An investigation into the effects of intravenous (IV) injection of tetracycline on mineral status and homeostasis in the bovine

by

Lebogang E. Motsei

Bsc Agric, Bsc Agric Hn, Msc Agric

A thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

In the Centre for Animal Health Studies,

Faculty of Agriculture, Science and Technology

North-West University

Supervisor: Prof. D. E. Beighle

Co-Supervisor: Prof F. R. Bakunzi

Date: April 2013

Declaration

I **Lebogang Ezra Motsei,** hereby declare that the work on which this thesis is based is original, and neither the whole nor any part of it has been, or is to be submitted for another degree at this or any other university.

_____

ACKNOWLEDGMENTS

The author wishes to thank the North West University's Faculty Research Committee (FAST), the Institutional Research Division and the International Mission Board, SBC, for providing the funding and manpower for this research. **Professor D. E. Beighle - Promoter** (Professor on loan from the International Mission Board to the Centre for Animal Health Studies in the Faculty of Agriculture, Science and Technology at the North West University (NWU) for his support throughout the research, his patience, understanding, and moral support during the study. **Prof F. R. Bakunzi and Prof B. M. Dzoma** for their encouragements and advice on some aspects of this research**, Dr B. G. Kgobe, Mr V. Mjekula and Nonyan**e for their biotechnical assistance and **Mr S. N. Thwane,** for his ultimate help in sample processing and chemical analysis. **Dr D. Snell** (Life and Analytical Science – Perkin Elmer SA) for mechanical and method application with the AAS 700S. My wife (**Mrs D. S. Motsei**), my lovely son and daughter (**K. Motsei and O. Motsei**) for giving me more free time in favour of this research work. My Parents **(Solly and Mmamoleleki)** for the pressure they instilled on me to complete this work. Lastly, to the Almighty **God**, without whom I would not had been able to complete this work.

ABSTRACT

Blood Ca was chelated through the IV injection with tetracycline to investigate the outcomes of the mineral homeostatic mechanism in calves. To achieve this, Friesian and Bonsmara calves of less than 36 months of age were blocked according to sex and breed and randomly assigned to two groups of treated and controlled groups. Bone minerals were within the normal range on treatment groups with P concentrations ranging from 92 mg P g^{-1} - 145.18 mg P g^{-1}, Ca from 191.93 mg Ca g^{-1} - 231.87 mg Ca g^{-1} and Mg from 3.35 - 4.35 mg Mg g^{-1} all on dry weight basis. Faecal minerals (P, Ca and Mg) were also in the normal range from 4.87 - 6.29 mg P g^{-1}, 8.07 - 12.54 mg Ca g^{-1}, and 1.36 - 2.88 mg Mg g^{-1} regardless of the sex or breed or treatment groups on a dry weight basis. Blood minerals (P, Ca and Mg) were within the normal range from 6.71 – 8.49 mg/100ml serum, 6.26 – 10.76 mg/100ml serum and 1.26 – 1.71 mg/100ml serum respectively. Friesian calves had significantly higher overall means for P, Ca, Mg and cortical bone thickness compared with Bonsmara calves. Male animals had significantly less bone P but had more bone Ca and Mg than female animals throughout the experiment. Injection with tetracycline resulted in significant changes in bone, faecal and blood levels of P, Ca and Mg which led to changes in bone thickness and Ca:P ratio. In response to the chelation of the blood Ca by tetracycline, the body pulled Ca and Mg out of the bone in the first 3 hours to increase blood Ca but within the next 3 h, replacement of Ca taken out of the bone was observed due to the body homeostatic mechanism. There is evidence from this research that animals are able to respond quickly to disturbances in mineral homeostatic mechanisms by absorbing minerals from the colon back into the blood or by secreting minerals into the colon from the blood. Further research needs to be done to investigate the ability of animals to regulate loss of minerals by way of absorption and excretion via the colon.

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

1. INTRODUCTION

Tetracycline (TC) is a broad-spectrum antimicrobial drug with a long history of use in humans and animals. TC is used primarily for the short-term oral treatment of clinical diseases (WHO, 2005). Tetracycline is known to chelate calcium in teeth and bone, become incorporated into these structures, inhibit calcification (e.g hypoplastic dental enamel), and cause yellowish, then brown discoloration. At extremely high concentrations, the healing process in fractured bones is impaired (Aiello and Moses, 2003). Tetracyclines are transported primarily as Ca^{2+} and Mg^{2+} chelates through blood plasma (Martin, 1985). Once chelated, they can act as ionophores, capable of transporting bound Ca^{2+} and Mg^{2+} through lipophilic phases such as cellular membranes, delivering the ions and tetracycline into intracellular compartments (White and Pearce, 1982). The ability to bind di-cations such as Ca^{2+} probably accounts for their inhibitory effect on bone growth and their ability to discolour growing teeth.

The total Ca and Mg body content of an adult 600 kg dairy cow are 7000 g and 240 g, respectively (Georgievskii *et al.*, 1982). More than 99% of the total body Ca and approximately 70% of the Mg in the animal body is present in the bone. The remaining Ca (1%, 70 g) is in other tissues involved in cellular metabolism, blood clotting, enzyme activation and neuromuscular action. The remaining 29% (70 g) of total Mg is intracellular, acting as a soft tissue cation (Georgievskii *et al.*, 1982).

Mg is an important co-factor of many enzymes and is essential for the normal function of nerve tissues (Georgievskii *et al.,* 1982, Underwood and Suttle, 1999). Extracellular Ca and Mg account for 0.1% (7 g) of total Ca and 1% (2.4 g) of total Mg. Normal plasma Ca and Mg concentrations for cattle range from 2.11 to 2.75 mmol/l and from 0,50 to 1.10 mmol/l, respectively (Radostits *et al.*, 1994). Approximately 50% of total plasma Ca and 70% of total plasma Mg are ionic form while the remainders are primarily bound to proteins or organic and inorganic anions (Agnes *et al.*, 1993, Riond *et al.*, 1995).

Intracellular total Ca and Mg concentrations are typically 0.0001 mmol/l and 4-8 mmol/l, respectively (Martens, 1993).

About half of the body magnesium is in the bone at a concentration of 0.5 – 0.7% of the bone ash. Mg in soft tissues is concentrated within cells. The highest concentration is in the liver and skeletal muscles. About 75% of blood Mg is in the red blood cells (6 meq/L) and 25% in serum (1.1 - 2.0 meq/L) (Pond *et al.*, 1995).

Mg is required for normal skeletal development as a constituent of bone and also required for oxidative phosphorelation by mitochondria of heart muscle and probably by mitochondria of other tissues. It is required for activation of enzymes that split and transfer phosphatases and the enzymes concerned in the reaction involving ATP (Pond *et al.*, 1995; Berne *et al.*, 1998).

The bulk of the calcium in the bodies of adult animals (99%) is found in the bone and teeth tissue in the form of hydroxyapatite crystals (Phillis,1976; Lloyd *et al.*, 1978; Georgievskii *et al.*, 1982; McDonald *et al.*, 1995) where approximately 360g Ca/kg, 170g P/kg and 10g magnesium/kg are found in bone ash (McDonald *et al.*, 1995). The total pool of intracellular free calcium is estimated to be only 0.2 mg. An additional 9 g of intracellular calcium is present in a bound form in the endoplasmic reticulum, the mitochondria, and the plasma membrane. This intracellular calcium constitutes an immediately accessible storage pool and also contributes to the structural integrity of the cell (Berne *et al.*, 1998).

The level of total Calcium (free and bound) in the plasma ranges from 9 – 11 mg Calcium per 100ml (Bondi, 1987), 8.6 to 10.6 mg/dl (2.1 to 2.65 mmol/L = 4.3 to 5.3 mEq/L) (Berne *et al.*, 1998), between 2.1 and 2.5 mmol/L (8.5 and 10 mg/dL) (Goff, 2008). These values might change from laboratory to laboratory and might vary by as much as 0.5 mg/dl and this might depend on the analytical methods employed. The skeleton contains 80% of the total P. Changes in the rates of bone accretion or resorption therefore results in changes in retention of both Ca and P. Resorption of the

skeleton during pregnancy and lactation occurs also as a result of a low P intake and skeletal reserves are therefore replenished when demands for P fall in late lactation. The skeletal system serves as an important source for calcium demand in order to preserve its concentration (Mosel *et al.*, 1994). Calcium enters and exits from an extracellular pool of 1000 mg, which is in equilibrium with a rapidly exchanging pool of several times that size. This pool probably represents the surface of recently or partially mineralised bone (Berne *et al.*, 1998).

Low-serum Ca or blood P values are generally indicative of reduced skeletal reserves, but normal or near normal values are not always reliable guides to the mineral status of the skeleton. Motsei and Beighle, (2006) reported that when blood P values were low, the skeleton was severely resorbed, but the converse relationship was not always true. Approximately 50% of calcium is irreversibly removed from the extracellular space by bone formation and the same amount is returned to it by bone resorption in the steady state process that constitutes normal bone remodeling (Berne *et al.*, 1998).

While revising a model of P kinetics proposed by Vitti *et al.* (2000), Dias *et al.* (2006) injected growing sheep aged 8 months with an average bodyweight of 31.6 kg with ^{32}P and ^{45}Ca to trace the movement of P and Ca in the body. Phosphorus and Ca metabolism were assessed conjointly using the revised model. The results showed a closely related P and Ca metabolism as evidenced by the ratio of these minerals in the bidirectional flows between plasma, bone and tissue (Dias *et al.*, 2006). Serum P is closely related to serum Ca because both make up the hydroxyapatite lattice structure of bone (Chan *et al.*, 2006). A blood Ca concentration below 5 mg/dL typically results in parturient paresis, the condition more commonly known as milk fever. As cows age, the Ca homeostasic mechanism reacts more slowly to the Ca demands of lactation (Horst *et al.*, 1994) Beighle *et al.,* (1989) found that in calves subjected to a low level of P in the diet, those on the anionic diets had higher levels of P in the blood, and lower levels of P in the bones than those on the cationic diets. Feeding anionic diets for 21 days pre-partum was effective in preventing hypocalcaemia by inducing a mild metabolic acidosis (Chan *et al.*, 2006) as serum Ca never declined below 8 mg/dL

throughout the study of the treated groups. According to Beighle *et al.*, (1997), blood P was observed to fluctuate more than bone P or faecal P when concentrations of P in the blood, bone and faeces were compared in animals offered an anionic diet. Jackson *et al.*, (1992) stated that blood pH increased linearly with increasing dietary cation-anion balance.

Important to the regulation of circulating concentrations of Ca^{2+} is the net flow of Ca from the large pool within the skeleton. The balance between Ca accretion and resorption can be set to mobilise around one-fifth of Ca from the skeleton during late pregnancy and lactation. When Ca intake is between 10 and 15 g/d, the cow is totally dependent on skeletal Ca reserves. When Ca intake is above 50-60 g/d, the major part of Ca comes from the diet with only a minor part derived from the bone (Ramberg *et al.*, 1984).

Available literature has revealed different studies carried out by scientists in evaluating diets formulated to have similar, less or excess standard recommendations for P, either supplemented with anionic or cationic agents, infused hypocalcemia through standardised EDTA infusion, voluntary blood Ca chelation by the use of tetracycline (this research). All these studies have been conducted to improve the production of farm animals either by increased reproductive efficiency, increased milk and meat production and prevention of diseases.

1.1 Aims and objectives

1.1.1. Aims of the study:

- To assess the mineral response in the bovine to intravenous injection of tetracycline as a voluntary calcium chelating agent in the blood,
- To investigate the interaction of blood, bone and faecal minerals in response to voluntary hypocalcaemia caused by Ca chelation after intravenous administration of tetracycline.

1.1.2. Research objectives

- To measure the change in blood, bone and faecal Ca as a result of the intravenous injection of tetracycline,
- To measure the change in blood, bone and faecal P as a result of the intravenous injection of tetracycline,
- To measure the change in blood, bone and faecal Mg as a result of the intravenous injection of tetracycline, and
- To compare interactions in bone, blood and faecal Ca, P and Mg, bone Ca:P ratio, bone thickness over time after intravenous injection of tetracycline.

