more severe clinical signs of mastitis. Mastitis occurrence coincided with very low plasma Ca (< 1.8 mmol Ca/l) and plasma Zn (< 10 µmol). It has been reported that cows with hypocalceamia are susceptible to mastitis than if hypocalcaemia was absent (Curtis *et al.*, 1983).

Zinc plays an important role in the prevention of bacterial or viral infection by stabilising epithelial cells (Moynaham, 1981). Zinc deficiencies has been reported to reduce incorporation of several amino acids into the skin proteins resulting in parakeratosis with lesions commonly occurring at the teat skin (Miller, 1979). It may be speculated that the lowered Zn concentration in cows supplemented with P/Zn and Ca/P/Zn (Table 17) in the present study in March 1998 was contributed to the reduced immunity in those cows resulting in mastitis and parakeratosis. Parakeratosis was observed in cows, which had a prolonged low Zn concentration from September to January i.e. in Ca/P (3 cows), P/Zn (2 cows) and Ca/P/Zn supplemented cows (2 cows) (Table 17).

Aspartate aminotransferase activity is used in detecting hepatic or muscular injury in domestic animals including cattle (Kaneko, 1989). Among factors affecting the activity of this enzyme include nutritional factors such as different and unstable composition of ration (Kaneko, 1989). Mean plasma ASAT tended to be low in

Ca/P/Zn supplemented cows and high in Ca/Zn supplemented cows. Plasma Ca tended to be high in the Ca/Zn cows and low in the Ca/P/Zn possibbly indicating an interaction between Ca or Zn and P in the cows. The role of Ca and Zn in ASAT is not known but at low plasma Ca and Zn the activity of ASAT was reduced and *vice versa*.

Mean PCV and Hb were within normal range in most of the sampling periods but at the lower side in all groups (Fig. 40, 41 and Appendices 28 and 29) when compared to the control cows but high when compared to single mineral administration. This probably indicate that Ca, P, Zn are important in red blood cell formation a combination of minerals may improve Hb and remove the effects of single element interaction between elements or unknown factor (s).

There was no improvement in plasma protein when cows were supplemented with a combination of Ca and P compared to Ca supplementation alone the same was applied to plasma glucose. However, high TP was recorded in the Ca/Zn in most of the sampling periods indicating that the combination of Ca and Zn had a positive effect on total protein. Calcium, P and Zn are important in energy and protein metabolism, any reduction of one element may result in low energy or protein (Kaneko, 1989) as indicated by different correlation (Appendix 41, 42 and 43).

The average lactation length was low for both years for all groups of cows when compared to Ca and Zn supplemented cows probably because of the presence of mastitis and poor body condition. Mastitis and feeding management may modify the

lactation length. Significant reduction in milk yield was observed when mastitis occurred hence possible reason for low milk yield.

The average PCO, CAI was low in all cows when compared to Ca supplementation only. It is possible that poor body condition, presence diseases such as mastitis and anaplasmosis and negative energy balance was the reason for not observing any difference between the groups.

Reproductive performances determine the efficiency of the dairy enterprise, resulting into regular birth of replacement animals and continued milk production. Poor reproductive performance in dairy cattle has been characterised by late maturity and long calving interval and poor conception rates (Gerloff and Morrow, 1986). These parameters may be adversely influenced by poor management (Girou and Brochart, 1970), nutrition and presence of diseases (Masters and Fels, 1985; Masters, 1984). The present study supports these observations in that mineral nutrition if not handled with care may cause damage to reproductive performance of dairy cows. The recommended economic length of calving to conception interval for a dairy cow is between 75 to 85 days (Lean and Westwood, 1977; Horward *et al.*, 1985; Lamond, (1970) which was true for Ca 75 (days) and Zn (76) supplemented cows. Postpartum ovarian disorders (cysts, ovarian inactivity), abortions and retained placenta may prolong the postpartum anoestrus and service periods (Perdok *et al.*, 1988; Roberts, 1986; Sasser *et al* 1988), which might have been the reason for longer calving to conception period for other groups of cows (Table 15).

In the first month of the experiment, initial mean ceruloplasmin activity was above the normal range of 40 IU /l (Fig. 33 and 54). This was an indication that cows were in a positive Cu balance. Following supplementation with minerals, the activity of ceruloplasmin was maintained in the Ca/Zn and Ca/P/Zn supplemented cows in May 1997 to September 1997 but was reduced in July 1997 in the Ca/P and Ca/Zn supplemented cows. Levels were reduced in all groups during the rainy season (November 1997 to March 1998). Ceruloplasmin activity was high in the Ca/Zn supplemented cows in most of sampling period when compared to P/Zn or Ca/P/Zn supplemented cows. Ceruloplasmin activity is not affected only by minerals but also by other unknown factors. Ceruloplasmin activity can be reduced due to infections, high Ca, Zn and Fe, which may impair Cu absorption (Paynter 1987; McDowell, 1992). It is possible that these factors were in operation and may explain the fluctuation of ceruloplasmin in different groups creating different correlation as indicated in Appendix 40, 41 and 42.

Selenium balance

The normal range of GSH.Px in cattle is above 130 IU / gHb according to RANSEL kit manufacturers (RANSOD, 1994). In the present study the initial levels (133 - 138 IU / gHb) implies that all cows were in their normal GSH.Px (Table 16). In general following supplementation with minerals the levels of GSH.Px was increased in all groups except in P/Zn supplemented cows. Glutathione peroxidase activity remained constant in the P/Zn interaction possibly indicating protective mechanism against increases in selenium uptake.

In January 1998, GSH.Px activity almost remained high in Ca/P, Ca/Zn and Ca/P/Zn supplemented cows while it was slightly increased to (141 IU/ gHb) in the P/Zn supplemented cows (Table 16), the period coincided with the rainy season. The implication of these observations is not clear but it can be speculated that a combination of P/Zn may prevent adverse increase in plasma GSH.Px. activity by preventing Se absorption or utilization.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

Soil calcium concentration was within normal range (> 2.50 me/ 100 g Ca). Feed and pasture had marginal Ca contents (0.30 – 0.39 %) for maintenance requirements in cattle. Similarly soil phosphorus concentration was below normal range (< 15 ppm) in the wet season but high in the dry season, which was reflected in marginal to critical suggested level of pasture P (> 0.25%). Soil zinc concentration was below normal levels (< 1.0 ppm) during the wet season but marginal to critical levels during the dry season and was reflected in low pasture Zn (< 40ppm). Concentrates mixture of maize bran and sunflower seedcake at a ratio of 4: 1 contained high amount of P (0.43%) and Zn (48 ppm).

Soil and pasture K, Na and Fe were above suggested normal levels. Magnesium and Cu were adequate in the soil but inadequate in pasture in both the dry and the rainy season as well as high in concentrates.

The low levels of pasture Ca, P and Zn were reflected also in the animal tissues in term of low plasma Ca, Pi and Zn concentration in the cows, heifers and steers under restricted amount of concentrate feeding. In view of marginal Ca, P, Mg, Zn and Cu contents in pastures it is recommended to feed these minerals as supplements to dairy cows at ASAS Dairy Farm.

The low plasma Ca and Zn concentration in the dairy cows at ASAS Dairy Farm varied with season and physiological status. Low Ca and Zn were more prevalent during the dry season and during calving period. Mineral supplementation using different formulation of Ca, P and Zn in grazing crossbred Zebu cows did not meet

the demand during these periods. However, Ca supplementation in the form of $CaCO_3$ improved plasma Pi and Zn concentration. Supplementation of Zn in the form of ZnO alone resulted in an increase in plasma Zn but not above the level resulted from $CaCO_3$ supplementation. Improvement in body condition score and reduced mastitis and anaplasmosis incidences were observed after Ca supplementation in the form of $CaCO_3$ and Zn supplementation in the form of ZnO compared to the other sources of Ca and Zn.

Phosphorus supplementation in the form of sodium monophosphate indicated that P supplementation was not beneficial when controlled amount of concentrates containing high amount of P were given to the cows as indicated by high plasma Pi in all the experimental cows. Live weight gain, body condition score were negatively affected with P supplementation as indicated by high cases of mastitis, anaplasmosis and lack of improvement in energy and protein balance.

High milk yield with high fat and protein was observed after Ca supplementation in the form of $CaCO_3$ compared to other formulations. Milk yield, milk fat and protein were affected in a negative manner with P supplementation, indicating the detrimental effect of excessive P supplementation on the performance of grazing cows given concentrates with high P.

There was no difference in reproductive performance between the control and the mineral supplemented cows. However, the $CaCO_3$ supplemented cows tended to return to oestrus earlier and had short calving intervals compared to other

formulation.

Supplementation with Ca/Zn in dairy cows maintained a stable copper balance. Other formulations resulted in reduction in copper. Supplementation of P/Zn maintained stable glutathione peroxidase during both the dry season and the rainy season. Calcium and zinc supplementation reduced the activity of glutathione peroxidase during the rainy season. These observations need more research inorder to understand the mechanisms involved.

There was no difference in animal performance when various mineral combinations were given to the animals. A large excess of either Ca or P interfered with the absorption of the other leading to lower plasma mineral concentration resulting in poor performance.

Dry season supplementation with concentrate mixture of maize bran and sunflower seedcake, good quality hay and fresh legumes (alfalfa which contained high Ca (1.4%)) played a role in improving metabolism of these elements and should be encouraged to ASAS Dairy Farm and other farmers with similar problems. However, caution should be taken with type and amount given to the animals. The farmer also should use farmyard manure to improve soil and forage Ca and P.

Based on the results in this study, P supplementation aimed at complementing soil licks, water and pasture which are likely to be low in this mineral element should be handled in manner that would not affect other minerals in the animal body. At

present substantial amounts of concentrate are provided to dairy cows without justification. Many dairy farmers may oversupply or undersupply P both scenarios may have an impact on animal performance as observed in this study.

Mineral requirements may differ from one location to another as observed in this study. It is recommended that studies on mineral availability in soil, pasture and animal tissues be carried out in the different agro-ecological zones of Tanzania. Furthermore, mineral formulations available on the market should be checked if the animals' requirements are met.

CHAPTER SEVEN

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APPENDICES

Appendix 1. Procedure for clinical examination of lameness

Clinical lameness was examined as described by (Greenough *et al.*, 1981). The following stages of examination were used (a) A rapid initial examination of the limb as a whole, (b) Detailed re-examination of the limb as a whole (c) Observation of locomotion, (d) A detailed examination of the digital region.

Initial and detailed examination

This was a visual appraisal of every aspect of the limb, with the objective of eliminating obvious lesions and of forming a general impression of conformation. Examination commenced at the hip or shoulder and proceeds downward to the digital region. The following points were given particular consideration; muscular atrophy, joint enlargement, swellings, trauma, abnormal stance and conformation of the limbs. Finally the unseen part of the limb between the thigh and udder were palpated because dermatitis of this area does occur in dairy cattle and can cause lameness.

Abnormal locomotion

Moving the animals was helpful to determine the degree of lameness; a distance of at least 5-15 metres at normal pace either towards or away from the observer was used. Fore limb lameness was best observed as the animal approaches and usually the head moves up as the affected limb is on the ground. Hind limb lameness was best examined when the animal moved away from the observer and the quarter usually sinks on the sound side at each step.

Note: No clinicical lameness was observed and therefore no detailed hoof examination was carried out except for radiological photography.

Radiological examination

Radiological pictures of the claw were taken at the beginning of the experiment and thereafter at 4 months interval for the whole period of study. The radiograph were examined for alteration in position, size, contour, architecture and density. To maintain uniformity the left front and hind digit from two cows from each group was radiologically photographed. Dorsopalmer and lateral medial views were taken using normal x – ray film (Fuji RX universal) and standard intensifying screen in cassettes at focus film of one meter. Exposure of 70 kV and 25 mAs were employed using a portable x ray machine.

Appendix 2 Determination of plasma total calcium

Plasma total Ca was determined using modified method from Gitelman, (1967) and Kessler and Wolfman, (1964).

Principle

A coloured complex with calcium ions is formed after an alkaline medium is mixed with cresolpthalein from calcium reagents I which is acidic and 2 which is alkaline in the test plasma. The complex is measured at 574 nm. The 8-hydroxyquinoline in the reagent binds the magnesium ions, thus minimizing their interference in the calcium assay.

Reagent

1. Potassium chloride, (KCL, MW 74.56)
2. 8-hydroxyquinoline (e.g. FLUKA 55070), MW 145.16
3. O-cresolphthaleine complexone (e.g. FLUKA 64000), MW 636.62
4. 2-amino-2-methyl-1-propanole (e.g. FLUKA 08580), Mw 89.14
5. Tritisol ^{TM} (Merk art. 1.09943), 1.000 g Ca in HCl-solution

Reagents preparation:

KCl-HCl buffer

7.456 g KCl was dissolved in 950 ml distilled water and adjusted to pH 1.80 with N HCl. The flask was refilled to 1000 ml.The buffer was kept closed at refrigeration temperature and was stable for 4 weeks.

Calcium Reagent 1

0.820 g 8-hydroxyquinoline and 0.025 g O-cresolphthaleine complexone were dissolved in 480 ml KCl-HCl buffer. The pH of the solution was mantained at 1.80 refilled to 500 ml. Calcium Reagent 1 was only stable for one day.

Calcium Reagent 2:

14.26 g of 2-amino-2-methyl-1-propanole was dissolved in 180 ml distilled water and pH was adjusted to 10.3 with 3 N HCl-solution. The flask was refilled to 200 ml. The reagent was kept in closed bottle and was stable for two weeks.

Procedure:

90 µl of plasma was pipetted into a test tube containing 3.0 ml of 'Calcium Reagent 1' and mixed on a whirl-mixer.

1.0 ml 'Calcium Reagent 2' was added to the solution four minutes later, and the contents were mixed once more. The mixture was left to stand for 5 min before reading the absorbency in the spectrophotometer at 574 nm. Due to rapid decrease in absorbency, the reading was finished within 30 seconds in the spectrophotometer. All readings were done within 1 hour from the addition of 'Calcium Reagent 1'.Only Ca-free tubes were used, i.e. acid washed tubes.

Standards:

Tritisol Ca-stock solution: 1.000 g was mixed with redistilled water, the morality of this stock solution was 24.95 mmol/1.

Redistilled water	Ca-stock solution	Ca-standard
		mmol/1
5.0	1.0	4.16
7.0	1.0	3.12
9.0	1.0	2.50
12.0	1.0	1.92
15.0	1.0	1.56
20.0	1.0	1.19
25.0	1.0	0.96
50.0	1.0	0.49

90 µl standard(s) were used in the procedure substituting plasma.

Appendix 3 Determination of plasma inorganic phosphates

Plasma inorganic phosphate was determined using methods described by Fiske and Subarrow (1925).

Principles of the procedure

Inorganic phosphate is assayed by measurement of vanado-phosphomolybdate complex formed in acid. Absorbance of this complex is read at 420 nm. Absorbance data are converted into concentration values via a standard curve.

Chemicals needed:

Ammonium hepta molybdate tetrahydrate (e.g. Merck 1.01182.1000), MW 1235.86

Ammonium meta vanadate, (e.g. FLUKA 10028), mw 116.98

Dilute hydrochloric acid

Potassium di-hydrogen phosphate (e.g. Merck 2579201), MW 136.09

Trichloeacetic acid (TCA), (e.g. FLUKA 91228), Mw 163.39

Reagents Preparation:

1. 0.100 g of NH_4V0_3 was dissolved in 80 ml of distilled water and mixed well using magnetic stirring .
2. 4.000 g $(NH_4)6Mo_70_{24}$, 4 H_20 was added and dissolved.
3. 10 ml of 6 N HCl was added and the flask refilled to 100 ml.

The reagent was kept in dark and was stable for 2 month.

Procedure

One volume of plasma was added to four volumes of 10% TCA solution, mixed on a whirl mixer and centrifuged for 5 minutes at 3000*G to obtain the supernatants. 200 µl supernatants was pipetted into 200 µl reagent. 1.50 ml water was added the mixture mixed on a whirl mixer and allowed to stand for 18 hours in the dark before reading at 420 nm on the spectrophotometer. Analyses were performed in doublicate.

Precaution: Utensils used were kept free from phosphates (detergents) by washing in dilute acids.

Standard curve:

Standards were prepared using KH_2PO_4 as a P-source.

Stock-standard solution

1.361 g $KH2PO_4$ was dissolved in 8 % TCA solution ad 1000 ml (10 mM P).

8 % TCA solution	Stock solution	P-standard Mmol/1
50	0	0
49	1	1
48	2	2
47	3	3
46	4	4
45	5	5

Standards replaced supernatans (i.e. 200 µl) in the procedure. A standard curve was raised through (0.0) = 0 mmol/P litre. Absorbance datas were converted into concentration values via the standard curve.

Appendix 4 Determination of plasma zinc concentration

Plasma Zn concentration was determined by atomic absorption spectrophotometer as described by Milner and Whiteside (1984). Zinc was first liberated from the protein by precipitation using trichloroacetic acid. The supernatant was then used to determine Zn concentration using the Atomic absorption spectrometer.

Reagents preparation:

1. Trichloroacetic acid 10 % (TCA): the acid (p.a.) was melted in a heating cabinet at 60 °C, 100 g of the liquid was weighed out in a beaker, transferred to a 1000 ml volumetric flask with distilled water and diluted to volume.
2. Zinc stock solution I (100 mg/l): 100.0 mg of metallic zinc p.a. (coarse powder) was dissolved in 10 ml of 5 N HCl and diluted to 1000 ml with distilled water and was stored at 4 °C in a polyethylene bottle.
3. Zinc stock solution II (10 mg/l) was prepared fresh every day by diluting 2500 µl of stock solution I to 25 ml with distilled water.

Procedure

1 ml TCA was pipetted into a centrifuge tubes(s) and 1 ml of plasma was added, the tubes(s) was stoppered, shaken thoroughly and left for 10 minutes. The sample(s) were then centrifuged for 10 minutes at 4500 rpm whereafter the zinc concentration was measured by atomic absorption spectrophotometry at 213.9 nm. Standard containing 0 (blank), 20, 40, 60, 80, 100 and 120 µg % of zinc in 5 % TCA were prepared in 25 ml volumetric flasks by dilution of stock solution II in

the following way:

Standard	µl stock II	Ml of TCA 10 %
0 (blank)	0	12.5
20 µg %	500	12.5
40 µg %	1000	12.5
60 µg %	1500	12.5
80 µg %	2000	12.5
100 µg %	2500	12.5
120 µg %	3000	12.5

All solutions were diluted to volume with distilled water.

The instrument was set to zero with the blank and standards and samples were measured. Between each measurement the instrument was flushed with distilled water.

Calculation

The standard curve was drawn and then zinc concentration in the sample diluted was read from it, hence zinc in plasma, µg % = zinc in sample dilution (µg %) x 2.

Appendix 5 Determination of ceruloplasmin activity in plasma

The analysis was carried out according to the method described by Schosinky *et al* (1974).

Principle

Ceruloplasmin acts as a diamine oxidase and the quantitative determination was based on this activity.. In this method O-dianisidin dihydrochloride (4-4'-diamino - 3-3 dimethoxy-biphenyl) was used as a substrate and acetate as a buffer at pH 5.5.

Procedure

Acetate buffer (0.7 ml) at pH 5.5 and 0.05 ml of plasma was pipetted into each of two test tubes and placed in a water bath at 30°C. Five minutes were allowed for temperature equilibration, then 0.2 ml of substrate (7.88 mmol/l, o-dianisidin dihydrochloride) was pipetted into the tubes at timed intervals and mixed. The substrate solution was preheated to 30°C before pipetting into the sample.

The first tube was incubated for exactly 5 minutes before the enzymatic reaction was stopped by adding 2 ml of 9 mol/l sulphuric acid and mixed immediately. The second test tube was treated in the same way exactly 15 minutes after addition of the substrate. The absorbance was measured at 540 nm using a UV spectronic 1001 Cecil spectrophotometer. A cuvette with 1 cm light passage containing distilled water was used as reference.

Calculation

Ceruloplasmin activity = $(A_{15} - A_5) \times 614.6$ U/l

Where A_{15} and A_5 were the absorbances after 15 and 5 minutes of incubation respectively. The activity was expressed in international units, where one unit corresponds to 1 umol substrate oxidized per minute per litre of plasma.

The factor 614.6 was derived in the following way:-

$$F = \frac{59 \times 1000}{9.6 \times 10 \times 1} = 614.6$$

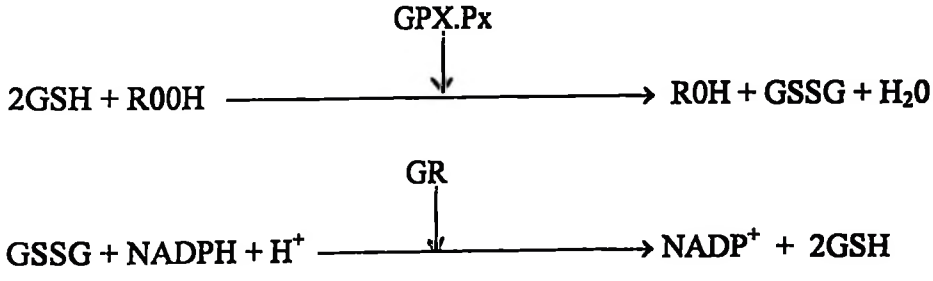
where 59 was a volume correction (0.05 ml of plasma diluted to a final volume of 2.95 ml), 9.6 was the molar absorptivity ceruloplasmin, 10 represented the incubation time in minutes and 1 was the optical path length in cm.

Appensix 6 **Determination of glutathione peroxidase activity**

Glutathione peroxidase activity in whole blood was determined using RANSEL reagent kit form RANDOX Laboratories, United Kingdom. The method was based on the method of Paglia and Valentine (1976) as described by RANDOX Laboratories (1994).

Principle

Glutathione peroxidase catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH the oxidase glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADPH +. The decrease is measured by absorbance of NADP at 340 nm.



Procedure

The method involved reconstituting reagents as recommended by the manufacturer of the kit. Heparinised whole blood (0.05 ml) was diluted with 2 ml of a diluting agent. In order to get linearity further dilution of the blood sample was performed using the diluting agent when the absorbance change per minute exceeded 0.1 at

Hg 340 nm as recommended by the manufacturer.

The semi-micro procedure was adopted in which 20 µl of reagent blank and test samples were pipetted into separate test tubes. To each 1000 µl of reagent 1 was added mixed and then 40 µl of cumene was added and mixed. Initial absorbance of sample and reagent blank was read at 340 nm wavelength after one minute of incubation at 37°C using a 1001 Spectronic Spectrophotometer. Simultaneously the timer was started and thereafter, absorbances was read at 1 and 2 minutes after the initial reading. The reagent blank value was substrated from that of the test sample.

Calculation

Glutathione peroxidase concentration was calculated from the following formula:

Unit/litre of haemolysate = 8412 x Absorption at 340 nm/minute

Unit/litre of haemolysate was multiplied by the appropriate dilution factor to obtain the result in Units/litre of whole blood. Then the Unit/litre of whole blood was converted to Units/gm Haemoglobin. 8412 is a factor given by the manufacturer of the kit.

For control of precision and accuracy a RANSEL control of whole blood provided with the kit was analysed together with the test samples for each analysis run.

Appendix 7 Determination of percentage packed cell volume

Parcentage packed cell volume (PCV) was determined by microhaematocrit method as described by Baker and Silverton (1976)

Principle

The capillary tubes are filled with blood by capillary action, the outside carefully dried with a piece of gauze, and the opposite end of the tube sealed. The sealed tubes are placed in a special high speed centriguge (Microcentrifuge) so that the sealed end is near the outside rim of the centrifuge, then the tubes are spun at 10,000 rpm for 5 minutes. The spumed tube was placed into the designed special reader for dtermining the per cent of red blood cells.

Procedure

1. The blood was collected in EDTA tubes mixed by inverting the tube about 10 times; vigorous agitation was avoided.
2. Blood was drawn up to two-thirds of a 75 mm x 1.00 mm microhaematocrit capillary tube.
3. Blood was wiped off the capillary tip and carefully the end was sealed by sealowax.
4. The closed end of the capillary was placed outwards, in a microhaematocrit centrifuge and spined at 10,000 revolutions for 5 minutes.
5. The capillaries were removed from centrifuge and read using a microhaematocrit reader.

Appendix 8 Determination of haemoglobin

Haemoglobin concentration was determined by cynmethaemoglobin method as described by Baker and Silverton (1976).

Principle

The haemoglobin was converted by the action of ferricyanide to methaemoglobin. This then was converted to cyanmethaemoglobin by the action of potassium cyanide. The concentration of cyanmethaemoglobin was then measured by spectrophotometer at a wavelength of 540 nm.

Reagents

1. Drabkin's solution

Potassium cyanide	0.2 g
Potassium ferricyanide	0.2 g
Sodium bicarbonate	1.0 g
Distilled water	1000 ml

2. Standard solution of cynamethaemoglobin (B.D.H.)

   The solution was regarded as dilution of whole blood. The figure on the label was multiplied by the dilution factor being used in the test. The label indicated 57.2 mg/ml, which was equivalent to 14.36 g Hb per dl in the above test.

Procedure

1. Blood was collected in EDTA tube mixed by inverting the tube 10 times, vigorous agitation was avoided.
2. With a 0.02 ml pipette, 0.02 ml of blood was drawn. The tip of the pipette was wiped and the contents washed into 5 ml Drabkin's solution* (1/251 dilution) and mixed well.
3. The solution was allowed to stand for 10 minutes and then the solution absorbency was read at 540 nm using a colorimeter. A tube of Drabkin's solution was used as a blank.
4. The standard solution of cyanmethamoglobin was read
5. The haemoglobin concentration was calculated as follows:

$$\frac{\text{Reading of test}}{\text{Reading of standard}} \times \text{concentration of standard} \times \text{dilution} = \text{g/dl}$$

Appendix 9 Dertermination of total white blood cell and differential counts

9.1 Total white blood cell counts using haemocytometer method

Principle

The haemocytometer consist of a counting chamber, coverglass, pipettes for diluting blood and a rubber tube with a plastic mouth piece for drawing the fluid into a pipette. Fluids used as diluents must be isotonic. The most common is Turk's fluid which destroys the erythrocytes and stains the leucocytes for easy identification.

Apparatus and reagents

1. Improved Neubauer counting chamber
2. White cell pipette
3. White cell diluting fluid

i.e. Turk's solution, 1 percent glacial acetic acid which destroys the erythrocytes, tinged with gentian violet, which stains the leukocyte.

Preparing white blood cell dilution

1. 4 ml of WBC diluting fluid was placed into a Bijou bottle .
2. The blood sample was mixed well by inverting approximately 20 times; vigorous agitation was avoided.
3. Using a white cell pipette blood was drawn to mark 0.5. The excess blood wiped off from the outside of the pipette. The contents of the pipette was mixed with the diluting fluid sucked at a mark 11. The blood and diluting fluid were

mixed by shaking the pipette for two or three minutes. After the diluted blood is thoughly mixed, few drops of fluid were discarded and the end of the pipette is dried with lint free absorbent material.

4. The tip of the pipette was touched to the side of the counting chamber and to let a drop of fluid run under the coverglass.
5. Counting of cells started after cells have settled for two to three minutes.
6. The dilution of blood was (1 : 20).

Counting of white blood cells

Using the improved neubauer chamber and the microscope cells were counted in the four square millimetres of the ruled area.

Calculation

Average number of cells counted per square mm x depth x dilution

e.g. (236 / 4) x 10 x 20

= 11 800 (11.8×10^3) per cubic millimeter or in S1 units 11.8×10^9/litre

9.2 Differential white blood cell count

Principle

A smear or film of blood is prepared and stained. The stain are special for differentiation of leucocytes.Giemsa stain will stain the nuclei of neutrophil, reddish purple; eosinophilic granules, red to orange; basophilic granules blue and lymphocyte, dark purple

Slide preparation

1. A drop of blood was placed at one end of a clean grease free slide.
2. Using a another clean glass, the drop top was allowed to spread along its edge.
3. The spreader was held at an angle of approximately 45 degrees and pushed along the slide, drawing the blood behind it until it was all been smeared. Too large drop, or to incline the spreader at too great an angle was avoided to prevent too thick films. The film was dried rapidly in air by vigorously shaking.The film was stained with Giemsa stain i.e.

(a) Smears were fixed in methyl alcohol for 3 minutes.

(b) Washed off and flooded with Giemsa stain:

   a. diluted 1 : 3 in buffered water pH 7.2 for 10 minutes

Counting

Battlement method was selected The film was examined systematically by being traversed three fields along the edge, two fields up, two fields along, two fields down, starting at the thin end of the smear. This sequence was continued until a

minimum of 200 cells had been enumerated.

The main types of leucocytes identified were:

Lymphocytes, neutrophils, eosinophils. Basophils and monocytes,

The respective counts was expressed as percentage of the total and the actual value calculated.

Appendix (10) Estimation of serum immunoglobulin

Serum immunoglobulin was determined as described by McEwan et al., (1970)

Principle

The serum immuno-globulin concentration is measured using a selective turbidity produced by zinc sulphate by the spectrophotometer. Turbidity becomes visible at an optical density of 0.4 to 0.5 which would be equal to 400 to 500mg of IgG/dl.

Reagents

1. Stock solution of zinc sulphate. 2.08 g (7.233 mmol) of $ZnS0_4.7H_20$ was disolved in de-ionised water and diluted to 1 litre. The solution was kept in a bottle fitted with a carbon dioxide trap.
2. Working solution. The above stock solution 1 was diluted in 10 to give a final concentration of 208 mg 723.3 mmol zinc sulphate per litre. The solution was diluted freshly for each batch of test.

Standard

3 ml of a 1.15 per cent (47.08 m mol/L) solution of barium cloride ($BaC1_2.2H_20$) was pipetted into a 100 ml flask and the volume made up to the mark using 0.1M (100 m mol/L sulphuric acid, the resulting turbidity when measured should read as 2.0 (using scale as 1-10).

Procedure

100 µl of serum was pipetted into two tubes. 6 ml of distilled water was added to one of the tube acting which acted as a blank and to the serum tube 6 ml of zinc sulphate solution was added. The solution was mixed by inversion and both tubes were left for one hour. The solution was mixed again and read in the spectrophotometer. The results were expressed as Z.S.T. units by multiplying the spectrophotometer reading by 10.

Notes

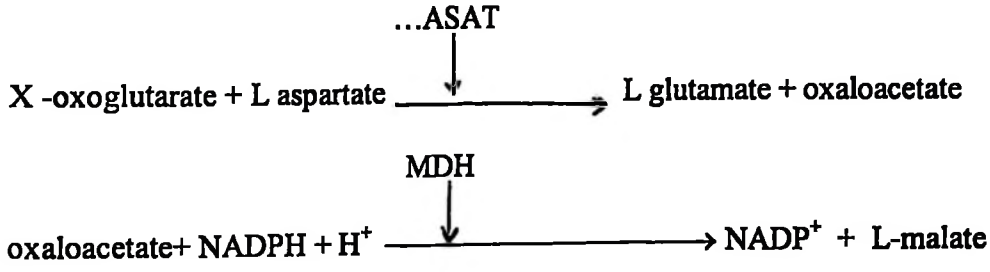
Since the test is very sensitive to temperatures above 20°C and also to the time allowed for the precipitation. The temperature of reagents was kept at 37°C and that the readings were taken within 60 min.

Appendix 11 Determination of aspartate aminotransferase activity

Aspartate aminotransferase activity in plasma was determined using a reagent kit form BOEHRINGER MANNHEIM Laboratories, Germany. The method was based on the method of Bergmeyer *et al* (1986) as described by BOEHRINGER MANNHEIM Laboratories (1994).

Principle

Aspartate aminotransferase catalyses the oxidation of x oxoglutarate by L aspartate to L glutamate and oxaloacetate. In the presence of malonil dehgrogenase (MDH) and NADPH the oxidase oxaloacetate is immediately converted to L malate with a concomitant oxidation of NADPH to NADPH +. The decrease is measured by absorbance of NADP at 340 nm.



Procedure

The method involved reconstituting reagents as recommended by the manufacturer of the kit. Plasma (0.2 ml) was diluted with 2 ml of a diluting agent mixed. Initial absorbance of sample and reagent blank was read at 340 nm wavelength after one minute of incubation at 37°C using Cecil 2000 spectrophotometer. Simultaneously

the timer was started and thereafter, absorbances was read at 1, 2 and 3 minutes. The mean absorbance change per minute was calculated and used for calculation.

Summary

Wavelength	Hg 340 nm:
Cuvette:	1 cm light path
Incubation temperature:	37°C
Measurement:	against air

Calculation

Unit/litre of ASAT = 1746 x Absorption at 340 nm/minute

For control of precision and accuracy a control of plasma provided with the kit was analysed together with the test samples for each analysis run.

Appendix 12 Determination of plasma alkaline phosphatase activity

Principle

The enzyme alkaline phosphate liberates ortho phosphate from p-nitophenyl phosphate in an alkaline solution. The reaction product p-nitrophenol (PNP) is measured spectrophotometric at a wavelength of 402.5 nm.

Reagents:

Buffer:

1 2-amino-2-methyl-1-propanole (e.g. FLUKA 08580), MW 89.14, 0.89 mol/1.

2-amino-2-methyl-1-propanole was preheatered to 30-35 °C, until liquid. 78.5 g of the liquid was measured into a 1000 ml cylinder and 500 ml distilled water was added and mixed. 200 ml 1 N HCl,was added to the mixture and mixed. The pH was adjusted to 10.31-10.35 with dilute NaOH or HCl solutions. The cylinder was refilled to 1000 ml mark with distilled water.The solution was stable for 2 weeks when stored in a closed container.

2 Co-factor: Magnesium solution, 1.5 mmol/1

300 mg $MgCl_2$, 6 H_20 was dissolved in a 1000 distilled water.

The solution was stable indefinitely.

3 Substrate solution: p-nitrophenyl phosphate (di-Na salt hexa hydrate, e.g. Merck 1.06850.0025), MW 371.15, 225 mmol/1. 1.670 g p-nitrophenyle phosphate was dissolved in 20 ml magnesium solution

and mixed by magnetic stirring.The substrate solution was not stable and it was prepared freshly every day.

Procedure:

The spectrophotometer was initially blanked to the value 0 using a mixture of 2.80 ml buffer and 0.20 ml substrate solution at wavelength 402.5 nm. 270 ml buffer was preheated in a water bath at 30 °C. 0.2 ml plasma was added. The enzymatic reaction was initiated by adding 0.20 ml substrate solution, the test tube was mixed gently on a whirl mixer. Approximately 2.5 ml of the mixture was sucked up into the 'flow cell' of the spectrophotometer, which was kept constant at 30 °C by a water circulating system.

The spectrophotometer was pre set on a kinetic program with the following specifications:

Wavelength	402.5 nm
Initial delay	60 sec.
Interval	30 sec.
Cycles	9
Factor	1592

The result is given directly in activity, i.e. concentration of reaction product mol PNN/min * liter in blood plasma (= I.U.)

Appendix 13 Determination of plasma total protein

Plasma total protein was determined using RANDOX reagent kit form RANDOX Laboratories, United Kingdom. The method was based on the method of Henry *et al* Valentine (1974) as described by RANDOX Laboratories.

Principle

A violet coloured complex is produced by reacting the serum proteins and peptides with an alkaline copper sulphate solution – the Biuret reaction. Other non-protein nitrogen compounds, creatinine and urea do not react.

Procedure

The method involved reconstituting reagents as recommended by the manufacturer of the kit. Plasma (0.02 ml) was diluted with 1 ml of a diluting agent. The mixture was incubated at 20 to 25 ^{0}C for 30 minutes. The absorbance of sample (AS) and of the standard (SD)and reagent blank was read at 540 nm wavelength using Cecil 2000 spectrophotometer.

Calculation

Total protein concentration (g/l) = AS/SD x standard concentration

To maintain linearity samples were diluted with 0.9 5 NaCl solution in ratio of 1:1. For control of precision and accuracy a RANDOX control of serum provided with the kit was analysed together with the test samples for each analysis run.

Appendix 14 Determination of plasma urea

Plasma urea was determined using RANDOX reagent kit form RANDOX Laboratories, United Kingdom.

Principle

Urea is hydrolysed to ammonium ions, the reaction is catalysed by urease enzyme.The ammonium ions formed react with salicylate and hypochloride to give a green dye which can be monitored by spectrophotometer.

Test principle

$$\text{Urea} + 2\,H_2O \xrightarrow{\text{urease}} 2\,NH_4^{+} + CO_3^{2-}$$

Reagents

1. Buffer/urease/salicylate

Phosphate buffer:	120 mmol/l; pH 7.0
urease:	5 IU/ml
sodium salicylate:	62.5 mmol/l
sodium nitroprusside:	1.48 mmol/l

2. Hypochloride

Sodium hypochloride:	6 mmol/l
Sodium hydroxide:	150 mmol/l

3. Standard Urea: 30 mg/dl (5 mmol/l)

4. Preparation and stability of solution

(a) One aluminium sachet was empted into a beaker to it 50 mls of distilled water was added and stirred vigorously to accelerate the dissolution. The solution was kept closed in amber glass and was stable for 4 weeks at at +2 to 8°C or 8 hours at +15 to 25°C

(b) The contents of bottle 2 was diluted with 450 ml distilled water and was kept slosed in amber glass bottles and was stable for 6 months at + 2 to 8° C or 3 months at + 15 to 25°C

(c) The contents of bottle 3 was used undiluted as instructed and was stable up to specified expiration date when stored at + 2 to 8°C

Procedure

Wavelength: Hg 578 nm

Incubation temperature: 20-25°C or 37°C

Measure against reagent blank (RB).

Summary

Pipette onto bottom of test tubes:			
	RB	Standard	Sample
Serum or diluted urine	-	-	0.02 ml
Solution 3	-	0.02 ml	-
Solution 1	2.50 ml	2.50 ml	2.50 ml
Mixed and incubated for 5 min (max. 30 min) at 20 to 25°C or for 3 min at 37°C. Add:			
Solution 2	2.50 ml	2.50 ml	2.50 ml
Mix and incubate for at least 10 min at 20 to 25°C or 5 min at 37°C. Read absorbances of sample (A_{sample}) and standard ($A_{standard}$) against the reagent blank within 60 min.			

One blank and one standard were sufficient for each assay series.

Calculation of the concentration of urea in the sample:

Plasma	mg/dl	$C = 30 \times \frac{A_{sample}}{A_{standard}}$
	mmol/l	$C = 5 \times \frac{A_{sample}}{A_{standard}}$

At higher urea concentrations, the assay was repeated using half the specified sample volume (result x 2). To avoid ammonium salts which are highly sensitive to the reaction, clean glassware were used.

Appendix 15 Determination of plasma glucose concentration

Plasma glucose was determined using RANDOX reagent kit form RANDOX Laboratories, United Kingdom

Principle

The enzyme glucose oxidase specifically catalyses the conversion of glucose in plasma filtrate to gluconic acid. The equivalent quantity of hydrogen peroxide formed oxidases ortho-diansidine in the presence of the enzyme peroxidase to a red-brown dye compound. The colour intensity is proportional to the glucose concentration and is measured spectrophotometrically.

Procedure

The method involved reconstituting reagents as recommended by the manufacturer of the kit. Plasma (0.02 ml) was diluted with 2 ml of a diluting agent. The macro procedure was adopted in which 20 µl of standard and test samples were pipetted into separate test tubes. To each 2000 µl of reagent 1 was added and mixed. The sample were incubated for 25 minutes at 25 °C . The absorbance of the standard (SD) and the sample (AS) against reagent blank was measured within 60 minutes.

Calculation

Glucose concentration (mg/dl) = AS/SD x 100.

For control of precision and accuracy a RANDOX control of serum provided with the kit was analysed together with the test samples for each analysis run.

Appendix 16 Determination of milk butter fat percentage

Milk butter fat was determined using Gerber Test.

Principle:

Concentrated sulphuric acid when mixed with milk will cause destruction and dissolving of milk components releasing the fat. Addition of amyl alcohol has the function of separating fat from other components. The mixture of milk, sulphuric acid and amyl alcohol is centrifuged for 4 – 5 minutes at 1100 revolutions per minute (r.p.m.) and after adjusting the temperature to 65°C the result of the test can be read directly.

Apparatus

a) Centrifuge with speed indicator,working speed 1100 r.p.m. (±100 r.p.m.)

b) Milk butyrometer tubes, reading 0% - 8% fat.

c) Rubber stoppers of acid resistant rubber.

d) A 10 ml pipette with safety bulbs for measuring the acid into the butyrometer tube.

e) A 10.94 pipette for delivering milk into the butyrometer tube.

f) Automatic measuring device to deliver 1 ml of amyl alcohol into the butyrometer tube

g) Rack for butyrometer tubes, acid resistant.

h) Water bath, 66-68°C, in which the butyrometer tubes can be placed vertically with the water level above the contents.

Reagents preparation:

1. Sulphuric acid, dairy grade for milk testing, specific gravity at 20°C 1.816 ± 0.003. Amount per sample 10.0 ± 0.2 ml at 20°C.
2. The milk sample : 10.94 ml at 20°C. The sample was inverted gently at least 5 times to distribute the fat. Chilled samples were slowly warmed to 40°C and then cooled to 20°C with periodic inversions..

Procedure

1. Sulphuric acid was measured into the butyrometer tube using automatic machine.
2. On top of the acid the milk was left to run in slowly allowing it to form an undisturbed layer without being charred by the acid.
3. Amyl alcohol was added on top of the milk.
4. The stopper were inserted fully without shaking.
5. The tubes contents were shaken by holding the tube stems with the stopper pointing upwards, later on the tube were inverted several times so that the contents are evenly distributed inside the butyrometer tubes.
6. Number were placed on each tube in the holding sleeves of the centrifuge with the stoppered ends outwards. The distribution of tubes was even so that the centrifuge was perfectly balanced.
7. The lid of the centrifuge was secured and then the centrifuge started and left to spin for 5 minutes at 1100 r.p.m.
8. After stopping the tubes were placed in the water bath at 65-67°C for 5 minutes.

9. The stem was wiped and reading done quickly to avoid fall in temperature. The lock stopper was adjusted up or down until the lower edge of the fat column was exactly on a whole number on the stem. With the stem at eye level the nearest tenth of a % was taken (example : 3.98 to 4.02 makes a reading of 4,00, 4.03 – 4.07 makes 4.05).

10. The test was repeated if there was and indications of :

    a Charred fat column, which suggests :

    The acid being too strong

    The mixing and dissolving may have been delayed.

    The mixing and dissolving having been interrupted.

    b The casein not having been completely dissolved which suggest :

    The acid being too weak.

    c A fluffy layer at the base of the column of fat which suggest :

    old rubber stoppers that require renewing.

Appendix 17 Determination of milk proteins

Milk protein was determined using Kjeldahl Method.

Principle

Milk is digested in H_2SO_4, using $5H_20$ as catalyst with K_2SO_4 as boiling point elevator, to release nitrogen from protein and retain nitrogen as ammonium salt. Concentrated NaOH is added to release NH_3, which is distilled, collected in boric (H_3BO_3) solution, and titrated.

Apparatus

1. Digestion flasks. – Kjeldahl. Hard, moderately thick, well annealed glass. Total capacity ca 500 or 800 ml.
2. Distillation flasks. – Same kjeldahl flask as in (a), fitted with rubber stopper through which passes lower end of efficient rubber bulb or trap to prevent mechanical carry over of NaOH during distillation. Connect upper end of bulb to condenser tube by rubber tubing.
3. Graduated 500 mL. Erlenmeyer titration flask to collect distillate. Trap outlet of condenser in manner to ensure complete absorption of NH_3 distilled into boric acid solution.
4. Digestion/distillation system.- Traditional apparatus with adjustable controls for individual flasks.
5. Titration burette. – 50 mL. Class A or equivalent.

Reagents preparation

1. Sulphuric acid. – 95 – 98% H_2S0_4 . Nitrogen free.
2. Copper catalyst solution.-$CuS0_4.5H_20$. Nitrogen free, Prepare solution 0.05 g/mL H_20.
3. Potassium sulfate.-K_2S0 . Nitrogen free
4. Sodium hydroxide solution.-50% w/w nitrate-free Na0H.
5. Boiling chips.-Mesh size 10 suggested. High purity, amphoteric alundum granules, plain.
6. Methyl red/bromocresol green indicator solution.-

   0.2 g methyl red was diluted to 100 mL in 95% ethanol. 1.0 g of bromocresol green was diluted to 500 mL in 95% ethanol. One part of methyl red solution was mixed with 5 parts of bromocresol green solution.
7. Boric acid solution.-4%, with indicator.

   40 g H_3B0_4 diluted to 1 L in water and 3 ml of methyl red/ bromocresol green indicator solution was added. The solution changed to light orange color.
8. Hydrochloric acid standard solution.-0.1N.
9. Ammonium sulfate.-99.9% $(NH_4)_2S0_4$.
10. Tryptophan of lysine hydrochloride.-99% $C_{11}H_{12}N_20_2$ or $C_6H_{15}CIN_20_2$.
11 Sucrose.-Nitrogen free.

Sample Preparation

15.00 g of K_2S0_4, 1 mL $CuS0_4.5H_20$ catalyst solution, and 8-10 boiling chips was added to digestion flask. Milk sample (5 ± 0.1 mL) warmed and immediately placed in the digestion flask. 25 ml of H_2S0_2 was added, rinsing any milk on neck

of flask down into bulb. The flask was stoppered.

Digestion burner setting.-

Digestion was conducted over heating device that was adjusted to bring 2.50 ml of H_20 at 25° to rolling boil.

Digestion-

The flask was placed in inclined position with fume ejection system on. Care was taken to make sure that sample does not foam up neck of Kjeldahl flask by starting slowly. Sample were digest at least 20 min or until white fumes appeared in the flask. Next, the burner setting was increased half way to maximum burner setting determined in (a) and heated for 15 minutes until the digest was clear (clear with light blue-green color).The digest was cooled to room temperature.

Distillation.-

The condenser water was turned on and 50 ml. H_3B0_3 solution with indicator was added to a graduated 500 ml Erlenmeyer titration flask. The flask was placed under the condenser tip so that the tip was well below H_3B0_3 solution surface. To the room temperature diluted digest, 75 ml 50% NaOH was carefully added down sidewalk of Kjeldahl flask with no agitation. NaOH formed a clear layer under the diluted digest. Immediately the flask was connected to distillation bulb on condenser. Vigorously the flask swirled to mix contents thoroughly; and heated until all NH_3 has been distilled (≥ 150 mL distillate; ≥ 200 ml total volume). The H_3B0 was titrated with standard 0.1000N HCl solution to first trace of pink and ml

used recorded.

G. Calculations

The results were calculated as follows:

Nitrogen, % $[1.4007 \times (V_a - V_b) \times N] W$

Where V_a and V_b =mL HCl titrant used for sample and blank, respectively;

N = normality of HCl solution; and W = sample weight, g.

The percent nitrogen was multplied by factor 6.38, to calculate percent "protein". This is "protein" on a total nitrogen basis. Maximum recommended difference between duplicates was 0.03% "protein".

H. Repeatability and Reproducibility Values

The intra and inter assay coefficients of variation was 2% and 3% respectively.

Appendix 18. Plasma total Ca (mmol /l) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at P > 0.05).

Month	n	Group 1 (Control)	Group 2 (Ca	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	P value
May 1997	6	2.27 ± 0.04^{a} (2.18 – 2.31)	2.26 ± 0.04^{a} (2.13 –2.44)	2.29 ± 0.04^{a} (2.16 – 2.40)	2.26 ± 0.04^{a} (2.00 –2.46)	2.25 ± 0.04^{a} (2.20 – 2.30)	2.27 ± 0.04^{a} (2.23 – 2.32)	2.27 ± 0.04^{a} (2.22 – 2.36)	2.25 ± 0.04^{a} (2.21 – 2.31)	0.9939
July 1997	6	1.73 ± 0.1^{a} (1.36 – 2.17)	1.39 ± 0.1^{c} (1.17 – 1.64)	1.52 ± 0.1^{b} (1.29 – 1.71)	1.68 ± 0.1^{a} (1.24 – 1.83)	1.51 ± 0.1^{b} (1.15 – 1.98)	1.35 ± 0.1^{d} (1.12 – 1.62)	1.41 ± 0.1^{c} (1.10 – 1.97)	1.55 ± 0.1^{b} (1.25 – 1.80)	0.0525
September 1997	6	1.72 ± 0.07^{b} (1.41 – 2.04)	1.77 ± 0.07^{a} (1.65 – 1.89)	1.72 ± 0.07^{b} (1.52 – 1.88)	1.70 ± 0.07^{b} (1.58 – 1.97)	1.51 ± 0.07^{c} (1.15 – 1.98)	1.90 ± 0.07^{a} (1.69 – 2.06)	1.85 ± 0.07^{a} (1.74 – 1.97)	1.81 ± 0.07^{a} (1.61 – 1.98)	0.0227
November 1997	6	2.55 ± 0.09^{c} (2.34 – 2.73)	2.88 ± 0.09^{a} (2.76 – 2.97)	2.75 ± 0.09^{b} (2.36 – 2.94)	2.74 ± 0.09^{b} (2.35 – 2.98)	2.51 ± 0.09^{c} (2.18 – 2.95)	2.72 ± 0.09^{b} (2.49 – 2.97)	2.52 ± 0.09^{c} (2.32 – 2.90)	2.36 ± 0.09^{d} (2.06 – 2.61)	0.0228
January 1998	6	2.34 ± 0.07^{c} (2.03 – 2.53)	2.52 ± 0.07^{a} (2.20 – 2.78)	2.21 ± 0.07^{d} (2.00 – 2.42)	2.30 ± 0.07^{d} (2.15 – 2.51)	2.35 ± 0.07^{c} (2.24 – 2.55)	2.25 ± 0.07^{d} (1.92 – 2.40)	2.46 ± 0.07^{b} (2.29 – 2.60)	2.27 ± 0.07^{d} (2.10 – 2.48)	0.0287
March 1998	6	2.09 ± 0.04^{a} (1.93 – 2.21)	1.70 ± 0.04^{c} (1.42 – 1.87)	1.96 ± 0.04^{b} (1.84 – 2.06)	1.79 ± 0.04^{c} (1.64 – 1.87)	1.80 ± 0.04^{c} (1.73 – 1.94)	1.76 ± 0.04^{c} (1.62 – 1.83)	1.74 ± 0.04^{c} (1.61 – 1.96)	1.72 ± 0.04^{c} (1.65 – 1.79)	0.0001
May 1998	6	1.83 ± 0.07^{a} (1.57 – 2.35)	1.46 ± 0.07^{d} (1.30 – 1.56)	1.60 ± 0.07^{c} (1.46 – 1.77)	1.44 ± 0.07^{d} (1.21 – 1.65)	1.59 ± 0.07^{c} (1.37 – 1.90)	1.62 ± 0.07^{c} (1.46 – 1.76)	1.62 ± 0.07^{c} (1.48 – 1.76)	1.71 ± 0.07^{b} (1.44 – 1.79)	0.0039
July 1998	6	1.55 ± 0.05^{a} (1.35 – 1.89)	1.49 ± 0.05^{a} (1.29 – 1.67)	1.52 ± 0.05^{a} (1.41 – 161)	1.49 ± 0.05^{a} (1.25 – 1.67)	1.49 ± 0.05^{a} (1.33 – 1.59)	1.55 ± 0.05^{a} (1.43 – 1.80)	1.58 ± 0.05^{a} (1.44 – 1.77)	1.46 ± 0.05^{a} (1.34 – 1.60)	0.6180
September 1998	6	2.02 ± 0.08^{b} (1.73 – 2.25)	2.45 ± 0.08^{a} (2.25 – 2.57)	2.45 ± 0.08^{a} (2.07 – 2.68)	2.48 ± 0.08^{a} (2.17 – 2.89)	2.46 ± 0.08^{a} (2.14 – 2.71)	2.44 ± 0.08^{a} (2.19 – 2.57)	2.55 ± 0.08^{a} (2.30 – 2.84)	2.41 ± 0.08^{a} (2.31 – 2.57)	0.0013
November 1998	6	2.29 ± 0.05^{c} (1.97 – 2.45)	2.46 ± 0.05^{a} (2.35 – 2.65)	2.28 ± 0.05^{c} (2.18 – 2.48)	2.35 ± 0.05^{b} (2.18 – 2.49)	2.40 ± 0.05^{b} (2.33 – 2.54)	2.36 ± 0.05^{b} (2.21 – 2.51)	2.40 ± 0.05^{b} (2.11 – 2.65)	2.34 ± 0.05^{b} (2.16 – 2.50)	0.0311
January 1999	6	2.23 ± 0.05^{c} (2.10 – 2.48)	2.43 ± 0.05^{a} (2.32 – 2.49)	2.20 ± 0.05^{c} (2.06 – 2.31)	2.13 ± 0.05^{c} (1.88 – 2.25)	2.29 ± 0.05^{b} (2.20 – 2.43)	2.24 ± 0.05^{b} (2.22 – 2.27)	2.21 ± 0.05^{b} (2.18 – 2.23)	2.24 ± 0.05^{b} (2.21 – 2.27)	0.0176
March 1999	6	2.41 ± 0.03^{a} (2.32 – 2.49)	2.18 ± 0.03^{d} (2.15 – 2.24)	2.27 ± 0.03^{c} (2.08 – 2.37)	2.31 ± 0.03^{b} (2.21 – 2.38)	2.44 ± 0.03^{a} (2.40 – 2.50)	2.45 ± 0.03^{a} (2.37 – 2.50)	2.43 ± 0.03^{a} (2.33 – 2.53)	2.33 ± 0.03^{b} (2.21 – 2.52)	0.0001