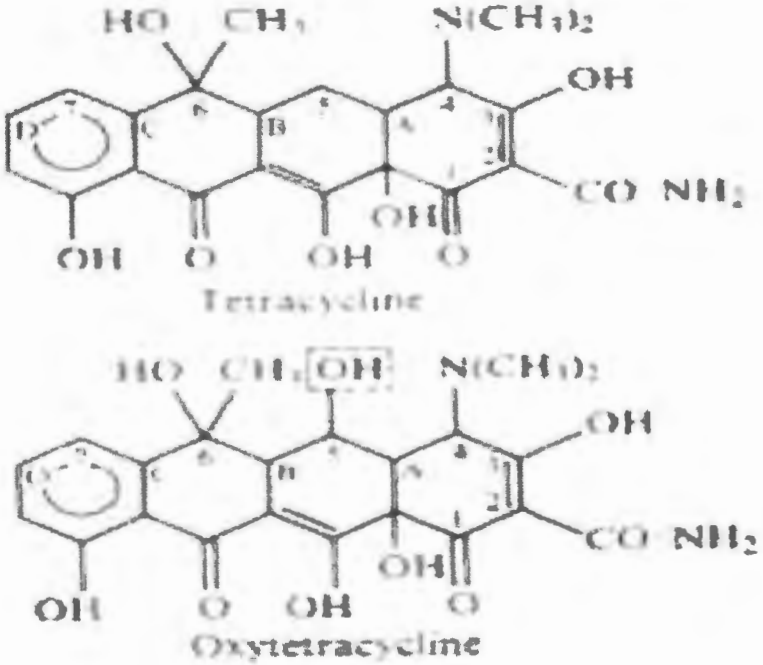
1.1.3. Research problem

Available literature concentrates mainly on the effect of Tetracycline as an antibiotic in animals. Little has been done to investigate the effect of Ca chelation by tetracycline. Not much has also been said in the literature concerning the interactions of Ca, P and Mg relative to induced Ca chelation by tetracyclines. The problem which this research sought to address was: What effect does the chelation of Ca by tetracyclines have on plasma, bone and faecal P, Ca and Mg homeostasis and their interactions in Bonsmara and Friesian Calves?

CHAPTER TWO
LITERATURE REVIEW

2.1. Tetracyclines: Overview

Tetracycline, an antibiotic obtained from *streptomyces*, was first discovered in 1948 as the fermentation product of an unusual golden-coloured soil bacterium aptly named *Streptomyces aureofacians*. Oxytetracycline (OTC) belongs to the tetracycline family of antibiotics and has been used widely to treat infectious diseases in both humans and farm animals. Below is the chemical structure of OTC (WHO, 2005).



The chemical structure of oxytetracycline

The tetracyclines (TCs) as a group was previously evaluated at the twelfth meeting of the committee of International programme on Chemical Safety in 1998, when a temporary ADI of 0 – 0.15 mg/kg body weight was established. Oxytetracyclines were re-evaluated at the thirty-sixth meeting of the committee of International programme on Chemical Safety when an ADI of 0 – 3 μg/kg body weight was established, based on

the human gut flora. Additional data have become available on Clortetracycline (CTC) and TC since then (WHO, 2005).

2.1.1. General properties

2.1.1.1. Molecular formula (USP Dictionary of USAN, 2002)

Chlortetracycline Hydrochloride= $C_{22}H_{23}C_1N_2O_{83}$.HCL
Oxytetracycline Hydrochloride = $C_{22}H_{24}N_2O_9$.HCL
Oxytetracycline = $C_{22}H_{24}N_2O_9.2H_2O$
Tetracycline = $C_{22}H_{24}N_2O_8$

2.1.1.2. Molecular weight (USP Dictionary of USAN, 2002)

Chlortetracycline Hydrochloride= 515.34
Oxytetracycline Hydrochloride = 496.89
Oxytetracycline = 496.46
Tetracycline = 444.43

2.1.2. Pharmacokinetic features

Unless otherwise noted, phamacokinetic values are based on a single intravenous dose of medication.

2.1.2.1. Mechanism of action / effect:

Tetracyclines are broad-spectrum bacteriostatic agents that inhibit protein synthesis by binding reversibly to receptors of the 30S ribosomal subunit of susceptible micro-organisms. Uptake appears to depend on passive diffusion and active transport (Barragry, 1994).

2.1.2.2. Absorption

Studies in humans have shown that the absorption of oxytetracycline or tetracycline is decreased when it is administered with food; the effect of food on doxycycline absorption is however insignificant. Doxycycline is also less likely than the older tetracyclines to form chelation complexes with divalent and trivalent metals and, therefore, there is less interference with oral absorption by calcium or other substances (Riviere *et al.*, 1999). The absorption of the long-acting formulations of oxytetracycline (with 2-pyrrolidone excepient) administered intramuscularly, has been described as having a rapid phase of 48 minutes for 14% of the dose and a slow phase of 18 hours for 38% of the dose in cattle administered a 20 mg/kg dose (Toutain and Raynaud, 1983). With a 10 mg/kg dose, the rapid phase is 16 minutes and the slow phase is 11 hours (Schifferli *et al.*, 1982).

The elimination or half-life of OTC is said to be 11.2 hours for the new born, 3.5 to 7.7 hours and 6.3 hours for an 8 months old animal (Burrows *et al.*, 1987; Schifferli *et al.*, 1982). The time taken by the body to reach a higher serum concentration for an intramuscular conventional formulation of OTC was found to be 6 hours with a serum concentration of 5.5 ± 1.25 mcg/mL using a dose of 18 mg/kg in the neck of calves that are 14 weeks old, 6.7 hours to higher serum concentration of 5.7 ± 2.39 mcg/mL (dose of 8 mg/kg in the neck) for cows (Nouws *et al.*, 1983). Time to higher serum concentration for pigs was found to be 1.5 hours with a concentration of 6.7 ± 3.4 using a dose of 20 mg/kg in the hind quarter (Hall *et al.*, 1989).

The time taken by the body to reach a higher serum concentration for a long-acting formulation of oxytetracycline was found to be 1-5 hours with a serum concentration of 4 mcg/mL (dose of 20 mg/kg in the gluteal muscle) for non-ruminating calves that were 5 weeks of age (Toutain and Raynaud, 1983). Non-ruminating calves 6 weeks of age, around 4.01 ± 2.84 hours to a higher serum concentration of 3.01 ± 0.72 mcg/mL (dose of 10mg/kg in the hind quarter (Schifferli *et al.*, 1982). In ruminating calves, the time taken to higher serum concentration was found to be 7.6 ± 4 hours at a concentration of

9.6 ± 2.6 mcg/mL using a dose of 40 mg/kg in the hindquarter (Terhune and Upson, 1989) and cows at 5 – 10 hours to reach a higher serum concentration of 4.5 – 6.8 mcg/mL using a dose of 10 mg/kg in the neck (Mevius *et al.*, 1986). The time taken to reach a higher tetracycline level in serum of pigs was found to be 0.5 hours at a concentration of 6 ± 2.2 mcg/mL using a dose of 20 mg/kg in the hind quarters (Hall *et al.*, 1989) and steers were found to be around 8 hours to a reach a peak serum concentration of 3.13 mcg/mL using a dose of 20 mg/kg in the hindquarters (Davey *et al.*, 1985).

2.1.3. Special clinical concerns: side effects and toxicity

High doses administered per os to ruminants seriously disrupt microfloral activity in the ruminoreticulum, eventually producing stasis. Elimination of the gut flora in monogastric animals reduces the synthesis and ability of the B vitamins and vitamin K to be absorbed from the large intestine. With prolonged therapy, vitamin supplementation is a useful precaution.

Tetracyclines chelate calcium in the teeth and bones. They become incorporated into these structures, inhibiting calcification and causing yellowish then brownish discoloration. At extremely high concentrations, the healing processes in fractured bones are impaired (Kahn and Line, 2005). Rapid IV injection of tetracycline can produce hypotension and sudden collapse. This appears to be related to the ability of tetracyclines to chelate ionised calcium, although a depressant effect by the propelene glycol carrier itself may also be involved (Kahn and Line, 2005).

2.1.4. Metal chelating properties of tetracyclines

Ca^{2+} and Mg^{2+} complexations of tetracyclines have been studied extensively, using a variety of spectroscopic techniques which have confirmed the powerful metal chelation properties of tetracyclines. The binding constants and spectroscopic studies of tetracyclines with metal show that they bind with extremely high affinity to the lower

peripheral region of the tetracycline molecule (Newman and Frank, 1976). Other studies show that the upper C4 dimethylamino group is also implicated in chelation (Lambs *et al.*, 1988). There are probably two theories that have emerged from studies concerning the location of the calcium-binding areas in the tetracycline molecule. The first theory postulates that chelation occurs within the lower peripheral region, where binding occurs between the O12 and O1 oxygen groups in a 2:1 ratio of Ca^{2+} to tetracycline (Newman and Frank, 1976). The second theory points to the lower peripheral region binding site, plus additional binding occurring between the O12a hydroxyl group and the C4 dimethyl-amino group (Lambs *et al.*, 1988).

Stability constants of cations for tetracycline in water refer to chelation at O11-O12 with N4 still protonated (Martin, 1985). Other studies with increasing Ca^{2+} concentrations showed similar spectra to 4-dedimethylamino tetracycline under the same condition (Newman and Frank, 1976). Both studies suggest that binding occurs primarily along the lower peripheral region. When the lower periphery forms ligands with Ca^{2+}, only the positions O10-O11, O11-O12, and O12-O1 are available. The probability that two Ca^{2+} ions would chelate directly next to each other along the lower periphery is negligible, due to the steric and electronic interactions that would develop. Therefore, the binding sites of Ca^{2+} can be narrowed down to those of the O10 and O11 oxygens of the DC rings and the O12-O1 sites on the BA rings. Calcium binding occurs in the sequential order, chelating to the DC ring site first, followed by binding at the BA ring site (Newman and Frank, 1976).

Tetracyclines are also said to display even higher affinity to other metals and transition metals. In bacterial resistance antiport protein systems, metal chelates of tetracycline with Mg^{2+} are required for transportation of tetracycline in the cell membranes of bacteria. Other cations also afford complexation with transport proteins, with Co^{2+} and Mn^{2+} ions displaying higher affinity than either Ca^{2+} or Mg^{2+}. The order of reactivity of metal ions with tetracyclines is in order of decreasing affinity (Martin, 1985).

Magnesium binds in a 1:1 ratio with tetracyclines, where the chelation site is believed to be primarily between the O11 carbonyl group and the O12 enol functional group (Newman and Frank, 1976). Tetracyclines can also form highly fluorescent metal complexes with transition metals such as molybdenum and zinc, where they induce complexation and intense fluorescence for the detection of trace amounts of tetracycline additives in foodstuffs (Speer *et al.*, 1984).

2.1.5. General properties

All the tetracycline derivatives are crystalline, yellowish, amphoteric substances that, form salts with both acids and bases in aqueous solution. The most common salt form is the hydrochloride, except for doxycycline, which is available as doxycycline hyclate. The tetracyclines are stable as dry powders but not in aqueous solution, particularly at higher pH ranges (7-8.5). Preparations for parental administration need to be carefully formulated, often in propylene glycol or polyvalent cations, particularly calcium, magnesium, aluminium and iron (WHO, 2005).

2.2. Calcium and Phosphorus regulating hormones

2.2.1. Parathyroid Hormone (PTH)

In the absence of inflammation, parathyroid hormone and 1,25-dihydroxyvitamin D ($1,25(OH)_2D_3$) are generally conservatory for the extracellular pool and are responsible for increasing intestinal absorption and renal reabsorption of Ca and bone resorption of Ca and P. However, it also increases renal Phosphate excretion and significantly increases salivary phosphate secretion (Overton and Waldron, 2004). Parathyroid hormone related proteins may also be important for the secretion of Ca, Mg and P into the milk of lactating animals (Thiede, 1994). Serum $1.25(OH)_2D_3$ levels are correlated inversely with the serum P and directly with Ca^{2+}. Using stepwise linear regression, the correlation between P and $1.25(OH)_2D_3$ accounted for the majority of the variance contributed by both P and Ca^{2+} (Bushinsky *et al.*, 1989). Increased Ca^{2+} induced by chronic metabolic acidosis does not inhibit the rise in serum $1.25(OH)_2D_3$ during low P

diet feeding of 0.1% P, indicating that during P restriction, Ca^{2+} does not inhibit production of $1.25(OH)_2D_3$. Thus, the mechanism whereby PTH and P restriction stimulate $1.25(OH)_2D_3$ production differ; increased Ca^{2+} blocks the PTH-mediated rise in $1.25(OH)_2D_3$ but does not alter the $1.25(OH)_2D_3$ response to P restriction (Bushinsky *et al.*, 1989).

2.2.2. Calcitonin

Calcitonin is secreted by the thyroid gland in response to elevated serum Ca (Berne *et al.*, 1998) and results in increased bone mineral deposition (Sjaadstad *et al.*, 2003), decreased intestinal absorption and increased urinary Ca excretion. The hormone acts to reduce the concentration of Ca^{2+} in the extracellular fluid by inhibiting bone resorption and increasing urinary excretion of calcium (Berne *et al.*, 1998; Sjaastad *et al.*, 2003). Calcitonin also prevents hypocalcaemia. The primary target cells for calcitonin are the osteoclasts, where the hormone is bound to G-protein-coupled membrane – receptors. Calcitonin reduces the rate of bone resorption by reducing the surface population of active osteoclast on the bone surface. The cells shrink and detach from the bone surface (Sjaastad *et al.*, 2003).