Appendix 19 Plasma calcium ,plasma inorganic phosphate , plasma zinc, body condition score and body weight correlation in both non and mineral supplemented crossbred Zebu cows at ASAS Dairy Farm, Iringa Tanzania. (The first row for each parameter and season indicates r-value and second row indicates P value. D = dry season; W = rainy season)

Parameter	Season	N	Group 1 (Control)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)
Plasma Pi	D	42	-0.1006	0.1183	-0.0279	-0.2305	0.0867	0.0661	0.1125	0.1657
			0.5262	0.4557	0.8608	0.1420	0.58031	0.6776	0.4782	0.2942
	W	30	0.4245	0.3782	0.3104	0.4778	0.1122	0.3333	0.6600	0.1371
			0.0194	0.0393	0.0951	0.0076	0.5548	0.0719	0.0001	0.4702
Plasma zinc	D	42	0.3021	0.4582	0.3883	0.2308	0.3517	-0.0913	0.0911	0.2880
			0.0518	0.0023	0.0110	0.1414	0.0207	0.5655	0.5660	0.0644
	W	30	0.0457	-0.1578	-0.4222	-0.0184	0.1266	0.0327	0.8068	0.7677
			0.8105	0.4050	0.0201	0.9230	0.5051	0.8638	0.0001	0.0001
Body condition score	D	42	0.1499	0.1598	0.1601	-0.0568	0.1670	0.1184	0.1035	0.0019
			0.3435	0.3120	0.3111	0.7207	0.2759	0.4551	0.5142	0.9907
	W	30	-0.1121	-0.1511	-0.4520	-0.0953	0.2768	-0.3244	0.4287	0.4983
			0.5555	0.4255	0.0122	0.6164	0.1387	0.0803	0.0181	0.0051
Body weight	D	42	0.0495	0.0272	-0.0032	-0.1473	0.1995	0.1626	0.0649	0.0414
			0.7555	0.8640	0.9838	0.3521	0.1995	0.3034	0.6827	0.7944
	W	30	-0.1254	-0.0176	-0.2520	-0.0577	0.1618	0.0584	0.0460	0.1870
			0.5091	0.9665	0.1791	0.7620	0.3930	0.7591	0.8092	0.3225

Appendix 20 Plasma inorganic phosphate (Pi) (mmol /l) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at $P > 0.05$).

Month	n	Group 1 (Control)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	P value
May 1997	6	1.92 ± 0.09^a (1.88 – 1.96)	2.01 ± 0.09^a (1.94 – 2.17)	2.07 ± 0.09^a (1.83 – 2.28)	1.99 ± 0.09^a (1.79 –2.16)	2.10 ± 0.09^a (1.85 – 2.34)	2.15 ± 0.09^a (1.91 – 2.32)	2.11 ± 0.09^a (1.81 – 2.37)	2.12 ± 0.09^a (1.57 – 2.34)	0.5909
July 1997	6	1.79 ± 0.10^a (1.55 – 2.00)	1.86 ± 0.10^a (1.72 – 2.01)	2.03 ± 0.10^a (1.79 – 2.47)	1.94 ± 0.10^a (1.27 – 2.43)	1.85 ± 0.10^a (1.46 – 2.26)	1.87 ± 0.10^a (1.59 – 2.32)	2.14 ± 0.10^a (1.95 – 2.39)	2.10 ± 0.10^a (1.88 – 1.80)	0.1521
September 1997	6	2.22 ± 0.09^b (1.99 – 2.44)	2.43 ± 0.09^a (2.13 – 2.80)	2.20 ± 0.09^b (1.98 – 2.57)	2.18 ± 0.09^b (1.98 – 2.34)	2.26 ± 0.09^b (1.98 – 2.53)	2.36 ± 0.09^a (2.10 – 2.88)	2.34 ± 0.09^a (2.20 – 2.71)	2.40 ± 0.09^a (1.98 – 2.73)	0.0545
November 1997	6	2.23 ± 0.11^a (1.97 – 2.56)	2.43 ± 0.11^a (1.94 – 2.67)	2.27 ± 0.11^a (1.84 – 2.84)	2.24 ± 0.11^a (1.87 – 2.53)	2.26 ± 0.11^a (1.98 – 2.53)	2.35 ± 0.11^a (2.10 – 2.88)	2.34 ± 0.11^a (2.20 – 2.71)	2.40 ± 0.11^a (1.98 – 2.73)	0.8301
January 1998	6	2.08 ± 0.14^a (1.75 – 2.35)	2.28 ± 0.14^a (1.96 – 2.57)	2.28 ± 0.14^a (1.92 – 2.62)	2.07 ± 0.14^a (1.65 – 2.41)	2.21 ± 0.14^a (1.77 – 2.97)	1.82 ± 0.14^b (1.08 – 2.97)	2.08 ± 0.14^b (1.79 – 2.31)	2.27 ± 0.14^a (2.10 – 2.48)	0.0495
March 1998	6	1.92 ± 0.11^a (1.64 – 2.16)	2.03 ± 0.11^a (1.57 – 2.47)	2.04 ± 0.11^a (1.33 – 2.81)	1.87 ± 0.11^a (1.63 – 2.22)	1.98 ± 0.11^a (1.73 – 2.31)	1.97 ± 0.11^a (1.74 – 2.20)	1.74 ± 0.11^b (1.57 – 1.87)	2.03 ± 0.11^a (1.84 – 2.24)	0.0510
May 1998	6	1.99 ± 0.14^b (1.43 – 2.64)	2.25 ± 0.14^b (1.98 – 2.51)	2.05 ± 0.14^b (1.62 – 2.97)	2.29 ± 0.14^b (1.98 – 2.52)	2.57 ± 0.14^a (2.28 – 2.80)	2.49 ± 0.14^a (1.95 – 2.82)	2.37 ± 0.14^a (2.08 – 2.76)	2.44 ± 0.14^a (2.23 – 2.88)	0.0495
July 1998	6	2.05 ± 0.11^b (1.47 – 2.83)	2.12 ± 0.11^b (1.78 – 2.42)	2.21 ± 0.11^a (1.88 – 2.47)	2.26 ± 0.11^a (1.63 – 2.77)	1.82 ± 0.11^b (1.62 – 2.02)	2.00 ± 0.11^b (1.71 – 2.17)	1.97 ± 0.11^b (1.83 – 2.13)	1.64 ± 0.11^c (1.16 – 2.05)	0.0289
September 1998	6	2.02 ± 0.09^b (1.73 – 2.25)	2.38 ± 0.09^a (2.16 – 2.55)	2.11 ± 0.09^a (2.01 – 2.39)	2.17 ± 0.09^a (1.89 – 2.49)	2.19 ± 0.09^a (2.02 – 2.38)	2.30 ± 0.09^a (2.07 – 2.67)	2.38 ± 0.09^a (2.14 – 2.69)	2.33 ± 0.09^a (2.06 – 2.49)	0.0534
November 1998	6	1.89 ± 0.05^a (1.68 – 2.13)	1.98 ± 0.05^a (1.84 – 2.12)	1.99 ± 0.05^a (1.88 – 2.11)	1.95 ± 0.05^a (1.77 – 2.10)	2.10 ± 0.05^a (1.81 – 2.19)	1.94 ± 0.05^a (1.82 – 2.12)	2.08 ± 0.05^a (1.88 – 2.21)	2.00 ± 0.05^a (1.87 – 2.20)	0.1337
January 1999	6	1.70 ± 0.05^a (1.59 – 1.82)	1.76 ± 0.05^a (1.57 – 1.96)	1.82 ± 0.05^a (1.92 – 2.62)	1.78 ± 0.05^a (1.57 – 1.98)	1.78 ± 0.05^a (1.66 – 1.98)	1.80 ± 0.05^a (1.57 – 1.97)	1.82 ± 0.05^a (1.68 – 1.94)	1.69 ± 0.13^a (1.55 – 1.89)	0.4444
March 1999	6	1.67 ± 0.05^c (2.32 – 2.49)	1.83 ± 0.05^b (1.65 – 1.91)	1.80 ± 0.05^b (2.08 – 2.37)	1.74 ± 0.05^b (1.59 – 1.98)	1.59 ± 0.05^c (1.43 – 1.76)	1.71 ± 0.05^c (1.61 – 1.85)	1.96 ± 0.05^a (1.90 – 1.99)	1.66 ± 0.05^c (1.42 – 1.84)	0.0003

Appendix 21 Plasma zinc concentration (μmol/l) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at P > 0.05).

Month	n	Group 1 Con)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	P value
May 1997	6	12.4 ± 0.4^{a} (11.5 – 13.2)	12.2 ± 0.4^{a} (11.3 – 13.0)	12.5 ± 0.4^{a} (11.8 – 13.8)	12.5 ± 0.4^{a} (11.0 –14.2)	12.3 ± 0.4^{a} (10.9 – 13.2)	12.6 ± 0.4^{a} (11.3 – 13.8)	12.6 ± 0.4^{a} (11.9 – 14.1)	12.2 ± 0.4^{a} (11.5 – 13.8)	0.9775
July 1997	6	12.2 ± 0.3^{b} (10.8 – 13.3)	11.7 ± 0.3^{c} (11.3 – 12.2)	11.5 ± 0.3^{c} (11.2 – 11.9)	12.4 ± 0.3^{b} (11.0 – 14.0)	12.8 ± 0.3^{a} (11.2 – 14.4)	11.5 ± 0.3^{c} (10.7 – 12.4)	11.9 ± 0.3^{b} (11.0 – 13.0)	12.2 ± 0.3^{b} (11.6 – 12.9)	0.0110)
September 1997	6	11.4 ± 0.4^{c} (10.5 – 12.2)	13.4 ± 0.4^{a} (10.9 – 14.8)	12.3 ± 0.4^{b} (11.3 – 13.5)	12.3 ± 0.4^{b} (11.2 – 13.3)	10.2 ± 0.4^{d} (9.80 – 10.6)	10.8 ± 0.4^{d} (7.70 – 11.7)	11.2 ± 0.4^{c} (10.2 – 11.9)	12.3 ± 0.4^{b} (10.6 – 13.9)	0.0001
November 1997	6	10.6 ± 0.5^{d} (8.70 – 11.9)	12.5 ± 0.5^{b} (11.5 – 14.4)	9.93 ± 0.5^{e} (8.20 – 12.5)	13.3 ± 0.5^{a} (10.8 – 15.1)	11.4 ± 0.5^{d} (10.3 – 12.8)	12.5 ± 0.5^{b} (10.5 – 13.5)	12.2 ± 0.5^{c} (11.4 – 13.5)	12.1 ± 0.5^{c} (10.9 – 13.0)	0.0006
January 1998	6	11.8 ± 0.2^{c} (11.0 – 12.7)	14.5 ± 0.2^{a} (14.2 – 14.7)	10.8 ± 0.2^{d} (9.90 – 11.5)	11.6 ± 0.2^{c} (11.2 – 12.1)	12.3 ± 0.2^{b} (11.9 – 12.7)	12.2 ± 0.2^{ab} (11.6 – 12.9)	12.5 ± 0.2^{b} (11.9 – 13.0)	12.6 ± 0.2^{b} (12.1 – 13.0)	0.0001
March 1998	6	9.70 ± 0.5^{d} (8.50 – 10.9)	14.0 ± 0.5^{a} (12.5 – 15.7)	12.3 ± 0.5^{c} (10.2 – 13.3)	13.3 ± 0.5^{b} (11.4 – 15.8)	11.9 ± 0.5^{c} (11.0 – 12.9)	12.2 ± 0.5^{c} (10.0– 14.1)	7.80 ± 0.5^{e} (6.10 – 10.0)	8.8 ± 0.5^{d} (8.20 – 9.70)	0.0001
May 1998	6	13.0 ± 0.2^{c} (12.5 – 13.8)	14.0 ± 0.2^{a} (13.3 – 14.5)	12.5 ± 0.2^{d} (11.9 – 13.6)	13.5 ± 0.2^{b} (12.7 – 14.5)	11.4 ± 0.2^{e} (10.7 – 11.9)	12.1 ± 0.2^{d} (11.5– 12.4)	13.1 ± 0.2^{c} (12.4 – 13.6)	12.1 ± 0.2^{d} (11.6 – 12.5)	0.0001
July 1998	6	13.5 ± 0.2^{b} (12.1 – 14.2)	11.6 ± 0.2^{e} (11.2 – 12.1)	13.6 ± 0.2^{b} (12.7 – 14.2)	13.7 ± 0.2^{b} (13.0 – 14.2)	12.3 ± 0.2^{d} (11.9 – 12.9)	14.0 ± 0.2^{a} (12.7 – 14.8)	14.4 ± 0.2^{a} (14.2 – 14.7)	13.0 ± 0.2^{c} (12.2 – 13.6)	0.0001
September 1998	6	13.0 ± 0.3^{b} (12.7 – 13.5)	14.8 ± 0.3^{a} (13.8 – 15.6)	13.1 ± 0.3^{b} (12.7 – 13.6)	13.4 ± 0.3^{b} (12.4 – 13.9)	11.9 ± 0.3^{e} (10.7 – 13.3)	11.3 ± 0.3^{d} (10.7 – 11.9)	12.3 ± 0.3^{c} (11.3 – 13.8)	13.8 ± 0.3^{b} (12.9 – 14.7)	0.0001
November 1998	6	14.3 ± 0.2^{b} (12.8 – 14.9)	13.9 ± 0.2^{b} (13.2 – 14.4)	15.4 ± 0.2^{a} (14.8 – 15.8)	15.6 ± 0.2^{a} (15.4 – 15.8)	13.3 ± 0.2^{c} (13.0 – 13.5)	12.9 ± 0.2^{e} (12.5 – 13.3)	14.0 ± 0.2^{b} (12.4 – 14.5)	13.0 ± 0.2^{e} (12.7 – 13.2)	0.0001)
January 1999	6	13.2 ± 0.2^{a} (12.2 – 13.9)	11.4 ± 0.2^{d} (11.0 – 11.6)	12.3 ± 0.2^{b} (12.2 – 13.9)	11.8 ± 0.2^{e} (11.2 – 12.7)	12.1 ± 0.2^{e} (11.5 – 12.7)	12.0 ± 0.2^{e} (11.2 – 12.9)	12.0 ± 0.2^{e} (11.5 – 12.2)	11.8 ± 0.2^{e} (11.3 – 12.2)	0.0001
March 1999	6	12.1 ± 0.2^{b} (11.8 – 12.9)	12.1 ± 0.2^{b} (11.8 – 12.5)	13.4 ± 0.2^{a} (12.1 – 14.1)	12.6 ± 0.2^{b} (11.5 – 13.2)	12.5 ± 0.2^{b} (12.2 – 13.0)	12.2 ± 0.2^{b} (11.6 – 12.7)	12.0 ± 0.2^{b} (11.2 – 12.5)	12.2 ± 0.2^{b} (11.5 – 12.9)	0.0001

Appendix 22 Plasma Pi and plasma zinc, body condition score, body weight, packed cell volume % and haemoglobin in grazing correlation in both non and mineral supplemented crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (The first row for each parameter and season indicates r-value and second row indicates P value. D = dry season and W = rainy) season

Parameter	Season	N	Group 1 (Control)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)
Plasma Zn	D	42	-0.0699	0.4338	-0.0311	-0.0022	-0.2553	-0.3847	-0.2741	-0.1858
			0.6600	0.0041	0.8449	0.9890	0.0985	0.0119	0.0790	0.2388
	W	30	-0.3660	0.2473	-0.4001	0.1410	-0.2981	0.0488	0.5639	0.0260
			0.0467	0.1878	0.0285	0.4574	0.1097	0.7981	0.0012	0.8914
Body condition score	D	42	0.0544	0.3208	0.2295	0.0663	0.0882	0.1229	0.2468	0.0742
			0.7322	0.0383	0.1437	0.6767	0.5739	0.4381	0.1151	0.6406
	W	30	-0.2337	0.0972	0.0412	0.0724	0.0492	-0.1123	0.0991	-0.0074
			0.2138	0.6095	0.8291	0.7039	0.7962	0.5545	0.6022	0.9690
Body weight	D	42	0.1059	0.0015	-0.0357	0.1889	0.0212	0.1595	0.1229	-0.1360
			0.5044	0.9923	0.8224	0.2315	0.8928	0.3129	0.4380	0.3906
	W	30	-0.2610	-0.2753	-0.0146	-0.0267	0.1471	0.0289	0.1012	-0.1206
			0.1635	0.1409	0.9388	0.8885	0.4379	0.8794	0.5949	0.5255
Packed cell volume	D	42	-0.1400	-0.3378	0.0142	-0.0714	0.0893	0.2902	0.2367	0.0920
			0.3765	0.0287	0.9239	0.6533	0.5691	0.0623	0.1313	0.5623
	W	30	-0.2756	0.0149	0.2190	0.1074	-0.1251	-0.3219	0.1171	-0.1615
			0.1404	0.9377	0.2450	0.5723	0.5102	0.0828	0.5378	0.3939
Haemoglobin	D	42	-0.0977	-0.1943	0.2727	-0.0253	0.1190	0.02761	0.2436	0.1368
			0.5381	0.2176	0.0807	0.8728	0.4473	0.8622	0.1201	0.3878
	W	30	0.1060	0.1223	0.3461	0.0068	-0.1581	-0.2056	-0.1415	-0.0847
			0.5773	0.5195	0.0610	0.9715	0.4039	0.2756	0.4456	0.6565

Table 23 Incidences of mastitis cases in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania.

Month	Group 1 (Control)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	Total Research n = 48
May 1997	-	-	-	-	-	-	-	-	-
July 1997	3	2	3	1	3	2	1	2	17
September 1997	1	1	1	-	-	1	-	-	4
November 1997	2	-	3	1	-	1	3	1	11
January 1998	1	-	2	1	-	-	3	2	9
March 1998	2	1		1	2	-	1	2	11
May 1998	-	-	2	1	1	1	-	1	6
July 1998	-	2	-	-	3	3	1	2	11
September 1998	1	1	2	1	2	2	1	-	10
November 1998	2	-	-	1	-	-	1	-	4
January 1999	2	-	3	-	2	1	-	1	9
March 1999	1	-	-	-	2	2	3	3	11
Total Cases	15	7	16	7	15	13	14	14	

Appendix 24 Incidences of anaplasmosis cases in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy farm, Iringa, Tanzania.

Month	Group 1 (Con)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	Total Research n = 48
May 1997	-	-	-	-	-	-	-	-	-
July 1997	-	-	1	-	-	-	-	-	1
September 1997	1	1	2	-	-	1	-	-	5
November 1997	1	-	1	-	-	1	-	-	3
January 1998	-	-	-	-	1	-	-	-	1
March 1998	-	1	-	2	1	-	1	2	7
May 1998	-	-	-	-	1	-	-	-	1
July 1998	2	1	2	-	-	1	-	2	8
September 1998	1	-	1	-	-	2	1	-	5
November 1998	1	-	-	-	-	-	-	-	1
January 1999	-	-	-	1	-	-	-	-	1
March 1999	-	-	-	-	-	-	-	-	-
Total Cases	6	3	7	3	3	5	2	4	33

Appendix 25 Reproductive problems in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (Dates indicates time of problem).

	Group 1 (Con)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	Total cases n = 48
Embryonic death	1	-	-	1	-	1	-	1	4
Abortions	1	-	1	1	1	2	1	-	7
	20.12.97		20.3.98	15.6.98	25.9.97	2.8.97 20.3.98	16.8.97		
Still birth	1	-	1	1	1	-	2	2	8
	5.1.98		10.1.98	14.10.97	21.1.98		27.12.97 5.1.98	2.1.98 10.1.98	
Retained placenta	1	-	-	-	2	-	-	-	3
Pyometra	1	-	-	-	-	-	-	-	1
Cystic ovaries	1	-	-	-	1	-	-	-	2
Total cases	6	0	2	3	5	3	3	3	25

Appendix 26 Live body weight (kg) in both non and mineral supplemented grazing crossbred Zebu cows, at ASAS Dairy Farm, Iringa, Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at P > 0.05).

	n	Group 1 (Con)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	P value
May 1997	6	374 ± 21[a] (288 - 423)	370 ± 21[a] (273 – 428)	367 ± 21[a] (287 – 443)	369 ± 21[a] (276 –401)	371 ± 21[a] (307 – 447)	375 ± 21[a] (310 – 441)	374 ± 21[a] (275 – 430)	374 ± 21[a] (300 – 436)	0.9978
July 1997	6	381 ± 22[a] (292 – 429)	386 ± 22[a] (286 – 448)	375 ± 22[a] (303 – 450)	375 ± 22[a] (292 – 416)	384 ± 22[a] (315 – 455)	379 ± 22[a] (316 – 442)	395 ± 22[a] (280 – 450)	400 ± 22[a] (320 – 470)	0.9991
September 1997	6	388 ± 21[a] (305 – 426)	397 ± 21[a] (299 – 465)	375 ± 21[a] (293 – 451)	394 ± 21[a] (320 – 428)	385 ± 21[a] (305 – 470)	389 ± 21[a] (325 – 451)	395 ± 21[a] (281 – 461)	406 ± 21[a] (332 – 461)	0.9887
November 1997	6	375 ± 20[a] (285 – 426)	391 ± 20[a] (306 – 468)	366 ± 20[a] (285 – 423)	391 ± 20[a] (326 – 423)	388 ± 20[a] (320 – 446)	384 ± 20[a] (315 – 442)	391 ± 20[a] (280 – 470)	397 ± 20[a] (332 – 463)	0.9486
January 1998	6	389± 20[a] (275 – 458)	402 ± 20[a] (332 – 470)	373 ± 20[a] (290 – 421)	385 ± 20[a] (316 – 423)	383 ± 20[a] (321 – 466)	383 ± 20[a] (320 – 444)	383 ± 20[a] (295 – 460)	393 ± 20[a] (335 – 435)	0.9837
March 1998	6	379 ± 20[a] (306 – 420)	389 ± 20[a] (315 – 458)	375 ± 20[a] (273 – 431)	385 ± 20[a] (316 – 423)	389 ± 20[a] (340 – 470)	383 ± 20[a] (300– 458)	382 ± 20[a] (280 – 470)	385 ± 20[a] (300 – 440)	0.9983
May 1998	6	396 ± 20[a] (322 – 443)	398 ± 20[a] (329 – 458)	373 ± 20[a] (280 – 443)	391 ± 20[a] (326 – 435)	382 ± 20[a] (310 – 473)	391 ± 20[a] (320– 468)	394 ± 20[a] (305 – 475)	401 ± 20[a] (348 – 467)	0.9872
July 1998	6	390± 20[a] (300 – 436)	403 ± 20[a] (324 – 460)	382 ± 20[a] (305 – 465)	385 ± 20[a] (323 – 433)	387 ± 20[a] (330 – 440)	383 ± 20[a] (330 – 470)	392 ± 88[a] (300 – 460)	398 ± 20[a] (330 – 470)	0.9881
September 1998	6	392 ± 21[a] (275 – 456)	408 ± 21[a] (346 – 470)	384 ± 21[a] (323 – 453)	390 ± 21[a] (314 – 443)	395 ± 21[a] (340 – 438)	401 ± 21[a] (325 – 477)	405 ± 21[a] (315 – 465)	407 ± 21[a] (323 – 480)	0..9516
November 1998	6	391 ± 21[a] (280 – 470)	412 ± 21[a] (350 – 475)	394 ± 21[a] (341 – 458)	400 ± 21[a] (320 – 468)	397 ± 21[a] (350 – 446)	399 ± 21[a] (350 – 487)	408 ± 21[a] (310 – 485)	414 ± 21[a] (341 – 475)	0.9689
January 1999	6	395 ± 20[a] (316 – 463)	416 ± 20[a] (350 – 471)	395 ± 20[a] (355 – 443)	394± 20[a] (321 – 470)	384 ± 20[a] (352 – 458)	385 ± 20[a] (320 – 441)	404 ± 20[a] (325 – 495)	406 ± 20[a] (353 – 480)	0.8946
March 1999	6	399 ± 20[a] (310 – 452)	414 ± 42[a] (373 – 482)	376 ± 20[a] (323 – 431)	388 ± 20[a] (306 – 480)	398 ± 20[a] (358 – 472)	387 ± 20[a] (310 – 473)	403 ± 20[a] (350 – 460)	404 ± 20[a] (360 – 460)	0.9020

Appendix 27 Plasma Zn and body condition score, body weight, percentage packed cell volume and haemoglobin correlation in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy farm, Iringa, Tanzania. (The first row for each parameter and season indicates r-value and second row indicates P value. D = dry season; W = rainy season)

Parameter	Season	N	Group 1 (Control)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)
Body condition score	D	42	-0.0241	0.1772	-0.0390	0.0618	-0.2357	0.0557	0.1553	-0.0134
			0.8798	0.2616	0.8064	0.6976	0.1281	0.7261	0.3262	0.9328
	W	30	0.3454	-0.0127	0.1287	-0.0203	0.0072	0.1786	0.5866	0.5443
			0.0616	0.9469	0.4980	0.9151	0.9700	0.3449	0.0007	0.0019
Body weight	D	42	0.0266	0.2021	0.0595	0.2030	0.0180	-0.0821	0.0851	0.2232
			0.8674	0.1993	0.7080	0.1973	0.9086	0.6051	0.5919	0.1554
	W	30	0.1855	-0.0152	0.2517	-0.0965	0.1310	0.2945	0.2393	0.1674
			0.3264	0.9355	0.1798	0.6122	0.4903	0.1142	0.2028	0.3767
Packed cell volume	D	42	-0.0371	-0.1170	0.2946	0.1384	0.1139	0.1064	-0.1196	-0.2403
			0.8158	0.4605	0.0583	0.3822	0.4671	0.5025	0.4505	0.1253
	W	30	0.0160	0.2815	-0.1411	0.1356	0.1020	0.0821	-0.1566	0.0014
			0.9333	0.1318	0.4571	0.4751	0.5917	0.6664	0.4086	0.9942
Haemoglobin	D	42	-0.3781	-0.2543	-0.2684	-0.2532	-0.2285	-0.2012	-0.4415	-0.3034
			0.0136	0.1041	0.0857	0.1057	0.1405	0.2014	0.0034	0.0508
	W	30	-0.1941	0.4573	-0.0939	0.1396	0.0142	-0.0162	-0.1437	-0.3353
			0.3040	0.0111	0.6217	0.4618	0.9408	0.9323	0.4486	0.0701

Appendix 28 Body condition score in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy farm, Iringa, Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at P > 0.05).

Month	n	Group 1 (Con)	Group 2 (Ca	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	P value
May 1997	6	3.00 ± 0.12[a] (2.50 – 3.50)	3.00 ± 0.12[a] (2.50 – 3.50)	3.00 ± 0.12[a] (2.50 – 3.50)	2.83 ± 0.12[a] (2.00 –3.00)	2.92 ± 0.12[a] (2.50 – 3.00)	2.92 ± 0.12[a] (2.50 – 3.00	2.83 ± 0.12[a] (2.50 – 3.00)	2.92 ± 0.12[a] (2.50 – 3.00)	0.9239
July 1997	6	2.75 ± 0.13[b] (2.50 – 3.00)	3.17± 0.13[a] (2.50 – 3.50)	3.17 ± 0.13[a] (3.00 – 3.50)	2.83 ± 0.13[b] (2.00 – 3.00)	3.00 ± 0.13[b] (2.50 – 3.50)	2.92 ± 0.13[b] (2.50 – 3.00)	2.83 ± 0.13[b] (2.50 – 3.00)	3.17 ± 0.13[a] (3.0 – 3.5)	0.0536
September 1997	6	3.17 ± 0.17[a] (3.00 – 3.50)	3.42 ± 0.17[a] (3.00 – 4.00)	3.30± 0.17[a] (2.50 – 3.50)	3.33 ± 0.17[a] (3.00 – 3.50)	3.33 ± 0.17[a] (3.50 – 4.00)	3.17 ± 0.17[a] (3.00 – 3.50)	3.33 ± 0.17[a] (2.50 – 4.00)	3.42 ± 0.17[a] (3.00 – 4.00)	0.3010
November 1997	6	2.58 ± 0.16[c] (2.00 – 3.00)	3.42 ± 0.16[a] (3.00 – 4.00)	2.50 ± 0.16[c] (2.50 – 2.50)	3.08 ± 0.16[b] (2.50 – 4.00)	3.16 ± 0.16[a] (3.00 – 3.50)	2.67 ± 0.16[c] (2.50 – 3.00)	2.92 ± 0.16[b] (2.50 – 3.50)	3.25 ± 0.16[a] (2.50 – 4.00)	0.0027
January 1998	6	3.08 ± 0.15[b] (3.00 – 3.50)	3.42 ± 0.15[a] (3.00 – 4.00)	3.00 ± 0.15[b] (2.50 – 3.50)	3.08 ± 0.15[b] (3.00 – 3.50)	3.17 ± 0.15[a] (3.00 – 3.50)	2.92 ± 0.15[b] (2.50 – 3.50)	3.08 ± 0.15[b] (2.50 – 3.50)	3.25 ± 0.15[a] (3.00 – 4.00)	0.0543
March 1998	6	2.75 ± 0.13a (2.50 – 3.00)	3.17 ± 0.13[a] (3.00 – 3.50)	3.00 ± 0.13[a] (2.50 – 3.50)	3.25 ± 0.13[a] (3.00 – 4.00)	3.08 ± 0.13[a] (3.00 – 3.50)	3.25 ± 0.13[a] (2.00– 3.50)	2.50 ± 0.13[a] (3.00 – 3.50)	2.75 ± 0.13[a] (3.00 – 4.00)	0.8529
May 1998	6	3.17 ± 0.14[a] (2.50 – 3.50)	3.08 ± 0.14[a] (2.50 – 3.50)	3.00 ± 0.14[a] (2.50 – 3.50)	3.08 ± 0.14[a] (3.00 – 3.50)	3.17 ± 0.14[a] (3.00 – 3.50)	3.25 ± 0.14[a] (3.00– 4.00)	3.42 ± 0.14[a] (3.00 – 400)	3.17 ± 0.14[a] (3.00 – 4.00)	0.2334
July 1998	6	2.92 ± 0.17[b] (2.50 – 3.50)	3.33 ± 0.17[a] (3.00 – 4.00)	2.92 ± 0.17[a] (2.50 – 3.00)	3.42 ± 0.17[a] (3.00 – 4.00)	3.08 ± 0.17[a] (2.00 – 4.00)	3.00 ± 0.17[a] (2.50– 3.50)	3.17 ± 0.17[a] (2.50 – 3.50)	3.17 ± 0.17[a] (3.00 – 3.50)	0.3162
September 1998	6	3.08 ± 0.16[a] (3.0 – 3.50)	3.33 ± 0.17[a] (3.00 – 4.00)	3.17 ± 0.16[a] (3.00 – 3.50)	3.17 ± 0.16[a] (3.00 – 3.50)	3.50 ± 0.16[a] (3.00 – 4.00)	3.25± 0.165[a] (3.00– 4.00)	3.33 ± 0.16[a] (3.00 – 4.00)	3.33 ± 0.17[a] (3.00 – 4.00)	0.4361
November 1998	6	2.92 ± 0.20[a] (2.50 – 3.00)	3.42 ± 0.20[a] (3.00 – 4.00)	3.17 ± 0.20[a] (3.00 – 3.50)	3.33 ± 0.20[a] (3.00 – 4.00)	3.00 ± 0.20[a] (2.50 – 4.00)	3.17 ± 0.20[a] (2.50– 4.00)	3.33 ± 0.20[a] (2.50 – 4.00)	3.25 ± 0.20[a] (2.50 – 4.00)	0.7006
January 1999	6	2.92 ± 0.17[b] (2.50 – 3.50)	3.42 ± 0.5[a] (3.00 – 4.00)	2.92 ± 0.17[a] (2.50 – 3.00)	3.33 ± 0.17[a] (3.00 – 4.00)	3.17 ± 0.17[a] (2.50 – 3.50)	3.08 ± 0.17[a] (3.00 – 3.50)	3.33± 0.17[a] (2.50 – 4.00)	3.08 ± 0.17[a] (3.00 – 3.50)	0.3032
March 1999	6	3.00 ± 0.17[a] (3.00 – 3.00)	3.25 ± 0.17[a] (3.00 – 3.50)	2.75 ± 0.17[a] (2.50 – 3.00)	3.17 ± 0.17[a] (2.50 – 4.00)	3.17 ± 0.17[a] (2.50 – 4.00)	3.08 ± 0.17[a] (2.50– 3.50)	3.42 ± 0.2[a] (3.00 – 3.50)	3.17 ± 0.3[a] (3.00 – 3.50)	0.0965

Appendix 29 Packed cell volume (%) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at P > 0.05).

Month	n	Group 1 (Con)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	P value
May 1997	6	33.5 ± 1.1^{a} (30.0 – 35.0)	32.7 ± 1.1^{a} (31.0 – 35.0)	32.8 ± 1.1^{a} (27.0 – 39.0)	32.5 ± 1.1^{a} (31.0 –35.0)	32.3 ± 1.1^{a} (31.0 – 34.0)	33.2 ± 1.1^{a} (29.0 – 41.0)	33.2 ± 1.1^{a} (30.0 – 35.0)	32.7 ± 1.1^{a} (29.0 – 35.0)	0.9965
July 1997	6	33.2 ± 1.1^{a} (30.0 – 36.0)	30.7 ± 1.1^{a} (25.0 – 36.0)	27.8 ± 1.1^{b} (24.0 – 32.0)	29.7 ± 1.1^{b} (26.0 – 34.0)	28.7 ± 1.1^{b} (25.0 – 31.0)	27.2 ± 1.1^{c} (24.0 – 30.0)	30.3 ± 1.1^{b} (29.0 – 33.0)	29.8 ± 1.1^{b} (27.0 – 34.0)	0.0127
September 1997	6	31.7 ± 1.1^{a} (29.0 – 37.0)	28.8 ± 1.1^{b} (26.0 – 31.0)	28.2 ± 1.1^{b} (25.0 – 32.0)	29.8 ± 1.1^{b} (27.0 – 33.0)	27.2 ± 1.1^{b} (23.0 – 32.0)	28.5 ± 1.1^{b} (25.0 – 31.0)	28.0 ± 1.1^{b} (26.0 – 32.0)	27.5 ± 1.1^{b} (25.0 – 30.0)	0.0520
November 1997	6	32.0 ± 1.5^{a} (28.0 – 36.0)	31.2 ± 1.5^{a} (28.0 – 36.0)	30.5 ± 1.5^{a} (25.0 – 38.0)	32.3 ± 1.5^{a} (29.0 – 35.0)	27.0 ± 1.5^{b} (21.0 – 34.0)	29.8 ± 1.5^{a} (27.0 – 32.0)	30.3 ± 1.5^{a} (28.0 – 38.0)	28.7 ± 1.5^{a} (23.0 – 32.0)	0.0532
January 1998	6	35.0 ± 1.1^{a} (33.0 – 36.0)	32.3 ± 1.1^{a} (30.0 – 36.0)	32.7 ± 1.1^{a} (30.0 – 38.0)	33.3 ± 1.1^{a} (30.0 – 39.0)	34.2 ± 1.1^{a} (30.0 – 38.0)	31.7 ± 1.1^{a} (26.0 – 36.0)	32.8 ± 1.1^{a} (30.0 – 36.0)	32.5 ± 1.1^{a} (29.0 – 37.0)	0.5283
March 1998	6	35.2 ± 1.2^{a} (30.0 – 41.0)	34.5 ± 1.2^{a} (31.0 – 41.0)	33.2 ± 1.2^{a} (29.0 – 36.0)	30.0 ± 1.2^{c} (27.0 – 35.0)	29.0 ± 1.2^{c} (25.0 – 33.0)	32.3 ± 1.2^{b} (29.0– 34.0)	32.7 ± 1.2^{b} (28.0 – 36.0)	31.5 ± 1.2^{b} (28.0 – 34.0)	0.0110
May 1998	6	32.8 ± 1.1^{a} (29.0 – 38.0)	30.7 ± 1.1^{a} (28.0 – 34.0)	31.3 ± 1.1^{a} (27.0 – 36.0)	31.5 ± 1.1^{a} (30.0 – 35.0)	30.5 ± 1.1^{a} (28.0 – 33.0)	31.0 ± 1.1^{a} (27.0– 35.0)	32.0 ± 1.1^{a} (29.0 – 34.0)	33.2 ± 1.1^{a} (29.0 – 38.0)	0.5925
July 1998	6	30.8 ± 1.3^{a} (22.0 – 39.0)	29.2 ± 1.3^{a} (25.0 – 32.0)	31.2 ± 1.3^{a} (27.0 – 34.0)	30.2 ± 1.3^{a} (28.0 – 32.0)	29.7 ± 1.3^{a} (27.0 – 32.0)	27.8 ± 1.3^{a} (20.0 – 32.0)	24.8 ± 1.3^{b} (22.0 – 27.0)	29.2 ± 1.3^{a} (27.0 – 32.0)	0.0435
September 1998	6	28.7 ± 1.4^{b} (22.0 – 35.0)	28.7 ± 1.4^{b} (22.0 – 32.0)	30.5 ± 1.4^{b} (28.0 – 35.0)	26.8 ± 1.4^{b} (22.0 – 33.0)	32.2 ± 1.4^{a} (29.0 – 38.0)	28.2 ± 1.4^{b} (24.0 – 32.0)	29.3 ± 1.4^{b} (28.0 – 30.0)	27.7 ± 1.4^{b} (22.0 – 31.0)	0.0528
November 1998	6	32.8 ± 1.1^{a} (30.0 – 38.0)	31.0 ± 1.1^{a} (29.0 – 32.0)	31.3 ± 1.1^{a} (27.0 – 35.0)	31.8 ± 1.1^{a} (27.0 – 37.0)	28.3 ± 1.1 (24.0 – 31.0)	29.8 ± 1.1^{a} (27.0 – 32.0)	29.7 ± 1.1^{a} (26.0 – 37.0)	29.7 ± 1.1^{a} (27.0 – 31.0)	0.1660
January 1999	6	34.0 ± 1.0^{a} (31.0 – 36.0)	27.8 ± 1.1^{c} (24.0 – 32.0)	30.5 ± 1.1^{b} (25.0 – 34.0)	29.7 ± 1.1^{b} (24.0 – 34.0)	29.0 ± 1.1^{b} (26.0 – 31.0)	32.0 ± 1.1^{b} (31.0 – 33.0)	29.8 ± 1.1^{b} (27.0 – 32.0)	30.5 ± 1.1^{b} (27.0 – 33.0)	0.0061
March 1999	6	35.3 ± 1.3^{a} (31.0 – 39.0)	33.0 ± 1.3^{a} (30.0 – 39.0)	31.3 ± 1.3^{a} (24.0 – 36.0)	31.5 ± 1.3^{a} (27.0 – 35.0)	31.0 ± 1.3^{a} (28.0 – 36.0)	32.2 ± 1.3^{a} (28.0 – 35.0)	31.0 ± 1.3^{a} (29.0 – 32.0)	31.2 ± 1.3^{a} (28.0 – 34.0)	0.2416

Appendix 30 Haemoglobin concentration (g/dl) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at P > 0.05).

Month	n	Group 1 (Control)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	P value
May 1997	6	11.6 ± 0.5^{a} (10.4 – 12.6)	11.2 ± 0.5^{a} (8.5 – 12.3)	10.8 ± 0.5^{a} (8.9 – 12.3)	11.1 ± 0.5^{a} (8.9 –12.3)	10.9 ± 0.5^{a} (9.7 – 12.7)	11.2 ± 0.5^{a} (10.0 – 12.8)	11.1 ± 0.5^{a} (8.9 – 12.2)	11.1 ± 0.5^{a} (10.3 – 11.6)	0.9695
July 1997	6	12.5 ± 0.5^{a} (10.8 – 13.8)	12.6 ± 0.5^{a} (10.4 – 12.7)	11.7 ± 0.5^{a} (11.8 – 13.7)	12.1 ± 0.5^{a} (11.1 – 13.2)	11.3 ± 0.5^{a} (10.4 – 13.3)	12.3 ± 0.5^{a} (11.2 – 13.2)	11.6 ± 0.5^{a} (9.2 – 14.3)	12.2 ± 0.5^{a} (11.0 – 13.5)	0.5414
September 1997	6	11.4 ± 0.4^{a} (9.6 – 13.3)	10.0 ± 0.4^{c} (9.3 – 11.4)	9.8 ± 0.4^{c} (7.6 – 11.0)	10.7 ± 0.4^{b} (9.2 – 11.8)	11.4 ± 0.4^{a} (10.3 – 13.0)	12.2 ± 0.4^{a} (10.8 – 13.2)	11.6 ± 0.4^{a} (10.4 – 12.4)	11.4 ± 0.4^{a} (10.0 – 12.4)	0.0038
November 1997	6	11.5 ± 0.5^{a} (9.6 – 13.3)	10.6 ± 0.5^{a} (9.7 – 12.0)	10.5 ± 0.5^{a} (8.3 – 11.3)	11.0 ± 0.5^{a} (10.0 – 11.6)	10.4 ± 0.5^{a} (8.9 – 12.0)	11.0 ± 0.5^{a} (10.0 – 12.0)	10.6 ± 0.5^{a} (8.9 – 12.7)	11.1 ± 0.5^{a} (10.0 – 12.3)	0.7091
January 1998	6	12.0 ± 0.6^{b} (9.7 – 14.2)	13.0 ± 0.6^{a} (11.3 – 14.8)	11.4 ± 0.6^{b} (8.8 – 14.7)	11.9 ± 0.6^{b} (10.6 – 14.3)	11.3 ± 0.6^{b} (10.2 – 13.2)	10.3 ± 0.6^{c} (8.3 – 12.0)	9.8 ± 0.6^{d} (8.8 – 10.9)	10.4 ± 0.6^{c} (8.3 – 12.3)	0.0139
March 1998	6	13.7 ± 0.6^{a} (11.0 – 17.8)	12.4 ± 0.6^{a} (9.9 – 14.4)	12.0 ± 0.6^{a} (10.3 – 14.0)	11.7 ± 0.6^{a} (10.3 – 13.2)	10.8 ± 0.6^{a} (9.2 – 12.1)	12.0 ± 0.6^{a} (10.7– 13.2)	11.6 ± 0.6^{a} (10.30 – 13.2)	12.1 ± 0.6^{a} (9.6 – 14.4)	0.1167
May 1998	6	12.7 ± 0.6^{a} (10.7 – 14.1)	12.0 ± 0.6^{a} (11.0 – 14.6)	12.3 ± 0.6^{a} (8.5 – 14.7)	12.2 ± 0.6^{a} (11.0 – 13.5)	11.5 ± 0.6^{a} (8.8 – 14.0)	11.6 ± 0.6^{a} (10.3– 12.9)	12.0 ± 0.6^{a} (11.5 – 12.5)	11.2 ± 0.6^{a} (10.2 – 13.3)	0.6672
July 1998	6	9.2 ± 0.6^{b} (6.3 – 12.5)	8.3 ± 0.6^{c} (7.0 – 9.2)	10.8 ± 0.6^{a} (8.5 – 14.2)	8.0 ± 0.6^{c} (7.0 – 9.6)	9.6 ± 0.6^{b} (8.1 – 11.4)	10.4 ± 0.6^{a} (8.8 – 11.8)	9.6 ± 0.6^{b} (7.4 – 11.4)	11.0 ± 0.6^{a} (9.9 – 11.8)	0.0102
September 1998	6	9.5 ± 0.4^{b} (8.5 – 11.8)	8.1 ± 0.4^{c} (7.0 – 9.2)	9.7 ± 0.4^{b} (8.5 – 10.7)	8.3 ± 0.4^{c} (6.6 – 9.6)	8.2 ± 0.4^{c} (7.4 – 9.2)	9.2 ± 0.4^{b} (8.1 – 9.9)	9.7 ± 0.4^{b} (8.1 – 10.3)	10.9 ± 0.4^{a} (9.6 – 12.1)	0.0001
November 1998	6	9.8 ± 0.3^{a} (9.2 – 11.0)	9.0 ± 0.3^{b} (8.1 – 9.9)	9.1 ± 0.3^{b} (8.1 – 10.3)	9.1 ± 0.3^{b} (7.7 – 10.3)	8.6 ± 0.3^{c} (7.4 – 9.6)	9.3 ± 0.3^{b} (8.5 – 10.3)	9.1 ± 0.3^{b} (7.7 – 11.0	8.9 ± 0.3^{b} (7.7 – 9.8)	0.0538
January 1999	6	10.8 ± 0.7^{b} (9.2 – 12.1)	9.3 ± 0.7^{c} (7.3 – 11.4)	9.7 ± 0.7^{c} (7.7 – 12.1)	10.3 ± 0.7^{b} (7.0 – 13.6)	11.0 ± 0.7^{b} (8.8 – 12.5)	12.5 ± 0.7^{a} (11.8 – 13.3)	12.0 ± 0.7^{a} (8.1 – 14.7)	10.7 ± 0.7^{b} (8.5 – 13.3)	0.0521
March 1999	6	12.2 ± 0.5^{a} (9.2 – 13.7)	12.3 ± 0.5^{a} (11.1 – 14.2)	11.4 ± 0.5^{a} (9.6 – 13.0)	11.3 ± 0.5^{a} (10.7 – 13.0)	11.2 ± 0.5^{a} (10.0 – 13.7)	11.3 ± 0.5^{a} (10.8 – 12.3)	11.7 ± 0.5^{a} (10.3 – 14.1)	10.5 ± 0.5^{a} (9.6 – 11.5)	0.2023

Appendix 31 Total white blood cell counts (x 10^3 per µl) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at $P > 0.05$).

Month	n	Group 1 (Control)	Group 2 (Ca	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	P value
May 1997	6	7.37 ± 0.37^a (7.30 – 9.75)	7.31 ± 0.37^a (5.05 – 10.00)	7.28 ± 0.37^a (5.20 – 9.90)	7.44 ± 0.37^a (6.20 – 9.15)	7.44 ± 0.37^a (6.60 – 10.6)	7.68 ± 0.37^a (6.00 – 9.00)	7.61± 0.37^a (6.45 – 8.40)	7.15 ± 0.37^a (5.60 – 8.60)	0.5909
July 1997	6	7.69 ± 0.74^a (6.40 – 11.0)	8.23 ± 0.74^a (5.65 – 10.05)	8.21 ± 0.74^a (5.60 – 10.80)	7.87 ± 0.74^a (6.65– 0.95)	7.41 ± 0.74^a (5.60 – 9.80)	7.10 ± 0.74^a (5.10 – 11.35)	7.31± 0.74^a (5.05 – 9.30)	9.19 ± 0.74^a (7.35 – 12.0)	0.5739
September 1997	6	7.72 ± 0.60^a (5.05 – 9.50)	8.08 ± 0.60^a (5.05 – 9.40)	7.27 ± 0.60^a (3.50 – 9.50)	8.24 ± 0.60^a (6.85 – 9.15)	8.26 ± 0.60^a (7.45 – 9.80)	7.13 ± 0.60^a (3.85 – 8.45)	8.49± 0.60^a (7.30– 1.35)	9.28 ± 0.60^a (7.50 – 11.70)	0.2651
November 1997	6	5.21 ± 0.77^a (4.15 – 6.75)	6.13 ± 0.77^a (4.20 – 7.15)	5.92 ± 0.77^a (3.10 – 10.90)	5.38 ± 0.77^a (2.75 – 8.40)	6.08 ± 0.77^a (4.30 – 8.05)	7.35 ± 0.77^a (4.20 – 10.45)	6.87 ± 0.77^a (4.80 – 8.60)	6.48 ± 0.77^a (4.20 – 9.90)	0.5400
January 1998	6	8.20 ± 0.79^a (6.75 – 11.75)	8.48 ± 0.79^a (6.45 – 10.05)	9.63 ± 0.79^a (6.40 – 11.65)	7.88 ± 0.79^a (3.85– 1.25)	8.82 ± 0.79^a (6.00– 1.20)	9.29 ± 0.79^a (6.95 – 11.85)	10.3 ± 0.79^a (9.05– 1.80)	10.3 ± 0.79^a (8.25 – 11.85)	0.2529
March 1998	6	6.91 ± 0.79^a (3.50 – 8.00)	7.47 ± 0.79^a (5.20 – 9.70)	8.69 ± 0.79^a (5.50 – 11.70)	6.94 ± 0.79^a (3.80 – 8.85)	6.88 ± 0.79^a (5.90 – 8.60)	6.53 ± 0.79^a (3.20 – 11.55)	7.10± 0.79^a (5.85 – 9.45)	6.67 ± 0.79^a (5.35 – 7.65)	0.6330
May 1998	6	8.81 ± 0.48^a (6.65 – 11.15)	7.02 ± 0.48^b (6.75 – 7.60)	7.69 ± 0.48^b (5.30 – 10.00)	7.38 ± 0.48^b (6.50 – 8.40)	6.99 ± 0.48^b (6.00 – 7.85)	8.28 ± 0.48^a (7.00 – 9.80)	7.68± 0.48^b (6.50 – 9.50)	7.98 ± 0.48^b (6.65 – 10.20)	0.0448
July 1998	6	6.88 ± 0.45^a (5.40 – 8.25)	6.79 ± 0.45^a (5.75 – 8.25)	5.83 ± 0.45^a (3.85 – 7.25)	6.58 ± 0.45^a (5.35 – 8.25)	6.71 ± 0.45^a (5.40 – 8.60)	6.48 ± 0.45^a (4.90 – 8.00)	6.93 ± 0.45^a (5.55 – 8.85)	7.22 ± 0.45^a (6.35 – 8.10)	0.5762
September 1998	6	8.81 ± 0.36^b (7.45 – 10.80)	9.96 ± 0.36^a (8.60 – 10.80)	10.03 ± 0.36^a (8.00 – 10.65)	9.94 ± 0.36^a (9.30– 0.80)	9.83 ± 0.36^a (9.00– 0.85)	10.2 ± 0.36^a (8.85 – 10.75)	9.99± 0.36^a (9.35– 10.6)	9.78 ± 0.36^a (8.00 – 10.65)	0.0532
November 1998	6	9.03 ± 0.65^a (7.00 – 10.80)	9.39 ± 0.65^a (6.60 – 10.70)	9.16 ± 0.65^a (5.02 – 10.70)	8.89 ± 0.65^a (6.60– 0.80)	9.70 ± 0.65^a (6.90– 0.90)	9.06 ± 0.65^a (6.05 – 10.50)	9.84± 0.65^a) (8.00– 10.6)	9.66 ± 0.65^a (7.00 – 10.80)	0.9468
January 1999	6	8.15 ± 0.79^a (5.55 – 10.80)	8.47 ± 0.79^a (6.00 – 10.00)	8.41 ± 0.79^a (5.90 – 10.55)	6.64 ± 0.79^a (2.45 – 9.85)	8.50 ± 0.79^a (5.50– 0.00)	7.19 ± 0.79^a (4.85 – 10.55)	6.78± 0.79^a (5.00–10.75)	8.03 ± 0.79^a (6.00 – 10.05)	0.4759
March 1999	6	7.73 ± 0.42^a (5.08 – 9.25)	8.50 ± 0.42^a (6.05 – 10.55)	7.94 ± 0.42^a (7.20 – 8.90)	7.79 ± 0.42^a (7.2 – 8.65)	8.93 ± 0.42^a (7.85–10.05)	8.17 ± 0.42^a (7.45 – 8.90)	7.81± 0.42^a (6.65 – 9.40)	8.03 ± 0.42^a (6.80 – 9.00)	0.4605

Appendix 32 Plasma Ca, leucocyte counts and serum immunoglobulins correlation in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (The first row for each parameter and season indicates r value and second row indicates P value. D = dry season; W = rainy season).