2.2.3. Calcitriol

The cells of the proximal tubules of the kidneys add another hydroxyl-group to 25(OH)-Vitamin D through the means of an enzyme called 1-hydroxylase enzyme. It is stimulated when the plasma concentrations of phosphate and calcium fall below the normal levels and inhibited by hypocalcaemia and hyperphosphatemia. This reaction produces $1,25(OH)_2$-vitamin D_3 which is biologically active and considered to be a hormone (Sjaastad *et al.*, 2003). Calcitriol exerts its action by binding to intracellular receptors in cells that transport calcium, immune system, muscle, endocrine tissues and in bones.

2.2.4. Vitamin D

Bone mineralization is stimulated by vitamin D through its regulation of serum mineral concentrations and its direct action on osteoblasts. Therefore, impaired action of vitamin D due to either dietary vitamin D deficiency or hereditary disease results in rickets with hypocalcaemia, hypophosphatemia and impaired bone mineralization (Masuyama *et al.*, 2001; Sjaastad *et al.*, 2003). Vitamin D3 or vitamin D2 supplied by the diet is transported by a vitamin D-binding protein to the liver. The liver functions as a storehouse of vitamin D, typically preventing the circulation of high levels of vitamin D in the blood stream; the basal concentration of vitamin D in the blood of dairy cattle is 1-3 ng/ml of plasma (Horst and Littledike, 1982). Vitamin D is processed into an intermediate, 25-OH D, which is not the active form of the vitamin but is kept in the liver until it is needed (Reichel *et al.*, 1989). Plasma 25-OH D has been reported to be the best indicator of the vitamin D status of animals (Horst *et al.*, 1994).

2.3. Homeostasis

2.3.1. Phatho- Physiology of Ca-P-Mg metabolism and bone

2.3.1.1. Physiology of calcium in the body

Ionised Ca is involved in a variety of physiological processes, including muscular contraction, blood coagulation, enzyme activity, neural exitability, hormone secretion, and cell adhesion (Sjaastad *et al.*, 2003). Endocrine factors influence the level of calcium in the body. These factors include the parathyroid hormone (PTH) synthesised and released by the chief cells of the parathyroid gland, calcitonin secreted by the parafollicular or C cells of the thyroid gland and calcitriol (1,25-dihydroxychole-calciferol; 1,25-dihydroxyvitamin D), the bioactive vitamin D metabolite derived from cholecalciferol (vitamin D3) (Capen, 2004; Overton and Waldron, 2004).

2.3.1.2. Absorption of calcium

The upper small intestine appears to be the major site for Ca absorption whereas the reticulo-rumen is the main site for Mg absorption (Remond *et al.*, 1996). Ca along with other products of digestion enters the luminal fluid from the chyme as a result of mechanical and enzymatic action. It then crosses the intestinal membrane and cell junctions and enters the lymph and blood. Ca is also absorbed from the intestine by an active transport mechanism which takes place against anionic and concentration gradient (Geogievskii *et al.*, 1982). The transport is specific to Ca and a Ca-binding protein facilitates it. Vitamin D can increase the absorption of Ca by increasing the synthesis of Ca-binding protein (CaBP) in intestinal cells. CaBP transports Ca through the intestinal cell. Ca enters the extracellular fluid in an exchange mechanism involving sodium (Na) (Capen and Rosol, 1989).

2.3.1.3. Absorption of magnesium

Although there are many contradictory data in the literature about the main site of magnesium absorption from the digestive tract, the opinion prevails that the large intestine in monogastric animals and forestomachs in ruminants are the main sites of its absorption (Rahnema *et al.*, 1994). While non-ruminants absorb Mg primarily from the small intestine, ruminants are able to absorb much of their Mg requirement from the rumen. In fact, the reticulum and rumen can account for up to 80% of the Mg absorption along the entire digestive tract (Remond *et al*, 1996).

The mucous membrane of the swine caecum and the proximal colon is permeable for the transportation of magnesium in the absorption direction, which is from the epithelial towards the submucosal side of the mucous membrane. The absorption rate of magnesium depends on the kind of mucous membrane used and on the concentration of magnesium contained therein (Milinkovic-Tur *et al.*, 2000). Mg transport across the apical membrane of the ruminal epithelium occurs by two active transport processes against an electrochemical gradient (Leonhard *et al.,* 1989). The process is ruminal K

concentration dependent so that an increase in K intake reduces Mg absorption (Leonhard-Marek and Martens, 1996).

2.3.1.4. Phosphorus absorption, secretion and exchange in the body

Absorption of dietary phosphate is approximately 60 – 70% and occurs by active transport using a sodium-phosphate co-transporter and by passive diffusion. In ruminants, the transporter may be coupled to H+ rather than Na+ (Shirazi-Beechey *et al.*, 1996 from Capen, 2004). Absorption of phosphate from the intestine increases when the concentration of phosphate in the plasma falls and is stimulated by a hormone called calcitriol (Underwood and Suttle, 1999). Serum P is closely related to serum Ca because both of them make up the hydroxyapatite lattice structure of bone (Chan *et al.*, 2006). Calcium and phosphorus are lost from the body by excretion into the intestine and in the urine (Underwood and Shuttle, 1999). The rate of excretion of endogenous faecal P is directly related to P intake and rate of absorption, and is inversely related to the rate of Ca absorption. It has been suggested that this excretion may also be related to the plasma inorganic P concentrations and that it plays an important role in P homeostasis. 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 Ca. Most of the P excretion occurs through the kidneys and renal excretion appears to be the main regulator of blood P concentration (Pond *et al.*, 1995).

2.3.1.5. Site of P absorption

Excess P may interfere with feed digestibility, metabolism at the tissue level, or unknown effects (Carstairs *et al.*, 1980). The concentration of soluble P in digesta decreases until the 7 metre site in the small intestine and then remains stable. Carsrtairs et al., (1980) found a very low concentration of soluble P in the faeces. The upper small intestine (1-3 m from the pylorus) appears to be the major side of calcium and phosphorus absorption. A large net secretion of P occurs between the mouth and

the duodenum. Net absorption of P occurs along the section of the intestine between 0.05 and 15m from the pylorus, although the most active site for P absorption is the upper small intestine. Absorption of P from the GI tract occurs by active transport and passive diffusion. Vitamin D apparently has an effect on P absorption. *In vitro* work has shown that P may traverse the intestinal cell membrane against a concentration gradient in the presence of Ca and requires Na (Perk and Hill, 2008). It has also been shown that P absorption is related to dietary P concentration. An excess of dietary P in relation to Ca depresses Ca absorption (Pond *et al.,* 1995). Vitti *et al.,* (2000) demonstrated through a radioisotope study that P absorption from the GIT is rapid and much of labeled P is incorporated into phospholipids in the intestinal mucosal cells.

2.3.2. Bone Homeostasis

A combination of a mineral balance and a radioisotope technique was used by Braithwaite (1980) to study the relationship between the dose rate of 1-∝-hydoxycholecalciferol (1-∝-OH-D3), the magnitude and the duration of its effect on the various processes of Ca and P metabolism in adult weather sheep. The rate of absorption and retention of P were increased by treatment and maximum responses occurred at the lowest dose rate (Braithwaite, 1980). He further found out that although the loss of endogenous P in the faeces was unaltered by treatment, the secretion of P into the gut was increased, and the increase was directly related to increased serum inorganic P concentration.

Beighle (1999) reported an increase in cortical bone P values and bone thickness with increase in milk production of up to 20 kg/day. However, the cortical bone mineral values in animals producing over 20 kg of milk per day were greater and cortical bone thickness was lower compared to those of animals producing less than 20 kg.

2.3.2.1. Regulation of calcium and magnesium homeostasis

Cattle can absorb Ca from the gut according to their needs. They are able to alter the absorption efficiency to meet changes in Ca requirement. For example, when cattle consume more Ca than needed, the proportion of Ca absorbed is decreased (Horst 1986, Reinhardt *et al.*, 1988). The decrease in Ca intake stimulates the secretion of parathyroid hormone (PTH) from the parathyroid gland. PTH enhances renal reabsorption of Ca (Capen and Rosol, 1989) and promotes the synthesis of 1,25-dihydroxycholecalciferol (1,25-(OH)2D) from 25-hydroxycholecalciferol in the kidney (Allen and Sansom, 1985). As a result of stimulated 1,25-(OH)2D and PTH secretion, bone Ca resorption and intestinal Ca absorption increase (Horst 1986; Horst *et al.*, 1994). The action of PTH hormone is counteracted by calcitonin (CT) which is secreted by thyroid C cells. CT replenishes the body stores of bone Ca at the times of Ca adequacy. CT decreases the concentration of Ca in blood plasma by reducing the rate of bone resorption (Allen and Sansom, 1985; Sjaastad *et al.*, 2003).

Woven bone is the temporary tissue that is formed rapidly and has a higher Ca content than compact bone. It acts as a readily mobilisable reserve of Ca during late pregnancy and lactation. In the bone, Ca is available in the fluid surrounding bone cells and within bone canaliculi. Soluble Ca in the bone fluids is separated from the extracellular fluids of the body by a syncytium of bone lining cells (Wasserman, 2004).

Mg homeostasis is controlled by intestinal absorption and renal excretion of Mg. There is no primary hormonal regulation of blood Mg. In contrast to Ca metabolism, the skeleton is largely inert with respect to Mg mobilization. The regulation of blood Mg during hypomagnesaemia is critically dependent on daily Mg intake (Capen and Rosol, 1989). A linear relationship between Mg intake and plasma Mg even above requirements (Chester-Jones *et al.*, 1990), confirms that Mg metabolism is less rigorously controlled than Ca.

2.3.2.2. Bone resorption

Beighle (1999) found no statistical significances (P>0.05) in cortical bone P concentrations in rib bone during the lactation period, but calcium concentrations in cortical bone were higher at parturition and during the first 30 days of lactation compared to the next 30 days and between 90 and 120 days. This shows that the cow resorps cortical bone during the middle of the lactation period and not during the peri-parturient period as previously believed (Beighle, 1999).

When blood P values were low the skeleton was severely resorbed, but the converse relationship was not always true (Motsei and Beighle, 2006). Resorption of the skeletons of ewes fed a low P ration was much greater than resorption of the skeletons of ewes fed a low-calcium ration. The quantity of bone resorbed depends on the amounts of Ca and P required for maintenance and milk formation, the amount of Ca or P released per unit weight of bone resorbed, the daily intake of Ca and P and the availabilities of these elements (Georgievskii, 1982). Bones serve as a store of Ca and P which can be mobilised when the provision of these minerals is not sufficient to meet the body requirements. The rate of exchange is rapid in spongy zones of the bones (trabecular bone) than in the compact bone (cortex) (Bondi, 1987).

2.3.2.3. Increased bone mobilization

About 70% of the phosphate ingested is absorbed by the intestine and remains relatively constant. The relationship between intake and absorption of phosphate from the intestine is more linear than that of calcium. Of the daily filtered load of 6000 mg, the renal tubules can reabsorb from 70 – 100%, with an average of 90% (Berne *et. al.*, 1998).

2.3.2.4. Experiments done on phosphorus homeostasis

Metabolic alkalosis is largely the result of a diet that supplies more cations (K, Na, Ca, and Mg) than those providing anions (Cl, SO_4, and PO_4) to the blood. If a greater number of positively charged cations enter the blood than negatively charged anions this will results in a disparity in electrical charges. Positively charged hydrogen ions have to be lost, therefore, leading to an increase in the pH of the blood (Goff, 2000).

In evaluating the short-term effects of dietary cation:anion balance on bone mineral homeostasis in the bovine given diets containing 0.25% P or less and a dietary cation-anion balance of either -11.1, +16.5 or +25.6 mmol 100 g^{-1} diet dry matter, Beighle *et al.*, (1995) found the bone, faecal and blood P concentrations being significantly higher in the anionic treatment group as compared to cationic and control group, the later being only significant as compared to the cationic group. Measures of acid-base status of the cows were linearly related to DCAD, but the animals did not experience metabolic acid stress. Neither faecal nor urinary P was affected by DCAD, and there was no change in overall P balance. Beighle *et al.,* (1997) further showed that bone P responded more positively to dietary anions than Ca, with an increase in bone P and a decrease in bone Ca. Prolonged low levels of the dietary P which cause P deficiency may be better detected from the measurement of bone P since the bone P content significantly reflects a variation in P content of pasture. Plasma P tended to increase and blood concentrations of ionised Ca were enhanced as DCAD decreased (Borucki Castro *et al.*, 2004).

Beighle (2000) continued to use acidogenic agents to prove the effectiveness of NH_4Cl of a dicalcium phosphate lick (A+) compared to a lick of 5 parts dicalcium phosphate and 3 parts salt (A-) at different percentages; blood and faecal P were significantly ($P<0.05$) higher in the A+ than in the A- animals at different experimental stages but bone P did not increase.