Parameter	Season	N	Group 1 (Control)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)
WBC	D	42	0.3831	0.5289	0.4805	0.4350	0.5257	0.4385	0.5514	0.2354
			0.0123	0.0003	0.0013	0.0040	0.0003	0.0037	0.0002	0.1334
	W	30	-0.1116	-0.2539	-0.4273	-0.2990	0.0751	0.1310	0.2175	0.2810
			0.5430	0.1757	0.0185	0.1085	0.6932	0.4903	0.2482	0.1325
Lymphocyte	D	42	0.2555	0.5179	0.0887	0.2146	0.4841	0.5441	0.4627	0.2380
			0.1333	0.0004	0.5763	0.1723	0.0010	0.0002	0.0020	0.1291
	W	30	0.1121	-0.3209	-0.2669	-0.1729	0.0415	0.4137	0.2066	0.0971
			0.5557	0.0835	0.1539	0.3609	0.8275	0.0231	0.2734	0.6096
Neutrophils	D	42	0.2742	0.3051	0.3167	0.4303	0.2175	0.1474	0.4187	0.2180
			0.0789	0.0494	0.04243	0.0044	0.1613	0.3517	0.0005	0.1665
	W	30	-0.0379	0.0233	-0.3014	-0.1650	0.0827	-0.1674	0.2077	0.3416
			0.8425	0.9026	0.1056	0.3836	0.6640	0.3767	0.2708	0.0647
Eosinophils	D	42	-0.1469	-0.2135	-0.1917	0.4151	0.0664	-0.1756	-0.1325	-0.3127
			0.3531	0.1747	0.2238	0.0063	0.6723	0.2660	0.4030	0.0438
	W	30	-0.0616	-0.6627	-0.1923	-0.4898	-0.2022	-0.3250	-0.4499	-0.2605
			0.7464	0.0001	0.3086	0.0060	0.2840	0.0796	0.0126	0.1644
Monocyte	D	42	0.0959	0.0518	0.0606	0.4421	-0.0579	-0.2457	0.007	-0.1689
			0.5458	0.7448	0.7028	0.0034	0.7124	0.1168	0.9647	0.2851
	W	30	0.6110	-0.3067	0.0424	-0.1080	0.1139	-0.5500	0.2631	0.2722
			0.7483	0.0992	0.8241	0.5961	0.5490	0.7727	0.1601	0.1456
Serum immunoglobulin	D	42	0.3555	0.1065	-0.0887	-0.0704	0.0236	0.3800	0.1156	0.4612
			0.0209	0.5022	0.5763	0.6578	0.8804	0.0131	0.4658	0.0021
	W	30	-0.1466	-0.4302	-0.4232	0.0563	-0.2078	-0.2224	-0.5418	-0.3258
			0.4696	0.0177	0.0198	0.1768	0.4080	0.2376	0.0020	0.079

Appendix 33 Plasma inorganic phosphate, serum immunoglobulins and leucocyte counts correlation in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy farm, Iringa, Tanzania. (The first row for each parameter and season indicates r value and second row indicates P value. D = dry season; W = rainy season).

Parameter	Season	N	Group 1 (Control)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)
WBC	D	42	0.1352	0.0350	-0.1645	0.1278	0.0535	0.1631	0.1764	0.2965
			0.3933	0.8258	0.2979	0.4228	0.7333	0.3022	0.2639	0.0566
	W	30	-0.1268	-0.3346	0.1950	-0.1282	-0.3591	-0.0350	0.2354	0.1527
			0.5042	0.0708	0.3019	0.4989	0.0513	0.8543	0.2106	0.4206
Lymphocyte	D	42	0.4024	-0.0037	-0.1104	-0.0829	-0.0911	0.0255	0.2338	0.1769
			0.0082	0.9813	0.4866	0.6016	0.5611	0.8727	0.1362	0.2630
	W	30	-2912	-0.3238	0.0518	-0.0069	-0.2079	0.0350	0.1434	-0.1520
			0.1185	0.0809	0.7858	0.9713	0.2704	0.8542	0.4497	0.4226
Neutrophils	D	42	-0.3258	0.1370	0.1962	-0.0921	0.0836	0.3988	-0.0101	0.3486
			0.0353	0.3871	0.2989	0.5617	0.5942	0.0089	0.9451	0.0237
	W	30	0.1780	-0.0294	-0.2419	-0.1326	-0.2094	-0.1671	0.3276	0.4609
			0.3467	0.6166	0.1228	0.4848	0.2667	0.3776	0.0772	0.0104
Eosinophils	D	42	-0.03021	-0.2710	0.2694	0.0055	-0.6823	-0.2570	-0.2863	-0.1094
			0.8497	0.0826	0.7402	0.9726	0.6637	0.1004	0.0660	0.4903
	W	30	0.0966	-0.5339	0.0632	-0.4724	-0.3748	-0.1960	-0.4423	-0.4707
			0.6116	0.0024	0.8450	0.0084	0.0413	0.2992	0.0145	0.0087
Monocyte	D	42	0.1690	-0.0699	0.3447	-0.2292	-0.3872	-0.0788	-0.0146	-0.1443
			0.2846	0.6600	0.0621	0.3893	0.0103	0.6198	0.29268	0.3620
	W	30	0.1021	-0.2215	0.0455	-0.2182	-0.2432	-0.3032	0.0054	0.0282
			0.5913	0.2394	0.7749	0.2466	0.1954	0.1034	0.9774	0.8823
Serum immunoglobulin	D	42	0.0691	-0.2525	-0.4300	0.2821	-0.1539	-0.1317	0.1284	-0.2144
			0.6637	0.1067	0.0177	0.0703	0.3245	0.4056	0.1457	0.1727
	W	30	-0.6363	-0.2415	-0.1634	-0.3665	-0.6545	0.4525	-0.5934	-0.6746
			0.0001	0.1986	0.3013	0.0463	0.0001	0.0121	0.0005	0.0001

Appendix 34 Plasma Zn, leucocyte counts and serum immunoglobulins correlation in both non suppplemented and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (The first row for each parameter and season indicates r value and second row indicates P value. D = dry season; W = rainy season).

Parameter	Season	N	Group 1 (Control)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)
WBC	D	42	0.0569	0.4577	0.1526	0.1002	0.1121	0.0742	-0.0622	0.1969
			0.7204	0.0023	0.3346	0.5278	0.4741	0.6406	0.6958	0.2114
	W	30	0.3704	0.0515	0.3729	0.2474	0.5061	0.5437	0.2242	0.4579
			0.0439	0.7868	0.0424	0.1875	0.0043	0.0019	0.2337	0.0109
Lymphocyte	D	42	0.2442	0.3925	0.0392	0.0879	0.2566	0.1287	0.0817	0.2738
			0.1191	0.0101	0.8055	0.5800	0.0967	0.4166	0.6069	0.0794
	W	30	0.3644	-0.0363	0.2421	0.4846	0.0958	0.4534	0.0076	0.3556
			0.0477	0.8491	0.1974	0.0067	0.6147	0.0119	0.9681	0.0538
Neutrophils	D	42	-0.1469	0.4356	0.3301	0.0737	-0.0743	-0.1214	-0.2930	0.0297
			0.3533	0.0039	0.0325	0.6429	0.6361	0.3517	0.0597	0.8517
	W	30	0.1725	0.0953	0.1403	-0.2180	0.5516	0.2537	0.3687	0.3805
			0.3620	0.6166	0.4596	0.2471	0.0016	0.1761	0.0450	0.0380
Eosinophils	D	42	-0.3791	-0.3705	-0.1068	-0.1619	-0.2242	0.2983	0.0490	-0.2477
			0.0133	0.0157	0.5009	0.3058	0.1483	0.0550	0.7581	0.1137
	W	30	-0.1409	-0.0488	0.4413	0.1520	0.2791	-0.3250	-0.4056	-0.3523
			0.4576	0.7981	0.0146	0.4425	0.1353	0.0796	0.0262	0.0562
Monocyte	D	42	-0.2132	-0.1701	-0.2125	0.1363	0.0450	0.2671	0.1743	0.0609
			0.1753	0.7448	0.1789	0.3893	0.7744	0.0873	0.2696	0.7015
	W	30	0.0287	0.1377	0.0657	-0.0066	0.3423	0.2099	0.2917	0.3317
			0.8804	0.4681	0.7301	0.9723	0.0641	0.2655	0.1179	0.074
Serum immunoglobulin	D	42	0.2777	-0.0925	0.1990	0.1175	-0.0674	-0.2344	0.1156	0.4058
			0.0750	0.5601	0.2064	0.4585	0.6674	0.1352	0.4658	0.0077
	W	30	0.4509	-0.4003	-0.6422	-0.0325	0.2303	0.3355	-0.5072	-0.3243
			0.0124	0.0284	0.0001	0.8645	0.2208	0.0699	0.0042	0.0804

Appendix 35 Lymphocyte counts (x 10^3 per µl) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at P > 0.05)

Months	n	Group 1 (Control)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	P value
May 1997	6	5.29 ± 0.24^a (4.28– 5.98)	4.89 ± 0.24^a (4.32 –5.76)	4.78 ± 0.24^a (4.28–5.54)	4.87 ± 0.24^a (4.40 –5.50)	5.11 ± 0.24^a (4.49 – 5.90)	5.06 ± 0.24^a (4.06 – 5.85)	5.09 ± 0.24 (4.47 – 5.93)	4.82 ± 0.24^a (4.22 – 5.68)	0.7955
July 1997	6	4.33 ± 0.56^a (3.30– 5.72)	5.06 ± 0.56^a (2.83 – 7.20)	4.99 ± 0.56^a (2.80–7.02)	5.18 ± 0.56^a (3.99 – 7.88)	4.64 ± 0.56^a (2.91 – 6.27)	4.32 ± 0.56^a (2.65 – 5.68)	4.60 ± 0.56^a (2.78 – 6.09)	6.10 ± 0.56^a (4.78 – 8.40)	0.3899
September 1997	5	5.09 ± 0.40^a (3.53– 6.18)	5.15 ± 0.40^a (4.23 – 6.08)	4.76 ±0.40^a (2.63–7.08)	5.43 ± 0.40^a (4.52 – 5.95)	5.11 ± 0.40^a (3.98 – 6.11)	4.38 ± 0.40^a (2.08 – 5.40)	5.62 ± 0.40^a (4.82 – 8.17)	5.54 ± 0.40^a (4.20 – 6.34)	0.3943
November 1997	3	3.35 ± 0.53^a (3.16– 3.97)	3.85 ± 0.53^a (2.52 – 4.62)	4.18 ± 0.53^a (2.29–6.63)	4.04 ± 0.53^a (1.24 – 6.63)	4.63 ± 0.53^a (3.66 – 5.72)	4.71 ± 0.53^a (2.94 – 6.27)	4.46 ± 0.53^a (3.31 – 5.43)	3.94 ± 0.53^a (2.76 – 6.75)	0.6547
January 1998	3	4.40 ± 0.47^a (2.93– 5.88)	3.97 ± 0.47^a (2.58 – 5.41)	4.09 ± 0.47^a (2.80–6.85)	3.92 ± 0.47^a (1.85 – 5.95)	4.52 ± 0.47^a (2.54 – 6.27)	4.37 ± 0.47^a (3.06 – 6.05)	4.93 ± 0.47^a (3.62 – 6.15)	5.25 ± 0.47^a (4.66 – 6.16)	0.4631
March 1998	6	4.56 ± 0.50^b (2.77– 7.12)	5.12 ± 0.50^a (3.90 – 6.35)	5.80 ± 0.50^a (3.69–6.05)	4.39 ± 0.50^b (3.27 – 5.47)	4.23 ± 0.50^b (3.0 – 5.59)	3.13 ± 0.50^d (1.28 – 5.20)	4.32 ± 0.50^b (3.26 – 5.88)	3.87 ± 0.50^c (2.93 – 4.59)	0.0318
May 1998	6	5.51 ± 0.45^a (3.72 –7.39)	4.33 ± 0.45^b (2.90 – 5.40)	5.04 ±0.45^b (3.87–7.62)	4.79 ± 0.45^b (2.93 – 6.31)	3.93 ± 0.45^c (2.79 – 5.10)	4.39 ± 0.45^b (2.77 – 5.18)	5.49 ± 0.45^a (4.81 – 6.84)	4.91 ± 0.45^b (3.18 – 5.77)	0.0520
July 1998	6	5.45 ± 0.40^a (3.78 –7.05)	4.76 ± 0.40^b (3.84 – 5.40)	3.54 ± 0.40^c (2.46–4.55)	4.80 ± 0.40^b (3.00 – 6.03)	4.34 ± 0.40^b (3.51 – 5.33)	3.59 ± 0.40^c (2.30 – 4.96)	4.50 ± 0.40^b (3.27 – 6.11)	4.62 ± 0.40^b (3.46 – 6.56)	0.0326
September 1998	6	6.19 ± 0.34^a (5.12– 8.73)	6.61 ± 0.34^a (5.82 – 7.35)	6.82 ± 0.34^a (5.20– .06)	6.01 ± 0.34^a (5.30 – 6.63)	5.67 ± 0.34^a (4.55 – 6.47)	5.89 ± 0.34^a (4.60 – 6.99)	6.46 ± 0.34^a (5.88 – 7.13)	6.31 ± 0.34^a (5.12 – 6.89)	0.2777
November 1998	6	6.33 ± 0.54^a (4.48– 7.56)	6.16 ± 0.54^a (4.36 – 7.88)	5.39 ± 0.54^a (2.78–6.53)	5.97 ± 0.54^a (3.30 – 8.10)	6.20 ± 0.54^a (4.16 – 8.59)	6.37 ± 0.54^a (4.60 – 7.56)	7.04 ± 0.54^a (5.28 – 8.48)	6.49 ± 0.54^a (4.55 – 7.63)	0.6368
January 1999	6	5.66 ± 0.63^a (2.80 –7.43)	5.27 ± 0.63^a (3.42 – 7.00)	5.14 ± 0.63^a (2.95–8.44)	3.97 ± 0.63^a (1.68 – 6.11)	5.38 ± 0.63^a (3.03 – 6.93)	5.16 ± 0.63^a (3.75 – 8.02)	3.98 ± 0.63^a (2.74 – 6.13)	5.23 ± 0.63^a (3.90 – 7.64)	0.4742
March 1999	6	5.16 ± 0.50^a (3.00– 6.48)	4.78 ± 0.50^a (2.90 – 6.63)	3.77 ± 0.50^a (2.40–4.88)	4.59 ± 0.50^a (4.03 – 5.88)	4.57 ± 0.50^a (3.66 – 6.50)	4.99 ± 0.50^a (4.13 – 5.66)	4.75 ± 0.50^a (2.82 – 7.24)	4.46 ± 0.50^a (3.47 – 6.93)	0.6735

Appendix 36 Neutrophil counts (x 10^3 per µl) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at $P > 0.05$).

Months	n	Group 1 (Con)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	P value
May 1997	6	2.47 ± 0.12^a (2.19 – 2.93)	2.41 ± 0.12^a (2.06 –2.97)	2.36 ± 0.12^a (2.16 – 2.97)	2.28 ± 0.12^a (2.01 –2.75)	2.48 ± 0.12^a (2.04 – 3.06)	2.42 ± 0.12^a (1.99 – 2.70)	2.35 ± 0.12^a (2.12 – 2.53)	2.24 ± 0.12^a (1.98 – 2.54)	0.8035
July 1997	6	2.83 ± 0.27^a (2.36 – 4.40)	2.66 ± 0.27^a (2.00 – 3.42)	2.78 ± 0.27^a (2.39 – 3.43)	2.40 ± 0.27^a (2.07 – 2.74)	2.35 ± 0.27^a (1.21 – 3.04)	2.31 ± 0.27^a (1.16 – 4.77)	2.32 ± 0.27^a (1.90 – 2.98)	2.46 ± 0.27^a (2.04 – 3.07)	0.7327
September 1997	6	2.18 ± 0.27^a (1.26 – 3.04)	2.54 ± 0.27^a (1.88 – 3.29)	2.03 ± 0.27^a (0.42 – 2.85)	2.53 ± 0.27^a (1.94 – 2.93)	2.63 ± 0.27^a (1.63 – 4.12)	2.39 ± 0.27^a (1.62 – 2.74)	2.39 ± 0.27^a (2.19 – 2.71)	3.24 ± 0.27^a (2.63 – 4.91)	0.0553
November 1997	6	1.55 ± 0.37^b (0.89 – 2.57)	2.07 ± 0.37^a (1.55 – 2.65)	1.25 ± 0.37^b (0.58 – 2.18)	1.17 ± 0.37^b (0.77 – 1.64)	1.23 ± 0.37^b (0.47 – 2.30)	2.38 ± 0.37^a (1.09 – 3.76)	2.18 ± 0.37^a (1.20 – 3.27)	2.47 ± 0.37^a (0.63– 5.94)	0.0451
January 1998	6	3.41 ± 0.54^a (2.36 – 5.41)	4.14 ± 0.54^a (2.34 – 5.25)	4.84 ± 0.54^a (3.06 – 7.57)	3.46 ± 0.54^a (1.81 – 4.45)	3.69 ± 0.54^a (2.40 – 5.32)	4.28 ± 0.54^a (2.96 – 5.86)	4.79 ± 0.54^a (3.16 – 6.49)	4.30 ± 0.54^a (1.40 – 6.29)	0.4190
March 1998	6	1.66 ± 0.40^a (0.48 – 3.06)	2.02 ± 0.40^a (0.72 – 4.66)	2.09 ± 0.40^a (0.81 – 4.86)	1.59 ± 0.40^a (0.30 – 2.83)	2.04 ± 0.40^a (0.95 – 2.81)	2.47 ± 0.40^a (1.44 – 4.50)	1.89 ± 0.40^a (1.48 – 2.84)	1.94 ± 0.40^a (1.44 – 2.38)	0.8641
May 1998	6	3.04 ± 0.43^a (1.68 – 5.74)	2.59 ± 0.43^a (1.54 – 3.71)	2.50 ±0.43^a (1.33 – 3.80)	2.42 ± 0.43^a (1.14 – 3.45)	2.85 ± 0.43^a (1.92 – 3.85)	3.61 ± 0.43^a (1.78 – 5.38)	1.92 ± 0.43^a (1.24 – 2.42)	2.66 ± 0.43^a (1.46 – 4.59)	0.2605
July 1998	6	1.06 ± 0.25^a (0.41 – 1.92)	1.39 ± 0.25^a (0.12 – 2.34)	1.76 ± 0.25^a (0.92 – 2.76)	1.47 ± 0.25^a (0.91 – 2.09)	1.96 ± 0.25^a (1.67 – 2.67)	1.85 ± 0.25^a (1.27 – 2.88)	1.76 ± 0.25^a (1.32 – 2.21)	2.10 ± 0.25^a (0.88– 3.24)	0.1179
September 1998	6	2.37 ± 0.24^d (1.86 – 2.81)	2.94 ± 0.24^b (1.72 – 3.35)	2.66 ± 0.24^c (2.12 – 3.20)	3.45 ± 0.24^a (2.79 – 4.50)	3.62 ± 0.24^a (2.70 – 4.34)	3.49 ± 0.24^a (2.55 – 4.20)	3.10 ± 0.24^b (1.90 – 4.20)	3.14 ± 0.24^b (2.56– 3.57)	0.0094
November 1998	6	2.18 ± 0.41^b (1.64 – 2.74)	2.93 ± 0.41^a (2.11 – 4.49)	3.45 ± 0.41^a (2.12 – 4.82)	2.62 ± 0.41^b (2.16 – 2.97)	3.04 ± 0.41^a (1.38 – 5.82)	2.46 ± 0.41^b (1.21 – 3.36)	2.56 ± 0.41^b (1.00 – 4.37)	2.88 ± 0.41^b (2.31 – 3.24)	0.0483
January 1999	6	2.08 ± 0.30^a (0.22 – 3.46)	3.05 ± 0.30^a (2.28 – 3.80)	2.80 ± 0.30^a (1.79 – 3.91)	2.25 ± 0.30^b (0.78 – 3.25)	2.66 ± 0.30^b (2.30 – 3.00)	2.13 ± 0.30^b (1.66 – 2.37)	2.36 ± 0.30^b (1.20 – 3.87)	2.38 ± 0.30^b (1.92 – 2.84)	0.0528
March 1999	6	1.95 ± 0.34^a (1.63 – 2.51)	2.98 ± 0.34^a (2.44 – 3.59)	2.80 ± 0.34^a (1.51 – 5.70)	2.38 ± 0.34^a (1.64 – 2.97)	3.02 ± 0.34^a (1.45 – 4.22)	2.06 ± 0.34^a (1.27 – 2.68)	1.80 ± 0.34^a (0.95 – 2.47)	2.35 ± 0.34^a (0.99– 3.20)	0.0535

Appendix 37 Eosinophil counts (cells per μl) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at P > 0.05).

Months	n	Group 1 (Control)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	P value
May 1997	6	209 ± 32^{a} (113 – 322)	210 ± 32^{a} (124 –334)	213 ± 32^{a} (144 – 342)	216 ± 32^{a} (181 –304)	209 ± 32^{a} (186 – 378)	210 ± 32^{a} (123 – 320)	208 ± 32^{a} (114 – 328)	213 ± 32^{a} (162 – 300)	0.3129
July 1997	6	278 ± 76^{a} (72 – 438)	284 ± 76^{a} (92 – 600)	329 ± 76^{a} (157 – 792)	134 ± 76^{a} (67 – 219)	298 ± 76^{a} (79 – 955)	255 ± 76^{a} (116 – 454)	217 ± 76^{a} (136 – 348)	263 ± 76^{a} (74 – 510)	0.4292
September 1997	6	201 ± 57^{a} (95 – 338)	201 ± 57^{a} (134 – 282)	256 ± 57^{a} (178 – 350)	148 ± 57^{a} (82 – 206)	258 ± 57^{a} (163 – 459)	225 ± 57^{a} (80 – 338)	423 ± 57^{a} (210 – 885)	182 ± 57^{a} (106 – 210)	0.6579
November 1997	6	235 ± 43^{a} (89 – 635)	81 ± 43^{b} (42 – 122)	205 ± 43^{b} (78 – 476)	75 ± 43^{b} (28 – 168)	94 ± 43^{b} (51 – 162)	103 ± 43^{b} (42 – 209)	82 ± 43^{b} (48 – 155)	82 ± 43^{b} (42 – 198)	0.0524
January 1998	6	246 ± 54^{a} (68 – 975)	85 ± 54^{a} (65 – 111)	97 ± 54^{a} (64 – 117)	91 ± 54^{a} (39 – 138)	98 ± 54^{a} (64 – 120)	93 ± 54^{a} (70 – 119)	103 ± 54^{a} (91 – 118)	145 ± 54^{a} (90 – 330)	0.2665
March 1998	6	464 ± 161^{a} (105 – 1136)	279 ± 161^{a} (141 – 408)	684 ± 161^{a} (55 – 1872)	672 ± 161^{a} (179 – 1599)	539 ± 161^{a} (350 – 774)	682 ± 161^{a} (352 – 1155)	711 ± 161^{a} (322 – 1229)	670 ± 161^{a} (366 – 1224)	0.5330
May 1998	6	126 ± 10^{a} (94 – 161)	70 ± 10^{b} (68 – 76)	77 ± 10^{b} (53 – 100)	74 ± 10^{b} (65 – 84)	70 ± 10^{b} (60 – 79)	97 ± 10^{b} (70 – 164)	90 ± 10^{b} (65 – 160)	92 ± 10^{b} (67 – 148)	0.0039
July 1998	6	206 ± 96^{b} (54 – 300)	229 ± 96^{b} (146 – 431)	316 ± 96^{b} (56 – 792)	123 ± 96^{b} (62 – 348)	76 ± 96^{c} (59 – 108)	694 ± 96^{a} (137 – 1520)	372 ± 96^{b} (128 – 555)	263 ± 96^{b} (64 – 725)	0.0024
September 1998	6	167 ± 46^{b} (75 – 324)	168 ± 46^{b} (90 – 216)	289 ± 46^{b} (160 – 525)	245 ± 46^{b} (100 – 465)	282 ± 46^{b} (98 – 651)	423 ± 46^{a} (210 – 885)	182 ± 46^{b} (106 – 210)	116 ± 46^{b} (80 – 213)	0.0144
November 1998	6	204 ± 41^{a} (86 – 530)	120 ± 41^{a} (95 – 180)	158 ± 41^{a} (50 – 400)	118 ± 41^{a} (72 – 214)	182 ± 41^{a} (106 – 288)	113 ± 41^{a} (98 – 150)	200 ± 41^{a} (80 – 500)	97 ± 41^{a} (70 – 108)	0.3843
January 1999	6	151 ± 68^{a} (56 – 451)	134 ± 68^{a} (80 – 197)	116 ± 68^{a} (48 – 206)	318 ± 68^{a} (25 – 700)	234 ± 68^{a} (91 – 561)	209 ± 68^{a} (67 – 485)	265 ± 68^{a} (50 – 600)	220 ± 68^{a} (60 – 430)	0.4264
March 1999	6	329 ± 157^{a} (85 – 628)	302 ± 157^{a} (77 – 454)	915 ± 157^{a} (89 – 1540)	531 ± 157^{a} (360 – 720)	807 ± 157^{a} (236 – 1592)	767 ± 157^{a} (149 – 1602)	798 ± 157^{a} (282 – 1272)	900 ± 157^{a} (612 – 1352)	0.0817

Appendix 38 Monocyte counts (cells per µl) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at P > 0.05)

Months	n	Group 1 (Control)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	P value
May 1997	6	213 ± 35^{a} (123 – 348)	204 ± 35^{a} (141 –334)	253 ± 35^{a} (144 – 373)	241 ± 35^{a} (153 –373)	255 ± 35^{a} (136 – 532)	221 ± 35^{a} (83 – 270)	222 ± 35^{a} (141 – 252)	244 ± 35^{a} (82 – 340)	0.3723
July 1997	6	338 ± 61^{a} (143– 550)	211 ± 61^{a} (163 – 302)	337 ± 61^{a} (112 – 594)	188 ± 61^{a} (73 – 276)	288 ± 61^{a} (157 – 408)	221 ± 61^{a} (138 – 454)	166 ± 61^{a} (68 – 480)	375 ± 61^{a} (147 – 720)	0.2346
September 1997	6	248 ± 32^{a} (135 – 435)	198 ± 32^{b} (134 – 282)	243 ± 32^{a} (175 – 312)	120 ± 32^{c} (82 – 233)	268± 32^{a} (153 – 490)	134 ± 32^{c} (35 – 213)	173 ± 32^{b} (77 – 281)	228 ± 32^{ab} (174 – 285)	0.0145
November 1997	6	173 ± 48^{b} (126 – 267)	130 ± 48^{b} (72 – 183)	294 ± 48^{a} (78 – 714)	87 ± 48^{c} (52 – 138)	116 ± 48^{b} (51 – 218)	160 ± 48^{b} (58 – 368)	150 ± 48^{b} (71 – 273)	138 ± 48^{b} (52 – 265)	0.0414
January 1998	6	299 ± 113^{b} (139 – 550)	280± 113^{b} (195 – 442)	605± 113^{a} (306 – 1049)	404 ± 113^{a} (138 – 1463)	531 ± 113^{a} (64 – 970)	289 ± 113^{b} (92 – 593)	397 ± 113^{b} (279 – 590)	652 ± 113^{a} (464 – 830)	0.0524
March 1998	6	220 ± 51^{a} (105 – 368)	225 ± 51^{a} (130 – 388)	238 ± 51^{a} (119 – 385)	237 ± 51^{a} (114 – 420)	195 ± 51^{a} (70 – 383)	248 ± 51^{a} (60 – 693)	171 ± 51^{a} (95 – 246)	225 ± 51^{a} (61 – 348)	0.9713
May 1998	6	155 ± 29^{b} (99 – 242)	106 ± 29^{b} (68 – 152)	77 ± 29^{b} (53 – 100)	102 ± 29^{b} (65 – 252)	142 ± 29^{a} (69 – 296)	182 ± 29^{a} (71 – 294)	186 ± 29^{a} (69 – 380)	111 ± 29^{ab} (67 – 204)	0.0540
July 1998	6	171 ± 86^{a} (54 – 405)	414 ± 86^{a} (120 – 1238)	210 ± 86^{a} (73 – 383)	214 ± 86^{a} (58 – 390)	333 ± 86^{a} (108 – 560)	307 ± 86^{a} (70 – 539)	293 ± 86^{a} (56 – 495)	242 ± 86^{a} (72 – 486)	0.5622
September 1998	6	199 ± 46^{a} (75 – 324)	343 ± 46^{a} (90 – 688)	266 ± 46^{a} (212 – 320)	249 ± 46^{a} (100 – 324)	247 ± 46^{a} (98 – 434)	185 ± 46^{a} (89 – 315)	250 ± 46^{a} (187 – 318)	212 ± 46^{a} (94 – 306)	0.3623
November 1998	6	314 ± 40^{a} (171 – 420)	218 ± 40^{a} (105 – 302)	166 ± 40^{a} (100 – 300)	228 ± 40^{a} (90 – 432)	260 ± 40^{a} (138 – 480)	149 ± 40^{a} (75 – 210)	184 ± 40^{a} (80 – 318)	197 ± 40^{a} (70 – 301)	0.1100
January 1999	6	177 ± 41^{a} (56 – 361)	189 ± 41^{a} (80 – 450)	183 ± 41^{a} (87 – 295)	172 ± 41^{a} (25 – 350)	225± 41^{a} (55 – 374)	197 ± 41^{a} (125 – 288)	146 ± 41^{a} (100 – 215)	216 ± 41^{a} (120 – 344)	0.9089
March 1999	6	220 ± 76^{b} (85 – 393)	263 ± 76^{b} (77 – 422)	342 ± 76^{a} (227 – 504)	305 ± 76^{a} (72 – 519)	527 ± 76^{a} (79 – 880)	351 ± 76^{a} (153 – 581)	467 ± 76^{a} (188 – 716)	445± 76^{a} (180 – 620)	0.0514

Appendix 39 Serum immunoglobulin (ZTU/l) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at P > 0.05).

Months	n	Group 1 (Control)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	P value
May 1997	6	19.1 ± 0.04^{a} (18.5 – 19.5)	19.8 ± 0.04^{a} (18.6 –20.3)	19.2 ± 0.04^{a} (18.3 – 20.1)	19.5 ± 0.04^{a} (18.5 –19.8)	19.6 ± 0.04^{a} (19.2 – 20.2)	19.8 ± 0.04^{a} (19.4 – 19.8)	19.7 ± 0.04^{a} (18.9 – 19.9)	19.9 ± 0.04^{a} (19.0 – 20.1)	0.551
July 1997	6	18.8 ± 0.4^{b} (17.8 – 19.6)	21.0 ± 0.4^{a} (19.1 – 22.0)	20.5 ± 0.4^{a} (18.8 – 22.5)	20.0 ± 0.4^{a} (18.3 – 21.1)	20.1 ± 0.4^{a} (19.0 – 21.2)	20.5 ± 0.4^{a} (19.2 – 22.0)	20.0 ± 0.4^{a} (19.5 – 20.8)	19.9 ± 0.4^{a} (18.6 – 20.8)	0.0556
September 1997	6	20.3 ± 0.4^{b} (19.0 – 21.3)	21.2 ± 0.4^{a} (18.8 – 22.5)	21.0 ± 0.4^{b} (20.5 – 21.4)	19.9 ± 0.4^{c} (18.8 – 21.1)	21.5 ± 0.4^{a} (19.1 – 22.6)	22.2 ± 0.4^{a} (20.5 – 22.8)	22.3 ± 0.4^{a} (21.8 – 22.6)	20.0 ± 0.4^{c} (19.3 – 20.9)	0.0002
November 1997	6	21.8 ± 0.0.4^{a} (21.1 – 22.9)	20.2 ± 0.4^{a} (18.7 – 21.0)	19.0 ± 0.4^{a} (16.5 – 20.5)	19.7 ± 0.4^{a} (19.0 – 21.1)	19.1 ± 0.4^{a} (18.0 – 20.8)	19.9 ± 0.4^{a} (18.7 – 21.0)	19.2 ± 0.4^{a} (18.2 – 20.8)	19.0 ± 0.4^{a} (18.6 – 19.3)	0.2990
January 1998	6	19.8 ± 0.3^{b} (19.2 – 20.6)	21.7 ± 0.3^{a} (21.0 – 22.5)	20.1 ± 0.3^{b} (19.5 – 20.8)	19.0 ± 0.3^{c} (18.4 – 19.4)	19.7 ± 0.3^{b} (17.8 – 20.8)	20.3 ± 0.3^{b} (19.3 – 21.5)	19.7 ± 0.3^{b} (18.9 – 20.9)	19.7 ± 0.3^{b} (19.1 – 20.7)	0.0001
March 1998	6	20.3 ± 0.3^{c} (19.1 – 21.6)	18.2 ± 0.3^{d} (18.0 – 18.6)	21.0 ± 0.3^{b} (20.0 – 22.2)	19.0 ± 0.3^{d} (18.0 – 19.5)	20.5 ± 0.3^{c} (19.4 – 21.6)	21.1 ± 0.3^{b} (19.6 – 21.8)	22.1 ± 0.3^{a} (21.2 – 22.8)	21.8 ± 0.3^{a} (20.1 – 22.8)	0.0001
May 1998	6	20.8 ± 0.4^{a} (18.6 – 21.8)	19.8 ± 0.4^{b} (18.5 – 20.8)	21.4 ±0.4^{a} (20.2 – 22.5)	19.9 ± 0.4^{b} (18.6 – 20.9)	19.4 ± 0.4^{b} (18.2 – 21.0)	18.5 ± 0.4^{c} (17.9 – 19.4)	19.1 ± 0.4^{c} (17.8 – 20.0)	20.1 ± 0.4^{b} (19.0 – 20.9)	0.0001
July 1998	6	18.8 ± 0.3^{d} (17.9 – 19.5)	21.0 ± 0.3^{a} (20.4 – 21.8)	19.3 ± 0.3^{c} (18.3 – 20.7)	21.7 ± 0.3^{a} (21.0 – 22.4)	21.2 ± 0.3^{a} (20.2 – 22.5)	19.2 ± 0.3^{c} (18.4 – 20.5)	19.9 ± 0.3^{b} (19.1 – 20.8)	20.7 ± 0.3^{a} (19.6 – 21.8)	0.0001
September 1998	6	19.8 ± 0.4^{b} (19.1 – 20.8)	21.0 ± 0.4^{b} (20.2 – 21.7)	20.2 ± 0.4^{b} (18.5 – 21.5)	20.8 ± 0.4^{b} (17.9 – 21.9)	20.4 ± 0.4^{b} (19.0 – 21.8)	21.0 ± 0.4^{b} (18.5 – 22.1)	21.0 ± 0.4^{b} (19.3 – 22.0)	21.9 ± 0.4^{a} (20.3 – 22.5)	0.0507
November 1998	6	20.9 ± 0.3^{b} (20.0 – 21.5)	21.9 ± 0.3^{a} (21.5 – 22.1)	21.6 ± 0.3^{a} (21.0 – 22.3)	20.0 ± 0.3^{c} (19.4 – 20.5)	20.7 ± 0.3^{b} (20.1 – 21.7)	22.0 ± 0.3^{a} (20.9 – 22.6)	20.2 ± 0.3^{c} (18.9 – 22.7)	21.6 ± 0.3^{a} (20.6 – 22.9)	0.0001
January 1999	6	22.9 ± 0.3^{a} (22.3 – 23.5)	23.2 ± 0.3^{a} (22.5 – 23.8)	22.1 ± 0.3^{b} (21.4 – 22.8)	21.3 ± 0.3^{b} (20.2 – 22.2)	23.1 ± 0.3^{a} (21.5 – 23.8)	23.1 ± 0.3^{a} (22.0 – 23.8)	21.0 ± 0.3^{c} (20.0 – 22.7)	22.6 ± 0.3^{a} (21.6 – 23.9)	0.0001
March 1999	6	21.6 ± 0.3^{a} (20.5 – 22.3)	21.3 ± 0.3^{a} (19.0 – 22.6)	21.3 ± 0.3^{a} (19.9 – 21.9)	22.1 ± 0.3^{a} (21.2 – 22.8)	22.0 ± 0.3^{a} (21.2 – 22.9)	21.4 ± 0.3^{a} (20.8 – 21.8)	21.2 ± 0.3^{a} (20.3 – 21.9)	21.8 ± 0.3^{a} (21.1 – 22.9)	0.3002

Appendix 40 Aspartate aminotransferase activity (IU/l) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at $P > 0.05$).

Month	n	Group 1 (Con)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	P value
May 1997	6	45.5 ± 2.2^{a} (32.1 – 54.5)	44.6 ± 2.2^{a} (39.4 – 50.6)	45.0 ± 2.2^{a} (39.6 – 50.0)	42.4 ± 2.2^{a} (33.6 –49.7)	49.0 ± 2.2^{a} (41.2 – 55.8)	45.3 ± 2.2^{a} (38.1 – 50.6)	44.9 ± 2.2^{a} (41.2 – 48.2)	45.7 ± 2.2^{a} (40.3 – 51.5)	0.7020
July 1997	6	34.9 ± 1.4^{c} (31.5 – 39.7)	33.3 ± 1.4^{c} (26.6 – 37.6)	34.0 ± 1.4^{c} (16.7 – 28.9)	38.2 ± 1.4^{b} (34.7 – 41.5)	31.7 ± 1.4^{d} (28.5 – 36.6)	42.6 ± 1.4^{a} (36.3 – 48.1)	38.7 ± 1.4^{b} (35.7 – 42.7)	26.8 ± 1.4^{d} (22.4 – 32.1)	0.0001
September 1997	6	33.7 ± 2.8^{a} (30.3 – 39.6)	31.0 ± 2.8^{a} (29.1 – 32.7)	26.6 ± 2.8^{b} (21.8 – 29.7)	30.4 ± 2.8^{a} (28.2 – 31.5)	33.5 ± 2.8^{a} (23.6 – 57.5)	24.0 ± 2.8^{c} (19.4 – 28.5)	28.1 ± 2.8^{b} (19.5 – 55.4)	29.5 ± 2.8^{b} (25.4 – 31.5)	0.0032
November 1997	6	36.7 ± 1.8^{b} (30.0 – 44.8)	24.7 ± 2.8^{c} (18.2 – 31.3)	31.8 ± 2.8^{b} (30.3 – 32.7)	37.3 ± 2.8^{b} (34.5 – 39.7)	32.0 ± 4.2^{b} (26.8 – 38.8)	41.4 ± 2.8^{a} (36.3 – 45.1)	33.3 ± 2.8^{b} (26.6 – 39.4)	29.5 ± 2.8^{b} (22.1 – 35.7)	0.0001
January 1998	6	30.4 ± 4.2^{c} (21.7 – 35.2)	31.0 ± 4.2^{c} (20.4 – 55.0)	39.6 ± 4.2^{b} (27.0 – 53.5)	37.8 ± 4.2^{b} (25.1 – 51.0)	48.1 ± 4.2^{a} (41.5 – 58.8)	48.4 ± 4.2^{a} (36.8 – 57.2)	51.7 ± 4.2^{a} (36.5 – 68.0)	55.8 ± 4.2^{a} (38.0 – 77.6)	0.0003
March 1998	6	45.4 ± 4.2^{a} (30.0 – 58.0)	45.0 ± 4.2^{a} (27.0 – 57.0)	34.5 ± 4.2^{a} (22.7 – 50.4)	44.2 ± 4.2^{a} (35.3 – 51.5)	37.4 ± 4.2^{a} (32.4 – 46.4)	43.0 ± 4.2^{a} (27.6 – 52.4)	49.2 ± 4.2^{a} (35.4 – 62.2)	45.8 ± 4.2^{a} (32.4 – 65.2)	0.2646
May 1998	6	42.7 ± 4.2^{a} (22.2 – 71.2)	44.6 ± 4.2^{a} (33.4 – 54.4)	49.2 ± 4.2^{a} (29.6 – 63.6)	45.1 ± 4.2^{a} (27.3 – 61.2)	43.1 ± 4.2^{a} (31.2 – 60.8)	59.0 ± 4.2^{a} (50.8 – 64.8)	49.6 ± 4.2^{a} (30.8 – 62.4)	50.5 ± 4.2^{a} (38.5 – 67.2)	0.2640
July 1998	6	48.5 ± 5.5^{b} (23.2 – 71.1)	44.6 ± 5.5^{b} (29.6 – 52.5)	50.2 ± 5.5^{b} (30.2 – 70.4)	67.3 ± 5.5^{a} (44.8 – 108)	55.7 ± 5.5^{b} (40.3 – 72.8)	53.3 ± 5.5^{b} (40.3 – 64.0)	52.4 ± 5.5^{b} (40.0 – 56.0)	41.3 ± 5.5^{b} (30.1 – 52.8)	0.0509
September 1998	6	51.5 ± 5.1^{c} (38.4 – 70.0)	59.8 ± 5.1^{b} (38.4 – 77.2)	59.6 ± 5.1^{b} (41.5 – 88.8)	72.9 ± 5.1^{a} (60.0 – 91.6)	59.2 ± 5.1^{b} (44.5 – 74.0)	63.7 ± 5.1^{b} (48.3 – 94.4)	48.8 ± 5.1^{c} (40.3 – 56.0)	43.7 ± 5.1^{d} (36.1 – 52.4)	0.0077
November 1998	6	59.8 ± 7.1^{b} (41.3 – 77.6)	60.5 ± 7.1^{b} (40.8 – 90.0)	69.4 ± 7.1^{a} (40.0 – 91.6)	52.8 ± 7.1^{b} (32.8 – 67.6)	73.2 ± 7.1^{a} (47.3 – 116)	65.9 ± 7.1^{b} (49.6 – 84.0)	60.4 ± 7.1^{b} (30.6 – 96.8)	44.2 ± 7.1^{c} (34.6 – 48.8)	0.0531
January 1999	6	59.9 ± 5.1^{b} (35.8 – 70.5)	52.4 ± 5.1^{b} (45.0 – 60.5)	67.8 ± 5.1^{a} (52.6 – 87.2)	54.6 ± 5.1^{b} (36.0 – 70.5)	58.7 ± 5.1^{b} (40.5 – 70.8)	53.8 ± 5.1^{b} (27.3 – 85.3)	42.8 ± 5.1^{c} (35.8 – 55.0)	48.7 ± 5.1^{c} (40.4 – 60.2)	0.0521
March 1999	6	65.5 ± 6.4^{a} (23.6 – 87.0)	56.1 ± 6.4^{b} (25.0 – 90.5)	72.2 ± 6.4^{a} (48.3 – 90.5)	79.2 ± 6.4^{a} (64.5 – 95.4)	69.2 ± 6.4^{a} (22.2 – 37.7)	42.2 ± 6.4^{c} (20.0 – 65.0)	32.2 ± 6.4^{d} (25.4 – 40.8)	59.8 ± 6.4^{b} (30.8 – 70.9)	0.0002

Appendix 41 Plasma Ca, total protein, plasma glucose, plasma urea and ceruloplasmin correlation in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (The first row for each parameter and season indicates r value and second row indicates P value. D = dry season; W = rainy season).

Parameter	Season	N	Group 1 (Control)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)
Plasma protein	D	42	-0.3457	-0.1733	-0.5274	-0.2909	-0.3803	-0.3308	-0.4216	-0.6625
			0.0249	0.2723	0.0003	0.0616	0.0119	0.0324	0.0054	0.0001
	W	30	0.3813	0.8028	0.5616	0.2986	0.6178	0.6003	0.4287	0.3399
			0.0376	0.0001	0.0012	0.1089	0.0003	0.0005	0.0182	0.0661
Plasma urea	D	42	-0.0728	-0.4000	-0.3649	-0.4793	-0.2682	-0.4239	-0.2813	-0.1008
			0.6440	0.0087	0.0176	0.0013	0.0820	0.0052	0.0692	0.5252
	W	30	0.0067	0.1993	-0.0998	-0.4125	-0.1422	-0.0202	-0.2761	0.0742
			0.9718	0.2919	0.5996	0.0235	0.4534	0.9157	0.1396	0.6969
Plasma glucose	D	42	-0.2906	-0.7563	-0.7001	-0.8177	-0.6113	-0.6806	-0.7395	-0.6483
			0.0619	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	W	30	-0.0442	0.2178	-0.5347	0.0986	-0.4989	-0.4036	-0.4655	-0.5127
			0.8166	0.2475	0.0023	0.6040	0.0050	0.0270	0.0095	0.0038
Ceruloplasmin	D	42	-0.0935	-0.2500	0.1688	0.3114	0.1410	-0.3246	-0.0873	-0.3547
			0.5559	0.1103	0.2852	0.0447	0.3671	0.0360	0.5825	0.0212
	W	30	-0.1483	0.0776	-0.2030	-0.2343	0.7152	-0.3632	-0.2768	0.1007
			0.4341	0.9582	0.2820	0.2127	0.0001	0.0485	0.1434	0.5965

Appendix 42 Plasma Pi, aspartate aminotransferase, alkaline phosphatase, plasma protein, plasma urea, plasma glucose and ceruloplasmin activity correlation in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (The first row for each parameter and season indicates r-value and second row indicates P value. D = dry season and W = rainy season).

Parameter	Season	N	Group 1 (Control)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)
Aspartate amino transferase	D	42	0.2010	0.1358	0.0155	0.3224	-0.0827	-0.0321	-0.0913	0.0476
			0.2019	0.3911	0.9226	0.0374	0.5980	0.8401	0.5654	0.7647
	W	30	-0.5469	-0.7435	-0.2122	-0.4593	-0.5240	-0.1246	-0.2486	-0.2013
			0.0018	0.0001	0.2603	0.0107	0.0030	0.5119	0.1856	0.2860
Alkaline phosphatase	D	42	0.0511	-0.1563	-0.1604	-0.2204	0.0290	-0.0482	0.2367	-0.0926
			0.7478	0.3228	0.3088	0.1607	0.8538	0.6819	0.1313	0.5396
	W	30	-0.1666	-0.1976	0.1070	-0.2309	0.2579	-0.1217	-0.0727	0.3495
			0.3789	0.2951	0.5735	0.2205	0.1689	0.5218	0.7026	0.0583
Plasma protein	D	42	-0.0592	-0.0975	-0.0806	-0.0360	-0.1853	-0.2202	-0.0319	0.0175
			0.7097	0.5392	0.6117	0.8212	0.2342	0.1612	0.8408	0.9121
	W	30	0.3056	0.4717	0.4346	0.2163	0.1577	0.2049	0.3834	-0.0453
			0.1006	0.0218	0.0164	0.2510	0.4052	0.2774	0.0365	0.8123
Plasma urea	D	42	0.0049	-0.0220	-0.0879	0.1650	0.3926	0.1573	0.0310	0.3929
			0.9754	0.8901	0.5797	0.2960	0.0092	0.3199	0.8453	0.0101
	W	30	0.4477	0.4090	-0.1449	0.0305	-0.4411	0.0533	-0.2172	-0.42791
			0.0131	0.0248	0.4450	0.8729	0.0147	0.7799	0.2490	0.0183
Plasma glucose	D	42	0.0660	0.0009	0.0217	0.1145	0.0532	-0.0843	-0.2127	-0.0974
			0.6778	0.9956	0.8917	0.4701	0.7350	0.5954	0.1763	0.5394
	W	30	-0.1122	0.4405	-0.1180	0.2103	-0.0153	-0.0768	-0.3694	-0.0369
			0.5551	0.0146	0.5345	0.2647	0.9361	0.6867	0.0445	0.8465
Ceruloplasmin	D	42	0.0280	0.4112	-0.0272	-0.2827	0.2775	0.1304	0.2675	0.0911
			0.8605	0.0068	0.8643	0.0697	0.0716	0.4105	0.0868	0.5661
	W	30	0.1151	-0.4688	-0.1288	-0.1434	-0.3080	-0.3559	-0.5665	-0.6505
			0.5447	0.0090	0.4975	0.4496	0.0978	0.0536	0.0011	0.0001

Appendix 44 Plasma alkaline phosphatase (IU /l) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at $P > 0.05$).