In 2004, Goff *et al.,* used blood and urine pH to monitor the effect on acid-base balance. The experiments were conducted to test the relative acidifying activities of various sulfate and chloride anion sources in nonpregnant, nonlactating jersey cows. Chloride proved to have approximately 1.6 times the acidifying activity of sulfate, whereas calcium and magnesium had a small but significant alkalinizing effect when accompanying chloride or sulfate.

Motsei and Beighle (2006) found bone P to be significantly higher on the animals given a lick rich in ammonium sulphate, compared to the control diet showing a beneficial effect of acidogenic agent in improving the bone P and general mineral status by significantly increasing the thickness of cortical bone and percentage ash.

2.3.3. Plasma responses

Beighle *et al.,* (1994) stated that P content of blood is not always a good indicator of the P status of the animal. The rate of bone remodeling (resorption and mineralization) is important in determining the concentration of serum P in the extracellular fluid pool.

The results of Block's (1984) trial indicates that the blood of cows responds to anion-cation balanced diets as shown by the response of plasma hydroxyproline (OHPRO) by cows offered anionic diets and lack of response by cows offered cationic diets. It appears that the anion (acidiogenic) diet allowed for easier bone mobilization during Ca stress even though the diets contained a high ratio of Ca:P (Block, 1984; Motsei and Beighle, 2006). Beighle (1999) found that P was removed from the bone as a result of the anionic excess and resulted in higher concentration of serum P and faecal P due to the anionic diets compared to cationic diets.

Block (1984) suggested that in addition to intestinal effects of a diet balanced for excess anions, there must be a systemic response of cows to this diet. He further stated that this systemic response may be as simple as a slight decrease of blood PH

and as complex as affecting liver and kidney function, which subsequently affects vitamin D metabolism. Because the kidney plays a major role in blood acid-base balance and in regulating blood ionic composition, these suggested effects of dietary ions are worth investigating (Block, 1984).

Plasma Ca and P concentrations were lower for cows offered the cationic diets at and around parturition. Cows offered the cationic diets that became paretic, had a more dramatic drop of in plasma Ca and P at calving than cows offered the cationic diet that were not paretic (Block, 1984).

Anionic diets were responsible for higher average daily gains, and higher concentration of serum P when compared to cationic diets. This was accomplished by the results of the experiment reported by Beighle *et al.*, (1989) whereby, P was removed from the bone as a result of anion excess and resulted in higher concentrations of serum P and faecal P due to the anionic diets a compared to the cationic diets.

Legradi *et al.*, (1997) reported that prolonged fasting is also associated with a number of changes in the thyroid axis manifested by low serum. He further said that fasted animals showed significant reduction in total plasma levels compared with the controls. Kiyma *et al.* (2004) reported that feed restriction may have altered the metabolic clearance of progesterone because fasting also increased serum concentrations of progesterone in ovariectomised ewes provided with an exogenous source of the hormone. Feed restriction did not affect overall or serum concentrations of growth hormone (GH) during fasting. They concluded that acute nutrient restriction (i.e. fasting) during the luteal phase of the oestrus cycle evoked endocrine changes that influenced the timing of the ensuing LH surge and presumably ovulation (Kiyma *et al.*, 2004).

2.3.4. Faecal responses

A greater total P excreted in faeces than intake leads to negative P retention (Dias *et al.*, 2006). Recent data from P feeding trials have demonstrated that the P concentration in dairy cattle faeces is directly affected by P levels in diets and that farm P surpluses as well as potential environmental losses can be reduced through dietary manipulation (Chapuis-Lardy *et al.*, 2004). Increasing dietary P resulted in increased faecal and urinary P excretion by cows in early lactation and did not consistently improve P retention (Knowlton and Herbein, 2002). A simple measure of inorganic P in a single water extract from the faeces is highly responsive to changes in the diet P concentrations and hence can be indicative of dietary P status (Dou *et al.*, 2002).

2.4. Sources and nutritional requirements of interest

2.4.1. Water

Water is absorbed by passive diffusion and depends on solutes, usually Na^+ absorption. Na^+ participates in absorption of bile salts, amino acids monosaccharides and pyrimidines (Beitz and Allen, 1984).

2.4.2. Minerals

2.4.2.1. Phosphorus (P)

The P status of forages differs widely and is influenced by the climate, soil and plant maturity (Underwood and Shuttle, 1999). Temperate forages generally contains more P than tropical forages (3.5 vs. 2.3gP kg^{-1} DM) and legumes slightly more than grasses (3.2 vs. 2.7gP kg^{-1} DM) (Minson, 1990). Phosphorus occurs abundantly and its approximate distribution in grains is as follows: soluble and insoluble phytates 50 – 80%, phospholipids, phosphoproteins, nucleic acids 20 – 30% and mineral phosphates 8 – 12%. The average concentration of phosphorus in grain is 3.5 – 4.5g/kg dry matter, 2.5 – 3.0g/kg dry matter in pasture plants and increases with plant fertilization and with

phosphorus fertilizers but decreases in late vegetative phases (Georgievskii *et al.*, 1982; Underwood and Suttle, 1999).

Phosphorus (P) occurs in the body as phosphate ions and as phosphate in the bone mineral. Phosphate groups are also present as activators or components of the structures of proteins, neucleotides and nucleic acids as well as organic components that play a major role in the intermediary metabolism of animals (Underwood and Suttle, 1999).

The bodies of adult animals contain 0.60 – 0.75% P calculated on fresh tissue basis, 1.9 – 2.5% calculated on dry tissue basis and 16 – 17% calculated on ash basis. The average weights of P contained in a 600kg cow, 100kg sow, 50kg sheep, 20kg dog and 2kg hen are 3600, 460, 280, 135, and 13g respectively (Georgievskii *et al.*, 1982). Lloyd *et al.*, (1978) outlined that calcium and phosphorus together account for over 70% of the total ash in the body and that 99% of the calcium, and about 80% of the phosphorus found in the body are located in the bones and teeth (Pond *et al.*, 1995).

The Ca:P ratio in animals depend on the degree of their physiological maturity at birth. The Ca:P ratio has a close-to-optimal value (1.7-1.8:1) in new born calves. In chicks, it attains this level only after 90 days and in rabbits even later (Georgievskii *et al.*, 1982). However, other researchers considerd 1:1 to 2:1 to be optimal values although there is evidence which suggests that ruminants can tolerate rather higher ratios provided the phosphorus requirements are met (McDonald *et al.*, 1995). Phosphorus in the bodies of adult animals is in the bone tissue in the form of hydroxylappetite and amounts to 83% (Georgievskii *et al.*, 1982).

Phosphorus enters the animals as mono-, di- and tri-substituted inorganic phosphates, and also as organic compounds – phytates, phospholipids e.t.c. The acid gastric juices dissolve the soluble and also some of the insoluble phosphates and split the phosphoric acid from the organic compounds in the presence of digestive juice phosphatases (Georgievskii *et al.*, 1982).

Whole blood contains 35 – 45 mg phosphorus per ml as orthophosphate, most of which is in the cells. The levels of plasma inorganic phosphorus are between 4 – 9 mg per 100 ml (Motsei and Beighle, 2006) where much of the plasma phosphate is ionised, but a small amount is complexed with proteins, lipids and carbohydrates (Bondi, 1987). According to Berne *et al.* (1998), the normal concentration of phosphate in the plasma was 2.5 to 4.5 mg/dl (0.81 - 1.45 mmol/L).

Serum phosphate is also measured as orthophosphate, since the organic forms are not routinely evaluated. Although inorganic phosphate is measured, it is often expressed as elemental phosphorus (P_i). Serum P_i ranges from 2.5 – 6.0 mg/dL (0.8 – 1.9 mmol/L) in adult animals (Capen, 2004).

Soft tissue stores of phosphate undergo rapid transfer with the extracellular fluid pool of phosphate. This transfer is an important factor in the regulation of plasma phosphate concentrations. About 250 mg, or half the total extracellular fluid pool of 500 mg, enters and leaves the bone mass daily in the process of bone remodelling (Berne *et. al.*, 1998).

2.4.2.2. Calcium (Ca)

Calcium is a constant component of animals and vegetables and is found in vegetable feeds (water-soluble, acid soluble and adsorbed fractions). The water soluble fraction has the greatest mobility while the acid-soluble is the least mobile (Georgievskii *et al.*, 1982). Calcium occurs in the water soluble fractions as organic acids and partly as calcium proprionate and a small proportion of the total calcium is bound to lipids (Georgievskii *et al.*, 1982).

Of the daily intake of 1000 mg of calcium, about 350 mg is absorbed, 150 mg is secreted back into the intestine and excreted in the stools along with 300 mg of unabsorbed fractions from the diet. The remaining 200 mg is excreted in the urine that

is filtered and provides a sensitive means of maintaining calcium balance (Berne *et al.*, 1998).

The content of calcium in the bodies of adult animals is 1.2 – 1.5 % on a fresh tissue weight basis, 3.5 – 4.0% calculated on dry tissue basis and 26 – 30% calculated on the ash weight basis (Georgievskii *et al.*, 1982). The average total calcium contents in the bodies of adult animals are : 600kg cow - 7000g, 100kg pig - 750g, 50kg sheep - 550g, a 20kg dog – 240g and a 2kg hen – 22g (Georgievskii *et al.*, 1982).

Approximately 50% of the plasma calcium is in the biologically active form (Bondi, 1987). Ten percent is complexed in non-ionic but ultra-filterable forms, such as calcium bicarbonate. Much of the plasma calcium is bound to albumin, and 40% is bound to proteins. It is due to this that the plasma calcium rises and falls with the albumin concentration even into the abnormal range (Berne *et al.*, 1998; Bondi, 1987).

The plasma ionised calcium concentration can drop below normal when there is a total body calcium deficit. A sudden shift in internal balance causes calcium to be taken up into bone faster than the extracellular calcium pool can be replenished or when the plasma protein binding to calcium increases (Berne *et al.*, 1998).

2.4.2.3. Magnesium (Mg)

The normal range of magnesium in plasma is 1.8 to 2.4mg/dl (1.5 - 2.0mEq/L). The body contains a total of about 25g of magnesium, of which 50% is present in the skeleton and almost all the rest is present in the intracellular fluid. The daily intake of magnesium ranges from 300 - 500mg. On average, 40% of this ingested magnesium is absorbed. In a steady state, the same amount, 120 - 200mg, is excreted in the urine (Berne *et al.*, 1998).

2.4.2.4. Iron (Fe)

Iron is widely distributed and encountered in plants and animals, and is an essential component. Its content in plants varies from species to species. Legumes contain more iron than cereal grasses and the iron concentration decreases with age on a vegetative stage (Georgievskii *et al.*, 1982). Seed coats, fish, meat and blood meals are also excellent sources of iron (McDonald *et al.*, 1995). Iron requirements are usually satisfied by natural feeds. However, under certain conditions, these elements may be limiting for example in intensified farming methods especially piggery, poultry and lactating cows fed mainly on roughage grown on iron-deficient soils (Georgievskii *et al.*, 1982).

The concentration of iron in the bodies of adult animals averages 0.005 – 0.006% calculated on fresh tissues basis (Phillis, 1976; Georgievskii *et al.*, 1982) and 0.14 – 0.17% calculated on ash weight basis. About 65% of the total iron is found in the blood stream, 10% in the liver, 10% in the spleen, 8% in the muscle, 5% in the skeleton and 2% in other organs (Lloyd *et al.,* 1978; Georgievskii *et al.*, 1982; McDonald *et al.,* 1995).

2.4.3. Vitamins

2.4.3.1 Vitamin A

Sources of carotene (the precursor of Vitamin A) in livestock diets are green feeds, dehydrated forages, and yellow corn. Normally, it is provided as esterified vitamin A, with the reactive hydroxyl group on the molecule esterified with an acid. Retinol acetate, propionate, and palmitate are the ester forms commonly used (Cheecke, 1991). However, vitamin A is the most likely to present toxic problems at levels that are 4 to 10 times the nutritional requirements in non-ruminants and approximately 30 times the nutritional requirements for ruminants (National Research Council, 1987).

The daily nutrient requirements for maintenance of lactating and pregnant cows are between 3000 to 6100 IU for animals between 400 to 800 kg liveweight (NRC, 1988). This also applies to animals on maintenance plus the last two months of gestation of

mature dry cows provided the cow is in calcium balance at the beginning of the last two months of gestation. If not, then the calcium balance should be increased from 25 to 33% (NRC, 1988).