Month	n	Group 1 (Con)	Group 2 (Ca	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	P value
May 1997	6	51.2 ± 5.5^{a} (36.7 – 73.8)	50.5 ± 5.5^{a} (31.7 – 67.0)	48.9 ± 5.5^{a} (21.5 – 69.2)	50.8 ± 5.5^{a} (45.6 –55.6)	50.6 ± 5.5^{a} (40.3 – 59.4)	49.8 ± 5.5^{a} (20.6 – 64.6)	51.5 ± 5.5^{a} (27.4 – 74.2)	48.9 ± 5.5^{a} (29.9 – 52.9)	0.9875
July 1997	6	43.4 ± 4.1^{a} (28.5 – 59.9)	21.7 ± 4.1^{b} (10.2 – 41.0)	22.2 ± 4.1^{b} (16.7 – 28.9)	26.6 ± 4.1^{b} (18.5 – 31.6)	25.2 ± 4.1^{b} (15.0 – 38.2)	24.4 ± 4.1^{b} (14.4 – 48.3)	29.6 ± 4.1^{b} (12.2 – 47.1)	23.5 ± 4.1^{b} (16.0 – 35.0)	0.0136
September 1997	6	36.5 ± 2.9^{a} (23.7 – 58.5)	22.6 ± 2.9^{b} (11.1 – 31.8)	18.4 ± 2.9^{b} (12.2 – 26.9)	16.0 ± 2.9^{b} (11.4 – 29.0)	18.2 ± 2.9^{b} (10.1 – 25.7)	20.1 ± 2.9^{b} (14.0 – 26.0)	19.5 ± 2.9^{b} (10.9 – 26.4)	16.6 ± 2.9^{b} (11.2 – 22.5)	0.0004
November 1997	6	36.2 ± 1.7^{a} (25.4 – 48.8)	25.5 ± 1.7^{c} (23.2 – 28.9)	23.7 ± 1.7^{c} (21.9 – 25.4)	27.5 ± 1.7^{c} (25.0 – 30.6)	32.8 ± 1.7^{b} (27.3 – 39.9)	28.7 ± 1.7^{c} (25.8 – 31.8)	28.6 ± 1.7^{c} (24.8 – 35.0)	33.8 ± 1.7^{b} (26.6 – 38.3)	0.0001
January 1998	6	44.8 ± 2.3^{a} (35.3 – 58.6)	33.1 ± 2.3^{b} (30.1 – 39.3)	31.0 ± 2.3^{b} (24.8 – 40.4)	32.3 ± 2.3^{b} (22.5 – 38.2)	36.0 ± 2.3^{b} (32.6 – 39.7)	35.1 ± 2.3^{b} (30.9 – 39.5)	37.9 ± 2.3^{b} (28.7 – 44.3)	33.7 ± 2.3^{b} (23.8 – 38.5)	0.0048
March 1998	6	35.4 ± 3.4^{a} (29.4 – 47.1)	36.4 ± 3.4^{a} (31.0 – 41.3)	24.9 ± 3.4^{c} (16.4 – 31.9)	29.7 ± 3.4^{b} (20.1 – 38.4)	26.1 ± 3.4^{b} (11.3 – 33.8)	34.1 ± 3.4^{a} (19.2 – 42.9)	36.2 ± 3.4^{a} (24.3 – 58.3)	31.2 ± 3.4^{b} (26.1 – 41.2)	0.0538
May 1998	6	42.9 ± 3.0^{a} (29.9 – 52.9)	35.0 ± 3.0^{b} (26.0 – 49.3)	27.3 ± 3.0^{c} (20.9 – 38.1)	25.7 ± 3.0^{c} (15.1 – 37.2)	26.6 ± 3.0^{c} (16.5 – 34.1)	27.7 ± 3.0^{c} (16.5 – 32.7)	27.8 ± 3.0^{c} (20.9 – 39.8)	23.2 ± 3.0^{c} (19.6 – 29.5)	0.0008
July 1998	6	31.1 ± 2.3^{a} (23.7 – 42.2)	22.0 ± 2.3^{b} (15.7 – 27.3)	20.2 ± 2.3^{b} (14.5 – 27.4)	16.9 ± 2.3^{b} (10.7 – 23.1)	17.4 ± 2.3^{b} (13.8 – 21.9)	19.0 ± 2.3^{b} (12.3 – 32.7)	21.5 ± 2.3^{b} (15.4 – 30.6)	19.0 ± 2.3^{b} (10.9 – 24.2)	0.0028
September 1998	6	47.6 ± 3.0^{a} (39.4 – 59.9)	30.8 ± 3.0^{b} (24.2 – 38.2)	33.7 ± 3.0^{b} (26.7 – 43.8)	36.9 ± 3.0^{b} (24.4 – 58.5)	40.2 ± 3.0^{b} (29.7 – 50.0)	30.4 ± 3.0^{b} (24.5 – 40.9)	34.2 ± 3.0^{b} (24.7 – 71.6)	30.6 ± 3.0^{b} (23.6 – 36.7)	0.0027
November 1998	6	53.8 ± 3.0^{a} (37.6 – 82.2)	38.7 ± 3.0^{b} (21.8 – 51.5)	50.3 ± 3.0^{a} (32.5 – 65.9)	33.5 ± 3.0^{c} (26.3 – 38.3)	46.6 ± 3.0^{b} (23.3 – 63.3)	34.6 ± 3.0^{c} (24.7 – 49.8)	46.1 ± 3.0^{b} (24.7 – 71.6)	33.3 ± 3.0^{c} (23.5 – 42.1)	0.0375
January 1999	6	51.4 ± 3.1^{a} (36.1 – 67.8)	27.1 ± 3.1^{b} (16.9 – 37.3)	30.6 ± 3.1^{b} (20.8 – 40.0)	30.5 ± 3.1^{b} (27.1 – 37.3)	29.4 ± 3.1^{b} (19.7 – 44.5)	29.4 ± 3.1^{b} (24.1 – 32.2)	28.7 ± 3.1^{b} (22.2 – 33.3)	30.1 ± 3.1^{b} (18.8 – 39.6)	0.0001
March 1999	6	43.9 ± 1.9^{a} (39.2 – 48.6)	33.1 ± 1.9^{b} (26.3 – 41.2)	36.2 ± 1.9^{b} (28.2 – 43.1)	30.1 ± 1.9^{b} (22.9 – 33.6)	30.4 ± 1.9^{b} (22.2 – 37.7)	34.3 ± 1.9^{b} (30.1 – 39.7)	33.4 ± 1.9^{b} (27.3 – 38.6)	28.6 ± 1.9^{b} (24.4 – 35.4)	0.0001

Appendix 44 Plasma alkaline phosphatase (IU /l) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at P > 0.05).

Month	n	Group 1 (Con)	Group 2 (Ca	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	P value
May 1997	6	51.2 ± 5.5^{a} (36.7 – 73.8)	50.5 ± 5.5^{a} (31.7 – 67.0)	48.9 ± 5.5^{a} (21.5 – 69.2)	50.8 ± 5.5^{a} (45.6 –55.6)	50.6 ± 5.5^{a} (40.3 – 59.4)	49.8 ± 5.5^{a} (20.6 – 64.6)	51.5 ± 5.5^{a} (27.4 – 74.2)	48.9 ± 5.5^{a} (29.9 – 52.9)	0.9875
July 1997	6	43.4 ± 4.1^{a} (28.5 – 59.9)	21.7 ± 4.1^{b} (10.2 – 41.0)	22.2 ± 4.1^{b} (16.7 – 28.9)	26.6 ± 4.1^{b} (18.5 – 31.6)	25.2 ± 4.1^{b} (15.0 – 38.2)	24.4 ± 4.1^{b} (14.4 – 48.3)	29.6 ± 4.1^{b} (12.2 – 47.1)	23.5 ± 4.1^{b} (16.0 – 35.0)	0.0136
September 1997	6	36.5 ± 2.9^{a} (23.7 – 58.5)	22.6 ± 2.9^{b} (11.1 – 31.8)	18.4 ± 2.9^{b} (12.2 – 26.9)	16.0 ± 2.9^{b} (11.4 – 29.0)	18.2 ± 2.9^{b} (10.1 – 25.7)	20.1 ± 2.9^{b} (14.0 – 26.0)	19.5 ± 2.9^{b} (10.9 – 26.4)	16.6 ± 2.9^{b} (11.2 – 22.5)	0.0004
November 1997	6	36.2 ± 1.7^{a} (25.4 – 48.8)	25.5 ± 1.7^{c} (23.2 – 28.9)	23.7 ± 1.7^{c} (21.9 – 25.4)	27.5 ± 1.7^{c} (25.0 – 30.6)	32.8 ± 1.7^{b} (27.3 – 39.9)	28.7 ± 1.7^{c} (25.8 – 31.8)	28.6 ± 1.7^{c} (24.8 – 35.0)	33.8 ± 1.7^{b} (26.6 – 38.3)	0.0001
January 1998	6	44.8 ± 2.3^{a} (35.3 – 58.6)	33.1 ± 2.3^{b} (30.1 – 39.3)	31.0 ± 2.3^{b} (24.8 – 40.4)	32.3 ± 2.3^{b} (22.5 – 38.2)	36.0 ± 2.3^{b} (32.6 – 39.7)	35.1 ± 2.3^{b} (30.9 – 39.5)	37.9 ± 2.3^{b} (28.7 – 44.3)	33.7 ± 2.3^{b} (23.8 – 38.5)	0.0048
March 1998	6	35.4 ± 3.4^{a} (29.4 – 47.1)	36.4 ± 3.4^{a} (31.0 – 41.3)	24.9 ± 3.4^{c} (16.4 – 31.9)	29.7 ± 3.4^{b} (20.1 – 38.4)	26.1 ± 3.4^{b} (11.3 – 33.8)	34.1 ± 3.4^{a} (19.2 – 42.9)	36.2 ± 3.4^{a} (24.3 – 58.3)	31.2 ± 3.4^{b} (26.1 – 41.2)	0.0538
May 1998	6	42.9 ± 3.0^{a} (29.9 – 52.9)	35.0 ± 3.0^{b} (26.0 – 49.3)	27.3 ± 3.0^{c} (20.9 – 38.1)	25.7 ± 3.0^{c} (15.1 – 37.2)	26.6 ± 3.0^{c} (16.5 – 34.1)	27.7 ± 3.0^{c} (16.5 – 32.7)	27.8 ± 3.0^{c} (20.9 – 39.8)	23.2 ± 3.0^{c} (19.6 – 29.5)	0.0008
July 1998	6	31.1 ± 2.3^{a} (23.7 – 42.2)	22.0 ± 2.3^{b} (15.7 – 27.3)	20.2 ± 2.3^{b} (14.5 – 27.4)	16.9 ± 2.3^{b} (10.7 – 23.1)	17.4 ± 2.3^{b} (13.8 – 21.9)	19.0 ± 2.3^{b} (12.3 – 32.7)	21.5 ± 2.3^{b} (15.4 – 30.6)	19.0 ± 2.3^{b} (10.9 – 24.2)	0.0028
September 1998	6	47.6 ± 3.0^{a} (39.4 – 59.9)	30.8 ± 3.0^{b} (24.2 – 38.2)	33.7 ± 3.0^{b} (26.7 – 43.8)	36.9 ± 3.0^{b} (24.4 – 58.5)	40.2 ± 3.0^{b} (29.7 – 50.0)	30.4 ± 3.0^{b} (24.5 – 40.9)	34.2 ± 3.0^{b} (24.7 – 71.6)	30.6 ± 3.0^{b} (23.6 – 36.7)	0.0027
November 1998	6	53.8 ± 3.0^{a} (37.6 – 82.2)	38.7 ± 3.0^{b} (21.8 – 51.5)	50.3 ± 3.0^{a} (32.5 – 65.9)	33.5 ± 3.0^{c} (26.3 – 38.3)	46.6 ± 3.0^{b} (23.3 – 63.3)	34.6 ± 3.0^{c} (24.7 – 49.8)	46.1 ± 3.0^{b} (24.7 – 71.6)	33.3 ± 3.0^{c} (23.5 – 42.1)	0.0375
January 1999	6	51.4 ± 3.1^{a} (36.1 – 67.8)	27.1 ± 3.1^{b} (16.9 – 37.3)	30.6 ± 3.1^{b} (20.8 – 40.0)	30.5 ± 3.1^{b} (27.1 – 37.3)	29.4 ± 3.1^{b} (19.7 – 44.5)	29.4 ± 3.1^{b} (24.1 – 32.2)	28.7 ± 3.1^{b} (22.2 – 33.3)	30.1 ± 3.1^{b} (18.8 – 39.6)	0.0001
March 1999	6	43.9 ± 1.9^{a} (39.2 – 48.6)	33.1 ± 1.9^{b} (26.3 – 41.2)	36.2 ± 1.9^{b} (28.2 – 43.1)	30.1 ± 1.9^{b} (22.9 – 33.6)	30.4 ± 1.9^{b} (22.2 – 37.7)	34.3 ± 1.9^{b} (30.1 – 39.7)	33.4 ± 1.9^{b} (27.3 – 38.6)	28.6 ± 1.9^{b} (24.4 – 35.4)	0.0001

Appendix 45 Plasma total protein (g /l) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at P > 0.05).

Month	n	Group 1 (Control)	Group 2 (Ca	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	P value
May 1997	6	83.6 ± 2.9^a (66.6 – 94.8)	87.8 ± 2.9^a (83.8 – 90.4)	83.0 ± 2.9^a (78.4 – 91.8)	86.5 ± 2.9^a (74.2 –95.8)	83.1 ± 2.9^a (73.8 – 96.4)	89.1 ± 2.9^a (81.6 – 101)	85.9 ± 2.9^a (67.9 – 101)	85.0 ± 2.9^a (77.0 – 93.4)	0.7928
July 1997	6	100 ± 1.8^a (92.8 – 112)	97.8 ± 1.8^a (93.0 – 102)	99.6 ± 1.8^a (95.4 – 106)	97.3 ± 1.8^a (94.21– 101)	104 ± 1.8^a (98.0 – 107)	101 ± 1.8^a (98.0 – 107)	102 ± 1.8^a (95.7 – 108)	98.6 ± 1.8^a (94.2 – 105)	0.2094
September 1997	6	94.4 ± 3.1^a (86.1 – 99.5)	102 ± 3.1^a (100 – 107)	97.4 ± 3.1^a (87.7 – 106)	91.8 ± 3.1^a (75.4 – 106)	92.3 ± 3.1^a (81.6 – 105)	101 ± 3.1^a (92.2 – 112)	99.1 ± 3.1^a (90.5 – 108)	99.3 ± 3.1^a (90.1 – 105)	0.1660
November 1997	6	102 ± 1.5^b (94.0 – 108)	109 ± 1.5^a (104 – 111)	107 ± 1.5^a (101 – 112)	95.5 ± 1.5^c (92.7 – 98.4)	99.2 ± 1.5^b (97.1 – 111)	108 ± 1.5^a (99 – 114)	94.9 ± 1.5^c (90.4 – 97.2)	91.6 ± 1.5^d (85.3 – 95.7)	0.001
January 1998	6	110 ± 1.7^a (109 – 112)	106 ± 1.7^a (101 – 113)	109 ± 1.7^a (105 – 112)	104 ± 1.7^a (96.2 – 111)	105 ± 1.7^a (97.1 – 111)	103 ± 1.7^a (100 – 107)	114 ± 1.7^a (110 – 116)	105 ± 1.7^a (99.7 – 115)	0.0.0515
March 1998	6	84.4 ± 2.4^a (78.2 – 92.6)	85.5 ± 2.4^a (79.4 – 89.8)	85.4 ± 2.4^a (82.4 – 90.4)	89.2 ± 2.4^a (82.0 – 93.6)	81.8 ± 2.4^a (74.2 – 86.2)	85.6 ± 2.4^a (77.6– 99.4)	82.7 ± 2.4^a (70.8 – 91.2	88.3 ± 2.4^a (81.0 – 96.2)	0.3930
May 1998	6	85.3 ± 3.5^b (76.4 – 92.2)	87.5 ± 3.5^b (82.0 – 94.0)	96.9 ± 3.5^a (85.4 – 104)	92.6 ± 3.5^a (84.8 – 97.4)	96.1 ± 3.5^a (80.8 – 119)	94.3 ± 3.5^a (83.0– 113)	92.0 ± 3.5^a (85.0 – 103)	97.3 ± 3.5^a (84.4 – 108)	0.0524
July 1998	6	93.6 ± 3.6^a (80.6 – 104)	92.8 ± 3.6^a (86.2 – 101)	96.2 ± 3.6^a (87.2 – 110)	105 ± 3.6^a (91.8 – 118)	100 ± 3.6^a (89.0 – 108)	96.2 ± 3.6^a (86.4 – 107)	95.1 ± 3.6^a (79.2 – 110)	97.1 ± 3.6^a (85.0 – 109)	0.3848
September 1998	6	81.4 ± 3.3^a (77.0 – 84.4)	85.9 ± 3.3^a (79.8 – 97.0)	80.7 ± 3.3^a (62.8 – 88.8)	90.6 ± 3.3^a (82.4 – 103)	85.5 ± 3.3^a (77.6 – 97.6)	85.8 ± 3.3^a (70.2 – 109)	82.7 ± 3.3^a (72.8 – 98.2	81.6 ± 3.3^a (75.6 – 84.4)	0.4415
November 1998	6	88.7 ± 3.0^b (85.6 – 92.8)	97.3 ± 3.0^a (90.6 – 108)	95.4 ± 3.0^a (88.8 – 103)	93.2 ± 3.0^a (76.4 – 109)	88.4 ± 3.0^b (75.6– 98.6)	94.7 ± 3.0^a (84.4 – 106)	91.9 ± 3.0^a (82.6 – 102	88.9 ± 3.0^a (81.4 – 97.0)	0.0542
January 1999	6	91.5 ± 3.0^b (81.4 – 98.4)	98.7 ± 3.0^a (89.8 – 108)	91.5 ± 3.0^a (87.0 – 99.0)	91.5 ± 3.0^b (80.2 – 104)	96.6 ± 3.0^a (83.8 – 110)	99,9 ± 3.0^a (95.4 – 106)	95.5 ± 3.0^b (85.4 – 110)	97.7 ± 3.0^a (91.2 – 104)	0.2452
March 1999	6	98.4 ± 3.2^a (93.0 – 104)	91.3 ± 3.2^b (79.9 – 100)	93.7 ± 3.2^a (85.2 – 105)	93.8 ± 3.2^a (74.6 – 106)	96.2 ± 3.2^a (86.1 – 105)	93.9 ± 3.2^a (72.4 – 108)	87.2 ± 3.2^b (81.3 – 93.8)	98.1 ± 3.2^a (92.0 – 102)	0.2712

Appendix 46 Plasma urea (mmol /l) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at P > 0.05).

Month	n	Group 1 (Con)	Group 2 (Ca	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	P value
May 1997	6	2.74 ± 0.09^{a} (2.1 – 3.09)	2.87 ± 0.09^{a} (2.53 – 3.20)	2.86 ± 0.09^{a} (2.68 – 3.06)	2.77 ± 0.09^{a} (2.46 –3.06)	2.68 ± 0.09^{a} (2.47 – 2.80)	2.86 ± 0.09^{a} (2.69 – 3.09)	2.68 ± 0.09^{a} (2.46 – 2.80)	2.82 ± 0.09^{a} (2.54 – 3.08)	0.5718
July 1997	6	2.34 ± 0.07^{d} (2.11 – 2.57)	2.46 ± 0.07^{d} (2.21 – 2.82)	3.36 ± 0.07^{b} (3.03 – 3.68)	2.93 ± 0.07^{c} (2.89 – 2.99)	3.02 ± 0.07^{c} (2.86 – 3.18)	3.79 ± 0.07^{a} (3.56 – 3.92)	2.89 ± 0.07^{c} (2.65 – 3.03)	1.96 ± 0.07^{d} (1.89 – 2.05)	0.0001
September 1997	6	2.06 ± 0.09^{c} (1.75 – 2.30)	2.01 ± 0.09^{c} (1.69 – 2.48)	1.78 ± 0.09^{d} (1.66– 1.87)	1.57 ± 0.09^{d} (1.42 – 1.80)	2.31 ± 0.09^{b} (2.01 – 2.66)	2.65 ± 0.09^{a} (2.21– 3.16)	2.22 ± 0.09^{b} (2.08 – 2.36)	2.17 ± 0.09^{b} (2.02 – 2.28)	0.0001
November 1997	6	6.28 ± 0.16^{c} (6.1 – 6.75)	6.08 ± 0.16^{c} (5.11 – 7.24)	5.81 ± 0.16^{d} (5.64 – 6.01)	5.54 ± 0.16^{e} (5.32 – 5.82)	7.23 ± 0.16^{a} (6.88 – 7.56)	6.44 ± 0.16^{b} (6.01 – 6.89)	6.55 ± 0.16^{b} (6.05 – 7.03)	5.83 ± 0.16^{d} (5.34 – 6.12)	0.0001
January 1998	6	6.23 ± 0.17^{b} (6.08 – 6.58)	6.34 ± 0.17^{b} (5.57 – 6.84)	6.09 ± 0.17^{c} (5.86 – 6.40)	6.45 ± 0.17^{b} (6.08 – 6.86)	5.69 ± 0.17^{d} (5.50 – 5.89)	8.19 ± 0.17^{a} (6.98 – 9.14)	5.93 ± 0.17^{c} (5.76 – 6.12)	5.79 ± 0.17^{d} (5.47 – 6.30)	0.0001
March 1998	6	6.20 ± 0.15^{b} (5.58 – 6.80)	5.98 ± 0.15^{c} (5.63 – 6.48)	5.70 ± 0.15^{d} (5.44 – 5.98)	6.24 ± 0.15^{b} (5.76 – 6.70)	7.57 ± 0.15^{a} (7.08 – 7.96)	6.49 ± 0.15^{b} (5.90 – 6.98	7.32 ± 0.15^{a} (6.80 – 7.80)	6.12 ± 0.15^{c} (5.26 – 6.85)	0.0001
May 1998	6	5.62 ± 0.13^{c} (5.24 – 5.98)	5.58 ± 0.13^{c} (5.22 – 5.98)	7.50 ± 0.13^{a} (7.12 – 7.68)	5.49 ± 0.13^{c} (5.12 – 5.90)	6.61 ± 0.13^{b} (6.12 – 7.05)	6.75 ± 0.13^{b} (6.38 – 7.08	5.45 ± 0.13^{c} (5.00 – 6.08)	7.28 ± 0.13^{a} (6.80 – 7.78)	0.0001
July 1998	6	2.37 ± 0.14^{d} (2.15 – 2.80)	2.49 ± 0.14^{d} (2.14 – 2.90)	2.66 ± 0.14^{c} (2.24 – 2.98)	2.34 ± 0.14^{d} (2.12 – 2.60)	3.14 ± 0.14^{b} (2.00 – 3.73)	3.66 ± 0.14^{a} (3.33 – 3.90)	2.89 ± 0.14^{b} (2.35 – 3.30)	2.21 ± 0.14^{c} (1.98 – 2.49)	0.0001
September 1998	6	1.50 ± 0.12^{c} (1.10 – 1.73)	1.60 ± 0.12^{c} (1.29 – 1.80)	2.07 ± 0.12^{b} (1.87 – 2.38)	1.59 ± 0.12^{c} (1.38 – 1.90)	1.55 ± 0.12^{c} (1.04 – 1.84)	2.13 ± 0.12^{b} (1.77 – 2.55)	2.26 ± 0.12^{b} (1.70 – 2.80)	3.01 ± 0.12^{a} (2.45 – 3.49)	0.0001
November 1998	6	2.40 ± 0.13^{d} (2.12 – 2.73)	2.50 ± 0.13^{d} (2.20 – 2.90)	2.65 ± 0.13^{c} (2.12 – 2.98)	2.21 ± 0.13^{e} (2.05 – 2.56)	2.43 ± 0.13^{d} (2.12 – 2.70)	4.22 ± 0.13^{a} (3.35 – 4.83)	3.51 ± 0.13^{a} (3.15 – 3.80)	2.15 ± 0.13^{e} (1.76 – 2.56)	0.0001
January 1999	6	6.21 ± 0.13^{b} (5.58 – 6.80)	5.85 ± 0.17^{b} (5.29 – 6.50)	7.11 ± 0.17^{a} (6.24 – 7.80)	6.02 ± 0.17^{b} (5.66 – 6.32)	7.25 ± 0.17^{a} (6.64 – 7.75)	5.91 ± 0.17^{b} (5.62 – 6.10)	6.34 ± 0.17^{b} (5.56 – 6.99)	6.08 ± 0.17^{b} (6.64 – 7.26)	0.0001
March 1999	6	4.91 ± 0.12^{e} (4.75 – 4.98)	3.89 ± 0.12^{f} (3.71 – 4.15)	5.61 ± 0.12^{d} (5.23 – 5.98)	5.56 ± 0.12^{d} (5.08 – 5.90)	7.86 ± 0.12^{a} (7.37 – 8.24)	6.27 ± 0.12^{c} (5.92 – 6.73)	7.70 ± 0.12^{a} (7.06 – 7.98)	6.99 ± 0.12^{b} (5.93 – 6.31)	0.0001

Appendix 47 Plasma glucose concentration (mmol/l) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at $P > 0.05$).

Month	n	Group 1 (Control)	Group 2 (Ca	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	P value
May 1997	6	3.62 ± 0.13^{a} (3.48 – 3.77)	3.62 ± 0.13^{a} (3.19 – 4.39)	3.69 ± 0.13^{a} (3.64 – 4.10)	3.61 ± 0.13^{a} (3.38 –4.30)	3.59 ± 0.13^{a} (3.15 – 3.86)	3.76 ± 0.13^{a} (3.38 – 4.21)	3.53 ± 0.13^{a} (3.05 – 3.96)	3.71 ± 0.13^{a} (3.33 – 4.23)	0.6222
July 1997	6	4.15 ± 0.14^{a} (4.10 – 4.21)	4.11 ± 0.14^{a} (3.76 – 4.67)	4.27 ± 0.14^{a} (4.12 – 4.48)	4.18 ± 0.14^{a} (4.06 – 4.77)	3.95 ± 0.14^{a} (3.19 – 4.51)	4.01 ± 0.14^{a} (3.46 – 4.77)	4.25 ± 0.14^{a} (4.13 – 4.35)	4.27 ± 0.14^{a} (4.17 – 4.35)	0.4976
September 1997	6	3.86 ± 0.12^{b} (3.43 – 4.17)	4.05 ± 0.12^{a} (3.58 – 4.56)	3.52 ± 0.12^{c} (3.22– 3.73)	3.85 ± 0.12^{b} (3.72 – 3.95)	3.75 ± 0.12^{b} (3.24 – 4.49)	3.52 ± 0.12^{c} (3.4– 3.64)	3.74 ± 0.12^{b} (3.14 – 4.24)	3.25 ± 0.12^{a} (2.98 – 3.95)	0.0019
November 1997	6	3.38 ± 0.15^{b} (2.96 – 3.54)	3.96 ± 0.15^{a} (3.70 – 4.39)	2.81 ± 0.15^{c} (2.16 – 3.35)	3.60 ± 0.15^{b} (3.40 – 3.84)	3.33 ± 0.15^{b} (2.84 – 3.87)	3.16 ± 0.15^{c} (2.55 – 3.47)	3.37 ± 0.15^{b} (2.84 – 3.78)	3.32 ± 0.15^{b} (2.72 – 4.14)	0.0008
January 1998	6	3.89 ± 0.1^{b} (3.72 – 4.05)	4.08 ± 0.1^{a} (3.87 – 4.26)	3.61 ± 0.1^{b} (3.55 – 3.79)	3.58 ± 0.1^{c} (3.15 – 3.97)	3.90 ± 0.1^{b} (3.63 – 4.18)	4.12 ± 0.1^{a} (3.74 – 4.63)	3.98 ± 0.1^{a} (3.67 – 4.33)	3.88 ± 0.1^{b} (3.80 – 3.95)	0.0031
March 1998	6	3.53 ± 0.08^{c} (3.12 – 3.94)	3.78 ± 0.08^{b} (3.64 – 4.09)	3.26 ± 0.08^{d} (2.89 – 3.56)	3.62 ± 0.08^{b} (3.52 – 3.90)	4.14 ± 0.08^{a} (3.87 – 4.30)	3.71 ± 0.08^{b} (3.61 – 3.89)	4.28 ± 0.08^{a} (4.03 – 4.62)	4.07 ± 0.08^{a} (3.88 – 4.29)	0.0001
May 1998	6	4.30 ± 0.06^{a} (4.02 – 4.56)	4.26 ± 0.06^{a} (4.06 – 4.49)	4.19 ± 0.06^{b} (4.05 – 4.50)	4.15 ± 0.06^{b} (4.00 – 4.33)	4.19 ± 0.06^{b} (4.09 – 4.31)	3.98 ± 0.06^{c} (3.88 – 4.17)	3.96 ± 0.06^{c} (3.77 – 4.15)	4.27 ± 0.06^{a} (4.18– 4.37)	0.0005
July 1998	6	4.32 ± 0.1^{a} (4.19 – 4.56)	4.07 ± 0.1^{b} (4.01 – 4.12)	4.08 ± 0.1^{b} (3.94 – 4.36)	4.17 ± 0.1^{b} (4.05 – 4.32)	4.17 ± 0.1^{b} (3.79 – 4.54)	4.03 ± 0.1^{b} (3.94 – 4.18)	3.96 ± 0.1^{b} (3.76 – 4.08)	4.09 ± 0.1^{b} (4.00– 4.21)	0.0053
September 1998	6	2.86 ± 0.04^{c} (2.80 – 2.96)	3.34 ± 0.04^{a} (3.23 – 3.58)	3.10 ± 0.04^{b} (3.05 – 3.16)	3.12 ± 0.04^{b} (3.01 – 3.32)	3.14 ± 0.04^{b} (3.01 – 3.33)	2.87 ± 0.04^{c} (2.73 – 2.94)	2.71 ± 0.04^{d} (2.64 – 2.78)	2.94 ± 0.04^{c} (2.86 – 2.98)	0.0001
November 1998	6	3.43 ± 0.09^{a} (3.32 – 3.57)	3.35 ± 0.09^{a} (3.22 – 3.59)	3.21 ± 0.09^{a} (3.17 – 3.31)	3.43 ± 0.09^{a} (3.00 – 3.81)	3.24 ± 0.09^{a} (2.97 – 3.57)	3.35 ± 0.09^{a} (3.12 – 3.63)	3.47 ± 0.09^{a} (2.71 – 3.80)	3.32 ± 0.09^{a} (3.04 – 3.78)	0.4331
January 1999	6	3.21 ± 0.11^{b} (2.89 – 3.83)	3.54 ± 0.11^{a} (3.22 – 3.79)	3.11 ± 0.11^{c} (2.67 – 3.42)	3.16 ± 0.11^{c} (3.04 – 3.27)	3.24 ± 0.11^{b} (3.07 – 3.47)	3.35 ± 0.11^{b} (3.12 – 3.63)	3.47 ± 0.11^{b} (2.71 – 3.80)	3.32 ± 0.11^{b} (3.09 – 3.59)	0.0525
March 1999	6	3.84 ± 0.07^{a} (3.65 – 3.96)	3.73 ± 0.07^{a} (3.63 – 4.01)	3.21 ± 0.07^{c} (3.03 – 3.37)	3.83 ± 0.07^{a} (3.50 – 4.30)	3.71 ± 0.07^{a} (3.52 – 3.99)	3.60 ± 0.07^{b} (3.50 – 3.78)	3.63 ± 0.07^{b} (3.51 – 3.84)	3.47 ± 0.07^{b} (3.32 – 3.66)	0.0001

Appendix 48. Milk yield (kg) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at P > 0.05; Number in brackets in the first row of each month indicates number of cows involved in computing the mean).

Month	Group 1 (Con)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	P value
May	304 [a](6)	310 [a](6)	316 [a](6)	326 [a](6)	316 [a](6)	308 [a](6)	317 [a](6)	323 [a](6)	0.9529
1997	(249 - 350)	(275 – 346)	(286 – 356)	(266 –379)	(278 – 351)	(250 – 350)	(284 – 380)	(287 – 350)	
July	230 [b](6)	278 [a](6)	246 [a](6)	289 [a](6)	273 [a](6)	267 [a](6)	260 [a](6)	299 [a](6)	0.0547
1997	(153 – 300)	(260 – 293)	(118 – 307)	(238 – 338)	(233 – 304)	(200 – 330)	(200 – 306)	(212 – 308)	
September	174 [a](5)	242 [a](5)	176 [a](5)	225 [a](6)	205 [a](6)	195 [a](6)	195 [a](6)	197 [a](6)	0.5975
1997	(106 – 256)	(226 – 264)	(123 – 207)	(116 – 280)	(100 – 280)	(140 – 290)	(125 – 257)	(100 – 250)	
November	188 [a](6)	227 [a](4)	167 [a](4)	212 [a](5)	235 [a](4)	182 [a](6)	164 [a](4)	183 [a](5)	0.5630
1997	(106 – 241)	(160 – 297)	(129 – 205)	(156 – 280)	(103 – 506)	(108 – 320)	(103 – 232)	(100 – 300)	
January	294 [a](3)	464 [a](1)	270 [a](3)	219 [a](4)	223 [a](4)	294 [a](3)	286 [a](5)	303 [a](4)	0.8452
1998	(230 – 381)	(464)	(123 – 411)	(120 – 390)	(117 – 456)	(120 – 414)	(129 – 395)	(138 – 404)	
March	263 [a](4)	195 [b](6)	258 [a](6)	287 [a](5)	276 [a](5)	315 [a](4)	371 [a](5)	334 [a](5)	0.0556
1998	(112 – 420)	(102 – 437)	(124 – 396)	(104 – 420)	(25 – 400)	(166– 380)	(328 – 437)	(178 – 400)	
May	277 [b](4)	427 [a](6)	327 [b](6)	328 [b](5)	324 [b](5)	340 [b](4)	345 [b](5)	338 [b](5)	0.0025
1998	(322 – 443)	(378 – 482)	(257 – 362)	(300 – 356)	(300 – 359)	(292– 362)	(250 – 460)	(250 – 400)	
July	276 [a](4)	364 [a](6)	317 [a](5)	256 [a](6)	254 [a](5)	290 [a](6)	290 [a](6)	275 [a](6)	0.2408
1998	(248 – 349)	(300 – 413)	(258 – 367)	(108 – 320)	(200 – 323)	(200 – 353)	(200 – 410)	(196 – 351)	
September	206 [b](5)	305 [a](6)	217 [b](5)	267 [b](6)	322 [a](3)	231 [b](5)	229 [b](5)	232 [b](5)	0.0534
1998	(140 – 282)	(264 – 364)	(181 – 306)	(192 – 396)	(255 – 411)	(162 – 350)	(100 – 330)	(150 – 300)	
November	193 [b](6)	273 [a](6)	163 [c](5)	238 [b](5)	257 [a](4)	232 [b](3)	204 [b](4)	118 [d](5)	0.0452
1998	(107 – 303)	(208 – 400)	(130 – 224)	(172 – 337)	(136 – 320)	(171 – 301)	(158 – 280)	(96 – 150)	
January	164 [a](5)	216 [a](4)	195 [a](1)	244 [a](2)	285 [a](5)	265 [a](5)	224 [a](3)	314 [a](3)	0.0987
1999	(103 – 254)	(90 – 380)	(355 – 443)	(200 – 287)	(155 – 411)	(200 – 337)	(156 – 307)	(263 – 372)	
March	241 [a](2)	379 [a](5)	256 [a](5)	250 [a](4)	372 [a](4)	256 [a](6)	257 [a](6)	326 [a](5)	0.0725
1999	(209 – 280)	(300 – 489)	(80 – 352)	(156 – 383)	(339 – 408)	(119 – 400)	(100 – 394)	(180 – 404)	

Appendix 49 Plasma Ca, milk yield, milk butter fat and protein correlation in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (The first row for each parameter and season indicates r value and second row indicates P value. D = dry season and W = rainy season).

Parameter	Season	N	Group 1 (Control)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)
Milk yield	D	42	-0.0834	-0.3645	-0.3521	-0.0423	-0.0060	-0.2391	-0.2687	-0.2762
			0.6287	0.0191	0.0302	0.7952	0.9723	0.1595	0.0981	0.0880
	W	30	0.0007	0.1396	-0.2805	-0.2489	0.0533	-0.3992	-0.5402	-0.4066
			0.9978	0.5573	0.2448	0.2900	0.8137	0.0533	0.0078	0.4702
Milk butterfat	D	42	-0.1949	0.0448	0.2578	0.1855	0.0044	0.0861	0.0223	-0.1366
			0.5056	0.7810	0.1410	0.2648	0.9807	0.6282	0.8973	0.4203
	W	30	0.6491	0.7986	-0.1100	0.3953	0.2078	-0.1596	-0.1221	0.1530
			0.0048	0.0004	0.6850	0.1297	0.4080	0.5016	0.6184	0.5318
Milk protein	D	42	0.1182	0.1614	0.0644	0.2463	-0.0400	-0.2503	-0.0597	-0.0096
			0.5056	0.3134	0.7173	0.1361	0.8279	0.1533	0.7294	0.9548
	W	30	0.6397	0.4293	0.1136	0.3813	0.2886	0.5005	0.8175	0.6784
			0.0057	0.1103	0.6754	0.1450	0.2455	0.0246	0.0001	0.0014

Appendix 50 Plasma inorganic phosphate, milk yield, milk butter fat and protein correlation in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (The first row for each parameter and season indicates r value and second row indicates P value. D = dry season and W = rainy season).

Parameter	Season	N	Group 1 (Control)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)
Milk yield	D	42	-0.0940	0.0239	-0.2470	0.2739	0.0891	0.0790	-0.2279	-0.0609
			0.5854	0.8819	0.1348	0.0872	0.6054	0.6468	0.1630	0.7127
	W	30	0.1860	-0.0821	-0.0407	-0.0669	-0.0773	-0.3240	-0.4507	-0.3908
			0.4459	0.7306	0.8688	0.7793	0.7326	0.1224	0.0309	0.0721
Milk butterfat	D	42	0.1556	0.2884	-0.3306	0.0855	-0.1233	-0.1691	-0.1221	-0.1209
			0.3796	0.0674	0.0562	0.6105	0.5014	0.3391	0.4780	0.4760
	W	30	0.2728	0.2831	0.0963	0.2796	0.7308	0.4526	-0.1776	0.3162
			0.2894	0.3065	0.7229	0.2942	0.0006	0.0451	0.4671	0.1872
Milk protein	D	42	-0.0370	-0.2774	-0.3700	-0.2559	0.0891	-0.4890	0.0001	-0.2221
			0.8352	0.0791	0.0313	0.1209	0.6054	0.0033	0.9994	0.1865
	W	30	0.3081	-0.2335	0.0097	0.0300	0.6950	-0.0585	0.4623	0.2348
			0.2289	0.4022	0.9717	0.9122	0.0014	0.8064	0.0463	0.3333

Appendix 51 Plasma Zn, milk yield, milk butter fat and protein correlation in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. (The first row for each parameter and season indicates r value and second row indicates P value. D = dry season and W = rainy season).

Parameter	Season	N	Group 1 (Control)	Group 2 (Ca)	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)
Milk yield	D	42	-0.0741	0.0273	-0.2672	-0.0376	0.1126	0.2551	0.1002	-0.3015
			0.6676	0.8653	0.1049	0.8180	0.5133	0.1333	0.5437	0.0621
	W	30	0.0869	0.1282	0.1264	-0.0977	0.0533	0.3289	-0.3155	-0.1524
			0.7237	0.5900	0.6064	0.6819	0.8137	0.1166	0.1882	0.4984
Milk butterfat	D	42	0.3586	-0.1954	0.0582	0.0341	0.0275	-0.3900	-0.0588	0.0610
			0.0376	0.2207	0.7439	0.8390	0.8813	0.0226	0.7336	0.7198
	W	30	0.1382	0.0289	0.5636	-0.6579	0.2078	-0.0397	-0.4556	0.0290
			0.5968	0.9187	0.0230	0.0056	0.4080	0.8679	0.0289	0.9062
Milk protein	D	42	-0.1152	-0.1358	-0.3550	-0.4355	0.0088	-2513	-0.0513	0.0705
			0.5165	0.3974	0.0394	0.0063	0.9617	0.1516	0.7665	0.6783
	W	30	0.1821	-0.6190	0.4739	-0.2739	0.2886	-0.0718	0.7473	0.5293
			0.4843	0.0139	0.0637	0.3047	0.2455	0.7635	0.0002	0.0198

Appendix 52 Plasma ceruloplasmin activity (IU /l) in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm Iringa, Tanzania. (Means with the same superscript letter within the same month and row do not differ significantly at P > 0.05).

Month	n	Group 1 (Con)	Group 2 (Ca	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)	P value
May 1997	6	44.2 ± 1.1^{a} (41.8 – 47.0)	42.9 ± 1.1^{a} (40.3 – 46.1)	44.0 ± 1.1^{a} (40.5 – 47.3)	44.6 ± 1.1^{a} (42.4 –47.9)	47.9 ± 1.1^{a} (42.3 – 51.9)	44.5 ± 1.1^{a} (40.6 – 49.0)	45.2 ± 1.1^{a} (40.6 – 49.0)	45.3 ± 1.1^{a} (42.7 – 47.4)	0.7331
July 1997	6	38.4 ± 1.6^{b} (28.3 – 41.9)	35.1 ± 1.6^{b} (30.0 – 41.0)	45.5 ± 1.6^{a} (39.5 – 49.7)	36.3 ± 1.6^{b} (30.7 – 40.1)	43.7 ± 1.6^{a} (40.0 – 48.1)	47.2 ± 1.6^{a} (43.8 – 49.8)	37.3 ± 1.6^{b} (33.8 – 44.1)	45.6 ± 1.6^{a} (40.4 – 50.3)	0.0001
September 1997	6	43.5 ± 1.6^{b} (40.0 – 47.9)	43.2 ± 1.6^{b} (40.6 – 45.8)	43.2 ± 1.6^{b} (39.5 – 47.0)	42.9 ± 1.6^{b} (37.5 – 46.7)	50.6 ± 1.6^{a} (48.0 – 55.9)	49.1 ± 1.6^{a} (47.6 – 50.8)	44.3 ± 1.6^{b} (40.3 – 49.6)	45.0 ± 1.6^{b} (38.3 – 49.6)	0.0003
November 1997	6	22.7 ± 1.6^{c} (18.3 – 28.3)	32.8 ± 1.6^{a} (28.8 – 39.3)	29.2 ± 1.6^{b} (26.1 – 35.0)	28.6 ± 1.6^{b} (24.6 – 32.7)	36.9 ± 1.6^{a} (30.3 – 43.0)	18.8 ± 1.6^{c} (17.4 – 19.7)	22.8 ± 1.6^{c} (20.4 – 30.6)	21.6 ± 1.6^{c} (18.0 – 30.6)	0.0001
January 1998	6	34.9 ± 1.7^{a} (32.8 – 37.1)	25.3 ± 1.7^{c} (19.1 – 32.9)	36.3 ± 1.7^{a} (29.5 – 45.5)	32.2 ± 1.7^{b} (27.2 – 38.0)	34.0 ± 1.7^{b} (32.6 – 35.8)	32.6 ± 1.7^{b} (28.9 – 39.3)	31.9 ± 1.7^{b} (28.3 – 41.2)	29.0 ± 1.7^{c} (25.4 – 32.6)	0.0020
March 1998	6	31.1 ± 2.1^{a} (20.6 – 35.1)	26.6 ± 2.1^{b} (22.3 – 32.1)	29.3 ± 2.1^{a} (20.0 – 38.6)	32.1 ± 2.1^{a} (21.2 – 39.8)	23.7 ± 2.1^{c} (21.2 – 28.9)	31.3 ± 2.1^{a} (20.0 – 38.4)	29.6 ± 2.1^{b} (27.3 – 32.0)	28.0 ± 2.1^{b} (20.6 – 36.0)	0.1111
May 1998	6	20.8 ± 1.7^{d} (15.0 – 25.2)	42.0 ± 1.7^{a} (36.0 – 46.8)	36.9 ± 1.7^{b} (27.8 – 48.6)	30.7 ± 1.7^{c} (26.7 – 35.7)	40.8 ± 1.7^{a} (37.6 – 48.0)	42.5 ± 1.7^{a} (39.0 – 46.1)	36.8 ± 1.7^{b} (32.7 – 41.0)	35.8 ± 1.7^{b} (30.5 – 40.2)	0.0001
July 1998	6	45.8 ± 1.3^{a} (42.3 – 49.8)	31.5 ± 1.3^{e} (29.8 – 33.8)	24.0 ± 1.3^{f} (20.7 – 27.2)	31.0 ± 1.3^{d} (25.0 – 36.8)	27.3 ± 1.3^{e} (20.7 – 30.2)	41.8 ± 1.3^{b} (37.8 – 46.8)	29.4 ± 1.3^{e} (24.5 – 34.8)	34.9 ± 1.3^{c} (31.7 – 37.9)	0.0001
September 1998	6	47.9 ± 1.1^{a} (42.5 – 52.3)	43.6 ± 1.1^{b} (40.6 – 47.4)	32.6 ± 1.1^{cd} (30.0 – 37.8)	31.9 ± 1.1^{d} (30.0 – 34.3)	35.9 ± 1.1^{c} (30.0 – 39.2)	43.3 ± 1.1^{b} (40.3 – 48.6)	33.9 ± 1.1^{cd} (30.4 – 35.9)	27.9 ± 1.1^{e} (26.3 – 30.1)	0.0001
November 1998	6	32.1 ± 0.1^{c} (30.4 – 34.5)	33.7 ± 0.1^{c} (30.7 – 37.8)	43.9 ± 0.1^{b} (40.0 – 46.6)	42.6 ± 0.1^{b} (40.4 – 49.6)	47.6 ± 0.1^{a} (44.9 – 49.6)	34.8 ± 0.1^{c} (32.3 – 36.8)	25.3 ± 0.1^{d} (22.7 – 27.4)	33.6 ± 0.1^{c} (31.2 – 38.6)	0.0001
January 1999	6	26.7 ± 1.1^{c} (23.7 – 29.8)	43.0 ± 1.1^{a} (40.6 – 47.6)	34.9 ± 1.1^{b} (32.5 – 38.0)	32.8 ± 1.1^{b} (30.3 – 36.8)	45.0 ± 1.1^{a} (40.4 – 49.2)	24.5 ± 1.1^{c} (20.1 – 28.7)	$33.9 \pm 2.1b$ (31.7 – 37.0)	45.7 ± 1.1^{a} (40.7 – 49.2)	0.0001
March 1999	6	37.1 ± 1.0^{b} (32.3 – 39.8)	43.9 ± 1.0^{a} (40.4 – 46.6)	24.1 ± 1.0^{b} (20.6 – 33.2)	35.2 ± 1.1^{b} (31.1 – 39.1)	42.2 ± 1.1^{a} (40.8 – 43.5)	34.8 ± 1.1^{b} (32.9 – 38.0)	28.3 ± 1.0^{c} (26.6 – 29.7)	34.7 ± 1.0^{b} (32.3 – 37.0)	0.0001

Appendix 53. Plasma Calcium/phosphorus ratio in both non and mineral supplemented grazing crossbred Zebu cows at ASAS Dairy Farm, Iringa, Tanzania. Supplementation started in May 1997 and ended in March 1999.

Month	Group 1 (Control)	Group 2 (Ca	Group 3 (P)	Group 4 (Zn)	Group 5 (Ca/P)	Group 6 (Ca/Zn)	Group 7 (P/Zn)	Group 8 (Ca/P/Zn)
May 1997	1.18:1	1.12:1	1.11:1	1.14:1	1.07:1	1.08:1	1.08:1	1.07:1
July 1997	0.98:1	0.75:1	0.75:1	0.87:1	0.82:1	0.72:1	0.66:1	0.74:1
September 1997	0.77:1	0.73:1	0.86:1	0.78:1	0.67:1	0.81:1	0.79:1	0.75:1
November 1997	1.14:1	1.19:1	1.13:1	1.22:1	1.11:1	1.16:1	1.08:1	0.98:1
January 1998	1.13:1	1.11:1	0.97:1	1.11:1	1.06:1	1.24:1	1.18:1	1:1
March 1998	1.09:1	0.84:1	0.96:1	0.96:1	0.91:1	0.89:1	0.97:1	0.85:1
May 1998	0.92:1	0.64:1	0.78:1	0.63:1	0.62:1	0.65:1	0.68:1	0.70:1
July 1998	0.76:1	0.70:1	0.69:1	0.66:1	0.82:1	0.76:1	0.80:1	0.89:1
September 1998	1.01:1	1.03:1	1.16:1	1.14:1	1.13:1	1.06:1	1.07:1	1.03:1
November 1998	1.22:1	1.24:1	1.15:1	1.21:1	1.14:1	1.22:1	1.15:1	1.17:1
January 1999	1.31:1	1.38:1	1.21:1	1.20:1	1.29:1	1.24:1	1.21:1	1.33:1
March 1999	1.44:1	1.19:1	1.26:1	1.33:1	1.53:1	1.43:1	1.24:1	1.40:1

Appendix 54 **Published papers from this work in referred journals, edited proceedings and conferences/workshops**

Referred Journals

1. Phiri, E.C.J.H. and Pereka, A.E. M.N. Mgasa and T. Larsen (1998). Clinical mastitis and bacterial isolates in Dairy cows at ASAS Dairy Farm, Iringa, Tanzania. Tanzania Veterinary Journal Vol. 18(3), 173 – 179.

2. Pereka, A.E, E.C.J.H Phiri, M.N. Mgasa and T. Larsen (2001). Milk production in relation to calcium, phosphorus and zinc supplementation in grazing crossbred Zebu cows. *Tanzania Veterinary Journal* Vol. 21, 43- 54.

Edited proceedings

1. Phiri, E.C.J.H., A.E. Pereka, M.N. Mgasa and T. Larsen (1997). Plasma calcium and inorganic phosphorus in grazing Dairy cattle at ASAS Dairy Farm, Iringa, Tanzania. *Tanzania Veterinary Journal* Vol. 17 (Supplementary 3), 44 – 49.

2. Phiri, E.C.J.H., A.E. Pereka, M.N. Mgasa and T. Larsen (1998). The effects of concentrate supplementation on plasma calcium and inorganic phosphate and other blood parameters in grazing dairy cattle at ASAS Dairy Farm, Iringa, Tanzania. *Tanzania Veterinary Journal* Vol. 18 (Supplementary 3), 105 – 110.

3. Phiri, E.C.J.H (1999). Effects of calcium, phosphorus and zinc supplementation on plasma calcium, inorganic phosphate and alkaline phosphatase in grazing cows, Iringa, Tanzania. *Proceedings of SUA- MU Enreca Project workshop on feed database*: 51 – 60.

4. Phiri, E.C.J.H., A.E. Pereka, and T. Larsen (2000).
Researcher- Farmer = Partnership in improving nutrition of grazing cattle: Iringa experience. *Proceedings of SUA- MU ENRECA Project on Farming System Research (FSR)* held on 1st August 2000, Dar es Salaam Tanzania, 28- 33.

5. Phiri, E.C.J.H., A.E. Pereka, M.N. Mgasa and T. Larsen (2000).
The effect of calcium, phosphorus and zinc supplementation on health performance of grazing dairy cattle. *TSAP Proceedings* Vol. 27, 35 - 50.

Conference/Workshop

1. Phiri, E.C.J.H., A.E. Pereka, M.N. Mgasa and T. Larsen (2000).
Calcium, phosphorus and zinc supplementation of grazing crossbred zebu cows in the dry season, Tanzania. A paper presented at the European Association of Animal Production (EAAP) Scientific conference 21- 24th August 2000, the Haag Holland.(In press).