2.4.3.2. Vitamin D

Vitamin D is known as "the sunshine vitamin' and is formed by the irradiation of sterols in plants and in the skin of animals. It occurs in two major forms: vitamin D_2 (ergocalciferol), and vitamin D_3 (cholecalciferol). Ergocalciferol is an activated plant sterol while cholecalciferol is an activated animal sterol that differs slightly in their chemical structure. Dietary sources of vitamin D are hay and other sun-cured forage and fish liver oil. Halibut liver and cod liver oils are rich sources of vitamin D_3 (McDonald *et al.*, 1995).

In animals, vitamin D_2 is converted to D_3 (the metabolically active form) but the efficiency of conversion is said to be very low in poultry (Cheecke, 1991). However, vitamin D plays an important role in calcium metabolism; bone mineralization and absorption. The metabolically active form of the vitamin is 1,25-dihydroxycholecalciferol (1,25-OHD_3) and is formed after the addition of two hydroxyl groups (-OH) to D_3. The first hydroxylation takes place in the liver to form 25-OH_3. It is then secreted into the blood and converted to 1,25-OHD_3 by the kidney and secreted back into the blood. It is in this form that it acts as a hormone that regulates calcium absorption and bone mineralization (Sjaastad *et al.*, 2003). The rate of formation of 1,25-OHD_3 is regulated by the parathyroid hormones (PTH). PTH is controlled by serum calcium level. When there is a need for more calcium, calcium absorption is increased by the formation of 1,25-OHD_3 (Cheecke, 1991; Puls, 1994; Pond *et al.*, 1995; McDonald *et al.*, 1995).

Daily nutrient requirements (maintenance) of lactating and pregnant cows for Vitamin D as outlined by the NRC (1988) are between 1200 to 2400 IU for animals between 400 to 800 kg liveweight. The same applies to animals on maintenance plus the last two months of gestation of mature dry cows provided the cow is in calcium balance at the

beginning of the last two months of gestation. If not, then the calcium balance should be increased from 25 to 33% (NRC, 1988).

2.5. Nutritional diseases caused by mineral imbalances

2.5.1. Calcium, Phosphorus, and Vitamin D3 Imbalances

A dietary deficiency of calcium and phosphorus and lack of vitamin D which impairs their absorption and utilization, results in abnormalities of the bones and teeth, subnormal growth and production, depressed appetite and poor feed efficiency. These deficiencies result in the reduction or failure in the mineralization process of the bone. Erickson *et al.*, (1999) reported that livestock producers are becoming increasingly aware of the challenges associated with nutrient management and perhaps, the largest challenge would be managing P inputs and outputs in livestock feeding operations. Supplementation is based on actual needs of the animals rather than the perceived needs.

2.5.1.1. Calcium (Ca)

If calcium is deficient in the diet, young animals suffer from rickets (Lloyd *et al.*, 1978). Characteristic symptoms of this disease include stunted growth, impaired or unnatural appetite, distorted spine, ribs and tubular bones, unsteady walking and lameness due to bone mineralization. Low calcium rachitis is manifested by hypocalcemia, 2-4 times higher level of alkaline phosphatase, lower citric acid concentration and sometimes decreased blood acid capacity. The bared bones are porous, soft and have deformed epiphyses, and much lower ash content. All these symptoms and biochemical changes have an intensity which is proportional to the calcium deficiency in the diet (Georgievskii *et al.*, 1982). Calcium deficiency in the diets fed to adult animals results in osteomalacia and osteoporosis. Growth of the long bones occurs primarily in the epiphyseal region through the synthesis of new cartilage. If the cartilage fails to calcify, the epiphyses become irregularly widened from the pressure of the body's weight. The primary defect

in rickets is not faulty calcification but rather the result of insufficient calcium and phosphorus in the serum which prevents calcification (Lloyd *et al.*, 1978).

2.5.1.2. Phosphorus (P)

Phosphorus exists exclusively as phosphates in the animal body; therefore, a phosphorus deficiency is a phosphate deficiency (Sjaadstad *et al.*, 2003). Rich supplies of phosphates are provided by the diet of carnivores through the bones and the soft tissues of the prey. Phosphate deficiency occurs mostly in herbivores in areas of the world where the soils contain low amounts of P. Animals with phosphate deficiency develop an abnormal appetite called pica and will in an effort to obtain extra phosphate, eat soil, bones and even soft tissues of dead animals. Severe phosphate depletion can lead to skeletal muscle weakness, cardiac and respiratory muscle dysfunction, loss of red blood cell membrane integrity, and abnormal formation of bone (Berne *et al.*, 1998). Impaired fertility has been reported in P-deficient cattle. Blood serum Ca is increased and serum P is decreased by P deficiency. Blood P is more complicated than blood Ca because blood P is in equilibrium not only with bone P but with several organic P compounds (Pond *et al.,* 1995).

2.5.1.3. Vitamin D3 imbalances

In rickets, a disease caused by vitamin D deficiency, the rate of absorption of Ca^{++} is very low leading to a low available Ca for bone growth. Bones are softer and more flexible than normal (Berne, 1998) and also have a low ash content (Bondi, 1987).

2.5.2. Hyperparathyroidism

Hypoparathyroidism can either be the result of subnormal secretion of PTH by pathological parathyroid glands or the hormone secreted is unable to interact normally with the target cells (Capen, 2004). Hyperparathyroidism results from a benign parathyroid neoplasm (adenoma). Hypercalcemia, hypophosphatemia, hypercalcinuria,

and renal calculi (stones) are typical manifestations. Long term secondary massive overproduction of PTH, typical of slowly developing renal failure, causes major bone effects. Areas of osteoclastic hyperactivity and rampant bone resorption are present next to areas of excessive and disorganised trabecular bone formation where the area becomes painful and fractures resulting to deformities (Berne *et al.*, 1998). Plasma Ca levels can become high enough from the direct action of PTH on bone and the indirect actions on intestine for the hormone's primary renal calcium-conserving action to be overwhelmed by the increased filtered load of calcium (Berne *et al.*, 1998). The result is hypercalcinuria and an increased frequency of renal calcium stone formation. Hypophosphatemia developed in response to hyperglycemia or hyperinsulinemia in dairy cows administered dextrose via continuous IV infusion, reaching a nadir in 24 hrs and remaining less than baseline value for 36 hrs, then increased after dextrose infusion was stopped, peaking in 6 hrs 9 (Grunberg *et al.*, 2006). The concentration of inorganic phosphate declined gradually in a fluctuating manner until recumbency and magnesium concentration remained constant in cows given intravenous Na_2EDTA infusion that allowed specific chelation of circulating Ca^{2+} leading to a progressive hypocalcemia (Mellau *et al.*, 2001).

CHAPTER THREE
MATERIALS AND METHODS

3. Experimental procedures

Oxy-tetracycline (Hi-tet 120 Bayer (Pty) Ltd, Reg. No. G1316, Act 36/1947) was infused intravenously into Bonsmara calves (beef) and Friesian calves (dairy) below 36 months of age. They consisted of 8 males and 8 female animals. Blood and faecal samples were collected before treatment (pre- treatment collection referred to as 0 hrs) and 30 minutes, 1 hr, 2 hrs, 3hrs, 5 hrs, 6 hrs, 24 hrs and 30 hrs thereafter as shown in Table A. Bone samples were collected before treatment (pre- treatment collection referred to as 0 hr) and 3 hrs, 6 hrs and 24 hrs post - treatment.

Table A. Experimental design of faecal and blood collected during the experiment using the intravenous injection of tetracycline in animals

Groups	0 h	1/2 h	1h	2h	3h	5h	6h	24h	30 h
Treatment groups									
Treatment	8	8	8	8	8	8	8	8	8
Control	8	8	8	8	8	8	8	8	8
Breed groups									
Friesian	8	8	8	8	8	8	8	8	8
Bonsmara	8	8	8	8	8	8	8	8	8
Sex groups									
Male	8	8	8	8	8	8	8	8	8
Female	8	8	8	8	8	8	8	8	8

3.1. Housing and diets

Eight Bonsmara and 8 Friesian calves of the same age (i.e less than 36 months old) were blocked according to sex and breed and randomly assigned to 2 treatments. The animals were later housed in experimental kraals designed to allow freedom of movement, with incorporated concrete water and feeding troughs and built in automatic water pipes for regular water supply. Roughage containing 50% *Medicargo sartiva* and 50% *Cenchrus ciliaris* was fed *ad-lib* to the animals with no feed supplementation throughout the experiment. The concentration of minerals in the diet ranges for P, Ca and Mg were 2.45 -2.96 mg P g^{-1}, 21.23 – 23.00 mg Ca g^{-1}, and 6.02 – 7.03 mg Mg g^{-1} respectively.

3.2. Collection of samples

The experiment consisted of two groups of experimental units on two breeds of animals (control and treatment groups) with 8 animals each. Each animal was used with a control in each time frame of collection. Rib bone samples were collected before treatment (0 hr) and 3, 6 and 24 hours post-treatment. Blood and faecal samples were collected before treatment (0hr) and 0.5 hr, 1 hr, 2 hrs, 3 hrs, 5 hrs 6 hrs, 24 hrs and 30 hrs post-treatment. This was done to try to monitor the changes made by the tetracycline-chelating effect in bone, blood and faecal mineral contents.

3.2.1. Blood samples

The animals were calmly driven into a crush pen where they were bled. The first baseline samples were collected from both groups at 0 hr from the jugular vein before the animals were treated. Samples were collected at 0.5 hr, 1 hr, 2 hrs, 3 hrs, 5 hrs, 6 hrs, 24 hrs and 30 hrs after treatment with tetracycline. Anticoagulant-free red stopper tubes (RST) were used in this study. The blood samples collected in the RST were stored at a temperature of 8°C for 28 hrs to allow clotting, centrifuged at 1000 rpm for

10 minutes and serum removed, stored in clean plastic tubes and immediately frozen at – 4°C for later analysis.

3.2.2. Rib bone samples

Rib bone samples were collected before treatment (0 hr) and at 3, 6 and 24 hours post-treatment. Rib bone samples were taken by using a trephine to remove a one half inch circular core. A local anesthesia was infused using 2% lignocaine. The operation was done aseptically to prevent infection in the bone site. The area over the site was cut using a scalpel blade and a ±3cm incision was made in the skin over the site to be sampled, carried down through the muscle to the bone and the periosteum over the rib. The trephine was introduced into the bone tissue until it was felt that the trephine was well seated into the bone. With proper movement of the trephine a core sample was collected from the rib and the muscles closed over the site using no. 1 chromic gut. The skin incision was closed using heavy vetafil. Rib bone samples were sanded using sandpaper to remove the trabecular bone that contains the red blood cells until only the outer core of the rib bone (cortical bone) was left for later analysis (Motsei and Beighle, 2006; Beighle *et al.*,1993).

3.2.3. Faecal samples

Faecal samples were collected into aluminium plates directly from the rectum and immediately dried by exposure to air and sunlight. Most of the samples were completely dry within a week. The air-dried samples were ground through a 2 mm screen after which they were stored in the screw-cap plastic containers for later analysis.

3.3. Preparation of samples for analysis

3.3.1. Blood samples

3.3.1.1. Serum inorganic P

Serum in the anti-coagulant free red-stopper tubes was aspirated using Pasteur pipettes. To precipitate the protein in serum, 0.7 ml of serum was added to 6.65 ml of stock tricloracetic acid (TCA) in clean test tubes, which were then covered, shaken individually on an electric stirrer and left to stand for 10 minutes. The samples were centrifuged at 2500 rpm for 10 minutes (Beighle *et al.*, 1995).

3.3.1.2. Blood Ca and Mg

The method used describes the determination of calcium and magnesium in blood serum and plasma where samples are diluted with lanthanum chloride. The presence of lanthanum controls chemical interferences (strong phosphate interferences) when determining calcium. If calcium is not to be determined, the dilution can be made with deionised water only.

For the determination of calcium and magnesium, serum was diluted with 0.1 % (w/v) lanthanum chloride diluent in a 1:5 ratio. If calcium was not to be determined, the dilution was made with deionised water. The dilution ratio was adjusted to insure that concentrations fall within a suitable absorbance range (Fernandez and Kahn, 1971).

3.3.2. Bone P, Ca and Mg

Bone samples were divided into 2 halves to provide duplicate samples. Empty crucibles were weighed (A) and the weight was recorded. The halved sample of bone was added and the crucible was reweighed and recorded (B). The difference between the weights A and B was the fresh weight of the sample. Another empty crucible was weighed and recorded, and the remaining of halved bone was added and the weight recorded. Crucibles with samples were placed in a drying oven at 106°C overnight, removed and

allowed to cool in a desiccator for 6 hours. Samples were then weighed and the weight recorded as dry weight. Samples were then ashed in a muffle furnace overnight for 16 hours at 600°C, removed and cooled in a desiccator for 6 hours, weighed and the weight recorded as ash weight. Two millilitres of 5 N hydrochloric acid (HCl) was added to each crucible containing bone, the bone was allowed to dissolve and transferred to 100 ml volumetric flasks, mixed properly and filled to the mark with distilled water. After the sediments had settled, the supernatant fluid was transferred into a McCartney bottle to be used in the analysis (Association of Official Analytical Chemists, 1980).

3.3.3. Faecal P, Ca and Mg

One gram duplicate air-dried faecal samples were weighed in dried, acid cleaned crucibles. The weight was recorded as fresh weight and then placed in an oven at 106° C for 16 hours. The crucibles containing the dried faeces and feed were then cooled in desiccators for 2 hours and weighed to determine the weight of the oven-dried faeces and feed and the weight was recorded as the dry weight. The crucibles were then placed in a muffle furnace for ashing at 800° C for 16 hours and the samples were allowed to cool in a desiccator for six hours. The crucibles were then weighed to determine the ash weight of the faeces and feed. One ml of concentrated nitric acid was added to the crucibles and evaporated to dryness on a medium heated hot plate. The crucibles were returned to the muffle furnace for a further 2 hours for ashing at 600° C, removed and cooled. Ten ml of 5 *N* HCl were added to each crucible and evaporated on very low heat until about 3 ml was left in the crucible. The solution was then transferred to a 100 ml volumetric flask ensuring that all contents of each crucible were completely transferred. After filling the volumetric flask with distilled water to the mark, the flasks were inverted several times for adequate mixing and left to stand overnight to allow sediments to settle at the bottom. Thirty ml were removed from the flask without disturbing the sediment at the bottom and stored in McCartney bottles for future analysis (AOAC, 1980).

3.4. Methods of analysis

3.4.1. Blood P

From each sample prepared as described in 8.3.1.1., 5 ml of the supernatant fluid was transferred to a clean test tube without unsettling the centrifuged material at the bottom. The sample solution was then transferred into a test tube containing 5 ml distilled water to make 10 ml and mixed with 1.5 ml each of ammonium molybdate, hydroquinone and sodium sulphite, thoroughly mixed and allowed to stand at room temperature for 40 minutes, poured into cuvettes and analysed using an Aquamate UV-Vis Spectrophotometer for the determination of blood P. The concentration of blood P was determined using the standards prepared as described in 8.5.2 and read at a wavelength of 686 nm.

3.4.2. Blood Ca and Mg

For the determination of Ca and Mg, serum was diluted with 0.1 % (w/v) lanthanum chloride diluent in a 1:50 ratio. If calcium was not to be determined, the dilution was made with deionised water (Fernandez and Kahn, 1971). The concentration of blood Ca and Mg were determined using the standards prepared as described in 8.5.1 and 8.5.3 below for the standard conditions and read at a wavelength of 820 nm for Ca and 282 nm for Mg using the AAS 700 Flame spectrophotometer.

3.4.3. Bone and faecal Ca and Mg

The presence of lanthanum controls strong phosphate interferences when determining bone Ca as in blood Ca above. For the determination of bone Ca and Mg, the supernatant fluid left after digesting the bone sample as in 8.3.2 was diluted with 0.1 % (w/v) lanthanum chloride ($LaCl_3$) diluent in a 1:5 ratio. For the determination of Mg the dilution was made with deionised water. The dilution ratio was adjusted to insure that concentrations fall within the absorbance range of the standards (Fernandez and Kahn, 1971).

Analysis of Ca and Mg was done on the supernatant fluid of the sample solution. To determine Ca and Mg fractions in faeces and bone, 1 ml of standards (200 ppm, 100 ppm and 50 ppm) and sample solutions were pippeted in respective test tubes and each mixed with 8 ml of 0.5 % $LaCl_3$. A blank which was a reagent blank of 0.5 % $LaCl_3$ was poured and from there onwards, sample solutions were poured in different cuvettes accordingly. The concentrations of blood Ca and Mg were determined using the standard dilutions listed in 8.5.1 and 8.5.3 and read at a wavelength of 820 nm for Ca and 282 nm for Mg using the AAS 700 Flame spectrophotometer (AOAC, 1980).

3.4.4. Cortical bone thickness

Rib bone samples were sanded using a sandpaper to remove the trabecular bone that consisted of red blood cells and only the cortical bone was used for analysis as described by Beighle *et al.*, (1993). The cortical bone was measured in millimetres using a calliper to determine the bone thickness.

3.5. Preparation of standards

3.5.1. Preparation of working calcium standards

A commercial stock standard containing1000 ppm Ca was used to make 50, 100 and 200 ppm Ca working standards. To prepare a 50ppm standard, 5ml of stock standard (1000 ppm) was mixed with 95 ml of 0.1% (w/v) lanthanum chloride, 10 ml of stock standard was mixed with 90 ml of 0.1% (w/v) lanthanum chloride to prepare a 100 ppm standard and 20 ml of stock standard was mixed with 80 ml of 0.1% (w/v) lanthanum chloride to prepare a 200 ppm standard (AOAC, 1980).

3.5.2. Preparation of working phosphorus standards

A stock standard of 1000 ppm P was used to make 50, 100 and 200 ppm phosphorus working standards. To prepare a 50 ppm standard, 5 ml of stock standard (1000 ppm)

was mixed with 95 ml of distilled water, 10 ml stock standard with 90 ml of distilled water to prepare a 100 ppm and 20 ml of stock standard to 80 ml of distilled water to prepare a 200 ppm standard (AOAC, 1980).

3.5.3. Preparation of working magnesium standards

A commercial stock standard containing 1000 ppm Mg was used to make a 5, 10, 20 and 30 ppm Mg working standards. To prepare a 5 ppm standard, 0.5 ml of the stock standard (1000 ppm) was mixed with 9.5 ml of distilled water, 1.0 ml of the stock standard mixed with 9.0 ml of distilled water to prepare a 10 ppm standard and 2 ml of the stock standard was mixed with 8.0ml of distilled water to prepare a 20 ppm standard (AOAC, 1980).

3.5.4. Preparation of a blank solution

The 0.1% (w/v) $LaCl_3$ solution was used as a blank for the determination of blood, bone, feacal and feed Ca and deionised water was used as a blank for blood, bone, faecal and feed Mg and P.

3.6. Preparation of laboratory equipment and reagents

All glassware laboratory equipment (crucibles, red-stoppered tubes, volumetric flasks, glass beakers, cylinders, pippets) were soaked in 36% HCl over night, rinsed with distilled water thrice and dried. Plastic containers and tubes were rinsed thrice with distilled water and dried. Rib biopsy equipment was washed and sterilised in an autoclave for 15 minutes and dried.

3.6.1. Aquamate Sequoia Spectronic Spectrophotometer

Blood P was analysed through the Aquamate Sequoia Spectronic Spectrophotometer machine. The inorganic phosphorus method used in the Aquamate Spectrophotometer

analytical instrument for the determination of inorganic P in serum was prepared as in the work study by Motsei and Beighle (2006).

3.6.2. Preparation of reagents for the determination of blood P using the aquamate spectrophotometer (Motsei and Beighle, 2006).

i. 10% Trichloracetic acid solution (TCA)

Exactly 10g of TCA was mixed with 100ml of distilled water to make a 10% TCA solution and used to precipitate the protein in serum.

ii. Ammonium molybdate reagent

Twenty-five grams of ammonium molybdate (A.R.) was dissolved in 300ml of distilled water. Seventy-five millilitres of concentrated sulphuric acid (A.R.) was slowly added to 125ml of distilled water and mixed. The 2 solutions were mixed together to form a final working solution.

iii. Sodium Sulphite solution

Ten gams of anhydrous sodium sulphite (A.R.) was dissolved in a 100ml of distilled water. A fresh solution was prepared daily.

iv. Hydroquinone solution

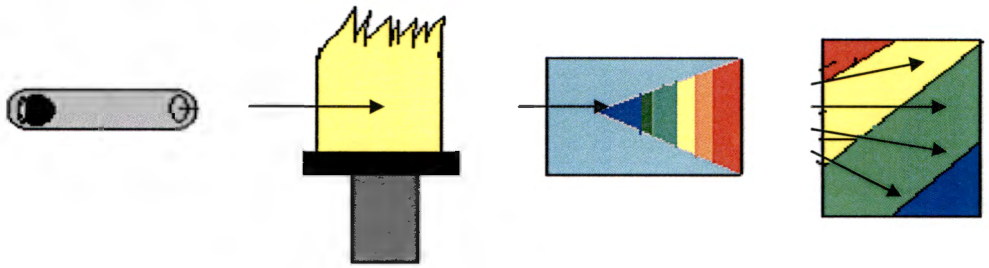
One gram of quinol (C.P.) was dissolved in a 100ml of distilled water and thoroughly mixed. A fresh solution was prepared daily.

3.6.3. Atomic Absorption Spectrophotometer (AAS-700)

Atomic spectroscopy is actually not one technique but three: atomic absorption, atomic emission and atomic fluorescence. Out of these techniques, atomic absorption and atomic emission (AE) are the most widely used. Atomic absorption is the process that occurs when a ground state atom absorbs energy in the form of light of a specific wavelength and is elevated to an exited state. The amount of light energy absorbed at this wavelength will increase as the number of atoms of the selected element in the light path increases. The relationship between the amount of light absorbed and the concentration of the analytes present in the known standards can be used to determine unknown sample concentrations by measuring the amount of light they absorb.

The basic instrumentation for atomic absorption requires a primary light source, an atom source, a monochromator to isolate the specific wavelength of light to be measured, a detector to measure the light accurately, electronics to process the data signal and a data display or reporting system to show the results. The light source normally used is either a hollow cathode lamp (HCL) or an electrodeless discharge lamp (EDL). In general, a different lamp is used for each element to be determined. In some cases, a few elements may be combined in a multi-element lamp (Perkin-Elmer, 2005).

The atom source used must produce free analyte atoms from the sample. The source of energy for free-atom production is heat, most commonly in the form of an air/acetylene or nitrous-oxide/acetylene flame. The sample is introduced as an aerosol into the flame by the sample introduction system, which consists of a nebuliser and spray chamber. The burner head is aligned so that the light beam passes through the flame, where the light is absorbed (Perkin-Elmer, 2005).



HCL or EDL

Simplified drawing of a basic flame atomic absorption system

Bone, faecal and blood Ca and Mg were analysed through an AAS-700 atomic absorption spectrophotometer using the current Winlab 23 AA Flame Software and methods supplied by Perkin-Elmer (2005).

3.6.4. Preparation of reagents for the determination of blood, bone and faecal Ca and Mg using the AAS 700 Spectrophotometer.

i. 0.1% lanthanum chloride ($LaCl_3$) solution

A solution of 0.1% of lanthanum as chloride was made by dissolving 1g of $LaCl_3$ in a 1000 ml of distilled water and mixed.

3.7. Experimental design and statistical analysis

3.7.1. Experimental design

The design of the experiment was planned as follows:

Hour	Time	Sample type
0	Day before or before 08H35	Blood, Faeces, Bone
	08H55 IV injection of Tetracycline	20mg/kg
0.5	09H30	Blood, Faeces
1	10H00	Blood, Faeces
2	11H00	Blood, Faeces
3	12H00	Blood, Faeces, Bone
5	14H00	Blood, Faeces
6	15H00	Blood, Faeces, Bone
24	09H00 Day 2	Blood, Faeces, Bone
30	15H00 Day 2	Blood, Faeces

This experiment was designed in such a way that the time counting began at the time when the injection with tetracycline was concluded. This was done to make sure that the collection of samples was at the correct time. The general design of this experiment calls for the division of subjects into two groups, one of which is randomly allocated to the control. The baseline measurements were done on all the experimental animals at a starting condition used to create balanced treatment groups. The experimental group was then exposed to treatment with the IV injection of tetracycline, while the controls were maintained at the starting condition under which the baseline measurements were made until the end of the experiment

3.7.2. Statistical analysis

The data collected was subjected to the Analysis of Variance to determine the effect of the drug on the bone, blood and faecal Ca, P and Mg, bone thickness and Ca:P ratio with the General Linear Model (GLM) procedures of the SPSS System 17 software.

The T-test and its associated P-Value were calculated to compare the mean difference between the two experimental groups. The results between the 2 sets of measurements were significant if the associated P-value was less than the alpha level established (i.e. $P<0.05$). Correlation analysis was also performed to see if there are associations among P, Ca and Mg of bone, faeces and blood and also bone Ca:P ratio and bone thickness between the experimental groups

3.7.3. Model

$y_{ijklm} = \mu + B_i + S_j + R_k + T_l + (B \times S)_{ij} + (B \times R)_{ik} + (B \times T)_{il} + (S \times R)_{jk} + (S \times T)_{jl} + (R \times T)_{kl} + (BSR)_{ijk} + (BST)_{ijl} + (SRT)_{jkl} + (BRT)_{ikl} + (BSRT)_{ijkl} + e_{ijklm}$

where :

B_i = Fixed effect of the i^{th} breed

S_j = Fixed effect of the j^{th} sex

R_k = Fixed effect of the k^{th} treatment

T_l = Fixed effect of the l^{th} time

$(BS)_{ij}$ = Interaction effect of the i^{th} breed type and the j^{th} sex

$(BR)_{ik}$ = Interaction effect of the i^{th} breed type and the k^{th} treatment

$(BT)_{il}$ = Interaction effect of the i^{th} breed type and the l^{th} time

$(SR)_{jk}$ = Interaction effect of the j^{th} sex and the k^{th} treatment

$(ST)_{jl}$ = Interaction effect of the j^{th} sex of the animal and the l^{th} time

$(RT)_{kl}$ = interaction effect of the k^{th} treatment and the l^{th} time

(BSR)ijk = interaction effect of the *i*th breed type, the *j*th sex and the *k*th treatment

$(BST)_{ijl}$ = interaction effect of the *i*th breed type, the *j*th sex and the *l*th time

$(SRT)_{jkl}$= interaction effect of the *j*th sex, the *k*th treatment and the *l*th time

$(BRT)_{ikl}$ = interaction effect of the *i*th breed type, the *k*th treatment and the *l*th time

$(BSRT)_{ijkl}$ = interaction effect of the *i*th breed type, the *j*th sex, the *k*th treatment and the *l*th time

e_{ijklm} = error term

CHAPTER FOUR
RESULTS AND DISCUSSION

INTRODUCTION

Cortical bone samples were taken from 16 calves less than 36 months old, eight of which were assigned to the control group and 8 assigned to the treatment group. Samples were collected in the middle of the 9^{th}, 10^{th}, 11^{th} and 12^{th} rib from both the left and right sides of the calves. Faecal samples were collected from the rectum and blood samples from the jugular vein from which the serum was used for analysis.

The animals were grouped according to treatment (treated and control), breed (Bonsmara and Friesian) and sex (male and female). The animals were not given any form of supplementation.

4. Results (time effect)

4.1. Group differences (treatment and control) through time

4.1. 1. Bone minerals (fresh weight)

The calves that had been injected with tetracycline had significantly ($P<0.05$) higher bone P at 6 h (101.02 mg P g^{-1}) post-treatment than the control calves (81.06 mg P g^{-1})(Table 1.). At 3 and 24 h post-treatment, concentration of P in the bones of both the treated and control groups were not significantly different. Bones taken from the treated calves showed a significant ($P<0.05$) increase in P 3 h post-treatment with tetracycline and observed to be the highest cortical bone P concentration compared with 0 h pre treatment and 6 and 24 h and thus showing a P sparing effect around that time on a fresh weight basis (Table 1.).

Treated calves lost Ca from their bones 3 h post-treatment with a concentration of 167.60 mg P g^{-1} as compared to the control calves (191.58 mg P g^{-1}) but regained the Ca lost to a level not significantly different from that of the control calves at 6

and 24 h post-treatment. Treated calves had significantly ($P<0.05$) less cortical bone Ca at 3 h post treatment compared with the control animals and also at 3 h compared with the same treated calves at 0 h pre-treatment and at 6 h post-treatment (Table 1.). This indicates that the tetracycline was tying up the Ca in the blood and the body responded by pulling Ca out of the bone at 3 h post-treatment. The body's homeostatic mechanisms must have reacted to this change so that by 6 h post-treatment there was no longer any significant difference in cortical bone Ca either between treated and untreated calves or between treated calves at 0 h before treatment and at 6 and 24 h post-treatment. This is further supported by the results of the blood analysis (Table 7.) in which there was significantly ($P<0.05$) less blood Ca at 1, 2 and 3 h among treated animals compared with the control animals.

Bone Mg concentrations of the treated and the control groups showed no significant differences at all experimental times. However, bones taken from the treated calves through time showed a significant reduction in Mg concentration 3 h post-treatment that also significantly decreased at 24 h post-treatment (Table 1.).

No significant differences were found in bone Ca:P ratio throughout the experiment between the treated and the control calves. The mean cortical bone Ca:P ratio was significantly ($P<0.05$) lower at 3 h compared with the pre treatment values. At 6 and 24 h post-treatment the mean cortical bone Ca:P ratio was significantly ($P<0.05$) higher compared with that at 3 h but the mean Ca:P ratio of the treated calves was significantly ($P<0.05$) less at all sampling periods compared with pre treatment values in the same treated calves (Table 1.). The required calcium was not available for bone formation hence the blood had lower levels of Ca at 3 h due to chelation by tetracycline (Table 7.). The body obtains Ca from wherever possible such as the storage depots in the bones. Table 1 indicates that calcium has been removed from the bone thus decreasing bone integrity, while P has been incorporated into the bone 3 h after treatment.

The mean thickness of the cortical bone in the treated group was significantly ($P<0.05$) higher at 6 (3.45 mm) and 24 h (3.16 mm) post-treatment as compared to the control calves with the means 2.60 mm at 6 h and 2.66 mm at 24 h post-treatment. The treated group had significantly ($P<0.05$) thicker bones at 6 h than at 3 and 24 h post-treatment. Also, the treated animals had significantly ($P<0.05$) thicker cortical bones at all three sampling periods compared with pre treatment values (Table 1.). These values are higher than the figure of 2mm reported by Bortolussi *et al.* (1996) in P deficient steers and 2.4 mm in supplemented calves. A bone thickness of 3 mm from bone biopsy samples of the 12^{th} rib was suggested to be indicative of adequate P in a diet by Little (1984), Motsei and Beighle (2006) reported bone thickness of between 1.36 and 2.14 mm from calves given ammonium sulphate offered as a lick whereas Beighle (1999) reported bone thickness between 2.41 – 3.33 mm while investigating the effect of stage of gestation on bone minerals in dairy cows.

4.1.2. Faecal minerals (fresh weight)

At 1h post-treatment the mean faecal P concentration of the calves that were injected with tetracycline was significantly ($P<0.05$) higher than that of the control calves but at 2 and 3 h post-treatment, the same calves had significantly ($P<0.05$) lower faecal P compared to the control calves (Table 2.). Also at 1 h post-treatment, the treated calves had significantly more faecal P (5.46 mg P g^{-1}) compared to the pre-treatment value of 5.01 mg P g^{-1} and one half hour post-treatment value of 5.00 mg P g^{-1}. Moreover, the treated calves had significantly ($P<0.05$) more faecal P at 1 h after treatment as compared with values observed at 2, 3, 6 and 24 h after treatment.

The treated calves lost significantly ($P<0.05$) more Ca through the faeces at 1 h post-treatment (9.59 mg P g^{-1}) and at 3 h post-treatment (11.25 mg P g^{-1}) compared to the controls (7.77 mg P g^{-1}) at 1 h and 8.52 mg P g^{-1} at 3 h post treatment. At 6 h, the treated calves absorbed more Ca from the gut into the blood

more than the control calves. As the concentration of Ca in the feaces of the treated calves were compared through time, the treated calves lost more Ca throughout the faeces half an hour post-treatment and at 1 h and 3 h post-treatment (Table 2.) but was significantly ($P<0.05$) resorbed from the gut 6 h post-treatment. The treated calves conserved more Mg from the body only at 6 h post-treatment as compared to the controls. The treated calves lost more Mg through the faeces at 24 h and 30 h as compared to values observed at ½, 1, 2, 3, 5 and 6 h post-treatment.

4.1.3. Bone minerals (dry weight)

The reated calves had significantly ($P<0.05$) more P in their bones at 6 h post treatment (114.32 mg P g^{-1}) compared to the controls (93.63 mg P ^{-}g). The treated calves also had significantly ($P<0.05$) more P in their bones at 3 h (145.18 mg P g^{-1}) as compared to the baseline bone sample value and post-treatment hours at 6 and 24 h (Table 3.).

There were no significant differences between the bone Ca concentrations of the treated and the control calves on a dry weight basis. However when the concentrations within treated group are compared over time, it was evident that at 3 h, the calves resorbed significantly ($P<0.05$) more Ca from their bones but regained more Ca at 6 h by reabsorbing more Ca from the gut (Table 3.) into the bones on a dry weight basis. At 24 h post-treated calves were able to pull out Ca from their bones as the Ca concentrations were significantly ($P<0.05$) lower than that at 6 h.

In contrast to bone Ca, bone Mg showed no significant differences between the treated calves and the controls. The treated calves had significantly ($P<0.05$) more Mg in their bones at 0 h than at any other time during the experiment , i.e at 3, 6 and 24 h post-treatment (Table 3.).

There were no significant differences (P>0.05) between the Ca:P ratio between the treated and the control group throughout the experiment. The treated calves had significantly (P<0.05) higher Ca:P ratio before treatment was given than any other time during the experiment.

4.1.4. Faecal minerals (dry weight)

Thirty minutes post-treatment, the mean faecal P concentration was significantly (P<0.05) lower than that of the control calves (5.00 vs 5.58 mg P g^{-1}) and at 1 h post-treatment, the faecal P concentration was significantly (P<0.05) higher than that of the control but the reverse was observed at 2, 3, and 6 h post-treatment, where the treated calves had significantly (P<0.05) less faecal P compared to the control calves. Calves in the treated group absorbed more P from their intestine therefore reducing the concentration of P in their feaces when compared with the control group (Table 4.).

Calves were able to conserve body P in response to the IV tetracycline injection because those calves had significantly (P<0.05) less P in the faeces compared with the control calves 30 minutes after treatment (5.00 vs 5.58 mg P g^{-1}). One hour post-treatment however, the treated calves had significantly (P<0.05) more faecal P compared with the control calves. This was probably due to a feedback response of the body's homeostatic mechanism because just one hour later, at 2 h, the mean faecal P concentration of the treated animals was again significantly (P<0.05) less than calves that were not treated. This was also true for faecal P concentration at 3 h and 6 h post-treatment. This would indicate that the IV injection of tetracycline stimulated the body's homeostatic mechanisms to conserve P and prevent it from being lost via the faeces. This is further supported by the fact that bone P concentration was significantly (P<0.05) higher in the treated calves compared to control calves at 6 h post-treatment (Tables 1, 3, 5) indicating that the P conserved when less P was lost in the faeces was deposited in the bone of the treated calves. Further evidence on the conservation of P as a

result of the iv injection of tetracycline is the significantly ($P<0.05$) lower overall mean faecal P the treated animals (5.07 mg P g^{-1}) compared with that of the control animals (5.30 mg P g^{-1}) on a fresh weight basis (Table 2.). This was also true on the ash weight basis (Table 6.).

Like P, Ca was also lost from the body by way of faeces 1 h and 3 h post-treatment. At 6 h, calves of the treated group absorbed significantly ($P<0.05$) more Ca from the gut, that led to the treated group having less Ca (10.29 mg Ca g^{-1}) in their faeces than the control calves (12.54 mg Ca g^{-1}). At 3 h post-treatment however, the treated calves had significantly ($P<0.05$) more faecal Ca (12.06 mg Ca g^{-1}) compared with pretreatment values at 0 h (9.57 mg Ca g^{-1}), one half hour (8.07 mg Ca g^{-1}), 1 h and 2 h (10.25 and 10.08 mg Ca g^{-1}) post-treatment. Moreover, the treated calves had significantly ($P<0.05$) more faecal Ca at 3 h after treatment compared to the values observed at 5, 6 and 24 h after treatment (Table 4.).

Only at 6 h post treatment did the treated calves have significantly ($P<0.05$) less faecal Mg (1.80 mg Mg g^{-1}) than the controls (2.34 mg Mg g^{-1}). The treated calves lost significantly ($P<0.05$) more Mg through their faeces at 24 h (2.57 mg Mg g^{-1}) and 30 h (2.80 mg Mg g^{-1}) as compared to the values observed at 0 h pre treatment, ½ , 1, 2, 3, 5 and 6 h post-treatment (Table 4.).

4.1.5. Bone minerals (ash weight)

At 6 h after treatment, the mean bone P concentration of the treated calves was significantly ($P<0.05$) higher than that of the control calves. This was the result of the storing by the body of the P conserved from faeces (Table 6.). Faecal P was significantly ($P<0.05$) lower in the treated calves compared to the control calves at 3 and 6 h. At the same time, blood P was also lower though not significantly different at 5 and 6 h. At 24 and 30 h post-treatment, blood P was significantly

(P<0.05) lower among treated calves compared to the control calves (Table 7.) as a result of P placed in the bone at 6 h (Table 5.).

Calves on the treatment had significantly (P<0.05) more P in their bones at 3 h as compared to 0h pretreatment and 6 and 24h post-treatment. That might be the same bone P at 3 h that was absorbed from the GIT into the bone via the blood stream, resulting in a reduction of faecal P concentration at 1 h until at 3 h post-treatment on the treated calves (Table 6.). Moreover, that resulted in faecal P being significantly (P<0.05) lower in the treated calves than the control calves at 3 h (Table 6.).

Bone Ca of the treated calves was significantly (P<0.05) lower than that of the controls 3 h after the treatment. This suggests that calves resorbed Ca out of the bone into the blood (Table 7.) and loose it through the faeces (Table 6) 3 h after an IV injection with tetracycline. At 6 h post-treatment, treated calves had significantly (P<0.05) more Ca in their bones as compared to the control calves. That is supported by a significantly (P<0.05) lower faecal Ca at 6 h (Table 6.) and more blood Ca that somehow was drawn from the gut, through the blood stream and stored in the bones as a Ca reserve. The treated calves tended to lose more bone Ca 3 h post-treatment compared to the pre-treatment value (324.45 mg Ca g^{-1}) which increased significantly (P<0.05) 6 h post-treatment and remained the same at 24 h post-treatment.

There was no significant difference (P<0.05) between bone Mg of calves on the treatment group and the control group. The treated calves tended to withdraw significantly (P<0.05) more Mg from their bones at 3, 6, and 24 h compared to the pre treatment value at 0h (Table 5.). This was supported by the loss through the faeces of Mg that increased significantly (P<0.05) at 3 h until at 30 h (Table 6.) and the blood Mg that showed the same characteristic at 2, 3, 5, 24 and 30 h post-treatment (Table 7.).

4.1.6. Faecal minerals (ash weight)

Treated calves conserved P by reducing the level of P lost throughout their faeces at 3 h and 6 h post-treatment as compared to the control calves. It has been stated before (Beighle, 2000, Motsei and Beighle, 2006) that P conserved from faeces is taken up by the blood into the bone to be stored by calves. The treated calves were able to pull out P out of the gut at the level of the colon (Table 6.). As faecal P concentration of the treated calves were compared through time, it was evident that at 1 h post-treatment, treated calves had significantly ($P<0.05$) more P in their faeces than at 0 h pre treatment and one half h after treatment. At 2 and 3 h post-treatment, the treated calves might have absorbed more P from the gut as shown by reduction in the faecal P (2 h = 27.36 mg P g^{-1} and 3 h = 23.67 mg P g^{-1}). At 5 h, calves continued to lose more P than values observed previously at 2 and 3 h post-treatment which remained non significant until at 24 h post-treatment. It was only at 30 h post-treatment that the calves lost more P in their faeces than all values observed previously from 0 – 24 h (Table 6.).

The treated calves lost significantly ($P<0.05$) more Ca through the faeces at 1h (60.24 mg Ca g^{-1}) and 3 h (61.31 mg Ca g^{-1}) as compared to the control calves [56.21 mg Ca g^{-1} (1 h) and 51.45 mg Ca g^{-1} (3 h)]. At 30 min and at 6 hr post-treatment, calves injected with iv tetracycline had significantly ($P<0.05$) less faecal Ca compared with control calves. This is an important finding because it indicates that the body's homeostatic mechanisms were able to respond rapidly and immediately reduce the loss of Ca by way of the faeces. This rapid response points to the ability of the animal to resorb Ca from the colon and thus reduce the amount of Ca lost in the faeces within 30 minutes. The significant loss of faecal Ca at 6 h coincides with the significantly ($P<0.05$) more bone Ca in treated calves compared with control calves at 6 h (Table 5.) and the significantly ($P<0.05$) higher blood Ca at 6 h in treated vs control calves (Table 7.). This would indicate that the Ca pulled out of the faeces was carried by the blood (Table 7.) and picked up by the bone which gave the significantly ($P<0.05$) higher bone values in the treated

calves compared with the control calves at 6 h (Table 5.). It was only at 24 and 30 h post-treatment that the treated calves had significantly ($P<0.05$) more Ca in their faeces compared with the pre treatment values of 57.46 mg Ca g^{-1} at 0 h . Only at 30 minutes did the treated calves have less faecal Ca than the pre-treatment hour in the experiment.

Treated calves were observed to have absorbed more Mg by pulling it from the gut half an hour after treatment and 5 and 6 h post-treatment as compared to the control calves. The treated calves tended to absorb more Mg from 30 min, 1, 2, and 3 h post-treatment. At 5 h, treated calves significantly ($P<0.05$) lost more Mg throughout their faeces compared to 3 h post-treatment. Faecal Mg of the treated calves continued to be significantly ($P<0.05$) more at 6, 24 and 30 h post-treatment. It was only at 30 h that the treated calves had more Mg in their faeces than at any point or time of the experimental hours (30 min, 1, 2, 3, 5, 6 and 24 h).

As observed from the Ca results, calves in the treatment group were conserving Mg 30 minutes after treatment. The treated calves had significantly less faecal Mg (11.88 mg Mg g^{-1}) compared to control calves (11.19 mg Mg g^{-1}) at 30 minutes post-treatment. In addition at 5 and at 6 h post-treatment, calves had significantly ($P<0.05$) less faecal Mg compared with control calves (Table 6.). The injections of tetracyclines had a very beneficial effect on the body's homeostatic mechanisms related to Mg as the treated calves lost less Mg through the faeces at 1/2, 5 and 6 h, (Table 6.) and had more blood Ca at 1/2, 5 and 6 h (Table 7.) compared with control calves. Further proof of the positive effect of the tetracycline on the Mg homeostatic mechanisms are the significantly ($P<0.05$) higher values for the overall mean faecal Mg and overall mean blood Mg in treated calves compared with control calves (Table 6. and Table 7.).

4.1.7. Blood minerals (mg/100 ml serum)

The treated calves had significantly ($P<0.05$) less P in their blood at 24 and 30 h post-treatment as compared to the control calves. Moreover, there were no significant differences in blood inorganic P between the treatment and the control calves from 30 min until at 6 h post-treatment of the experiment, and also when the inorganic P concentration of the treated calves were compared through time from 0 until at 30 h of the experiment. This might indicate that the calves maintained a constant P balance despite the increase or decrease in P levels of the GIT and the bone P. Mellau *et al.* (2001) found an increased concentration of inorganic P in only one out of 6 cows after 120 min of EDTA infusion while the cow was actually struggling. It was therefore hypothesised that such an increase in plasma inorganic P during infusion occurs in struggling cows in which increased muscular activity releases energy in the form of ATP and might have released inorganic P into the extracellular fluids (Mellau *et al.* 2001).

Blood Ca of the treated calves tended to be lower than that of the controls at 1, 2, and 3 h post-treatment. This might be because the treated calves significantly ($P<0.05$) lost more Ca through their faeces at 1, and 3 h post-treatment (Table 6.). At 6 h post-treatment, calves in the treated group significantly ($P<0.05$) had more Ca in their blood when compared to the control calves (Table 7.). Blood Ca concentration of the treated calves significantly ($P<0.05$) reduced from 30 min, until at 1, 2, and 3 h but was increased at 5 h post-treatment. At 6 h after treatment, the mean blood Ca concentration of the treated calves significantly ($P<0.05$) reduced and remained stable at 24 and 30 h post-treatment. At 5 h post-treatment, the treated calves had more blood Ca than concentrations observed at the previous periods of the experiment (30 min, 1, 2, and 3 h), and also those observed after (at 6, 24, and 30 h). These results agree with the findings of Goff and Horst (1993) who found that plasma Ca concentrations significantly ($P<0.05$) increased above pretreatment values within 30 min of treatment and remained

elevated for the next 3 h. They also found that Ca proprionate increased plasma Ca concentration less than did $CaCl_2$.

Blood Ca tended to decrease in treated calves compared to control calves up through 3 h but at 5 h, the trend was reversed and the treated calves tended to have more blood Ca compared with control calves with significantly ($P<0.05$) more at 6 h (Table 7.). This is in agreement with previous reports by Martin, (1985) that the injection of tetracyclines lowered blood Ca.

Calves in the treated group consistently had more blood Mg compared with the control calves and significantly more at ½, 1, 5, 6 and 30 h post-treatment (Table 7.). In addition, the overall mean blood Mg was significantly ($P<0.05$) higher among the treated (1.54 mg Mg g^{-1}) compared with control (1.39 mg Mg g^{-1}) animals (Table 7.). These higher concentrations of blood Mg among the treated calves were consistent with the lower values of faecal Mg among the same calves (Table 6.) and the significantly ($P<0.05$) lower overall mean faecal Mg concentration in the treated calves (10.86 mg Mg g^{-1}) compared with control calves (13.13 mg Mg g^{-1}).

Although the values were not significantly different, the overall mean bone Mg among treated animals was higher (5.71 mg Mg g^{-1}) compared with control animals (5.52 mg Mg g^{-1}). This indicates that the Mg pulled out of the gut and placed in the blood was eventually incorporated into bone but not with the efficiency observed in P. Perhaps, the body's homeostatic mechanisms for maintaining body stores and especially blood concentrations of Mg are not as efficient as those of Ca and P.

4.2. Mean differences between breeds (Friesian and Bonsmara) through time

4.2.1. Bone minerals (fresh weight)

The bone of Friesian calves had significantly ($P<0.05$) more P at 6h after treatment as compared to the Bonsmara calves (Table 8.). The Friesian calves were able to remove P from the faeces and deposit it in the bone. This is evident in Table 9. where the Friesian calves had less P in their faeces than the Bonsmara calves at 2, 3, 5 and 6 h even though the difference was significant ($P<0.05$) only at 5 h post-treatment. At 3 h, post-treatment the mean bone P concentration of the Friesian calves was significantly ($P<0.05$) higher than the pre treatment value at 0h and also at 6h and 24 h post-treatment (Table 8.). The Bonsmara calves also had significantly ($P<0.05$) more P in their fresh bones 3 h after treatment with tetracycline than the pretreatment value at 0 h and similarly at 6 and 24 h post-treatment (Table 8.).

Cortical bone of Friesian calves had significantly ($P<0.05$) more Ca than that of the Bonsmara calves at 6 and 24 h post-treatment (Table 8.). This means that at 6 and 24 h post-treatment the Friesian calves managed to store more Ca in their bones than the Bonsmara calves after treatment with tetracycline. Friesian calves had significantly ($P<0.05$) more Ca in their bones at 6 and 24 h post-treatment as compared to the previous hour (3 h). The Bonsmara calves had significantly ($P<0.05$) more Ca in their bones at 0 h pretreatment than at 3 and 6 h. Bone Ca concentration of the Bonsmara calves continued to be less at 24 h compared with the baseline samples at 0 h.

Cortical bone Ca:P ratio of the Friesian calves was significantly ($P<0.05$) lower than that of the Bonsmara calves at 6 h but at 24h post-treatment the cortical bone Ca:P ratio was reversed as the Friesian bone Ca:P ratio was significantly ($P<0.05$) higher (2.15) than the ratio of 1.72 observed in the Bonsmara calves (Table 8.). When the Ca:P ratio of the Friesian calves was compared through time, it was evident that at 3 h post-treatment, the ratio was significantly ($P<0.05$) lower than

the ratio observed at 0 h pretreatment and also at 6 and 24 h post-treatment (Table 8.).

4.2.2. Faecal minerals (fresh weight)

Findings from this experiment showed that the Friesian calves had significantly ($P<0.05$) less faecal P as compared to the Bonsmara calves 5 h after treatment with tetracycline, whereas, the Bonsmara calves conserved P at 24 and 30 h post-treatment. This shows that both the Friesian and Bonsmara calves can conserve P in terms of absorbing more P from their gut (Table 8.).

Friesian calves lost P at the same rate from pre treatment hour (0 h) until at 6 h post-treatment after the intravenous injection with tetracycline. At 24 h post-treatment, the Friesian calves lost significantly ($P<0.05$) more P (2.57 mg P g^{-1}) than at any time in the experiment while Bonsmara calves lost significantly ($P<0.05$) more P at 5 h (2.34 mg P g^{-1}) and at 6 h (2.23 mg P g^{-1}) post-treatment than anywhere else in the experiment (Table 9.).

At 3, 5 and 6 h post-treatment, Friesian calves had significantly ($P<0.05$) more Ca in their faeces than at 0 h pretreatment. Moreover, Bonsmara calves lost significantly ($P<0.05$) more Ca at 24 and 30 h post-treatment as compared to ½, 1, 2, 3, 5 and 6 h post-treatment (Table 9.). Faecal Mg of the Friesian calves was significantly ($P<0.05$) less than that of the Bonsmara calves throughout the experiment from pre treatment hour at 0 h until at 30 h after treatment with tetracycline (Table 9.).

At 24 h and 30h post-treatment, Friesian calves lost more Mg throughout their faeces than in previous periods (½, 1, 2, 3, 5 and 6 h). However, the Bonsmara calves lost significantly ($P<0.05$) more Mg through their faeces at 6, 24 and 30 h post-treatment as compared to values observed at ½, 1, 2, 3, and 5 h post-treatment (Table 9.).

4.2.3. Bone minerals (dry weight)

At 6 h post-treatment, Friesian calves had significantly ($P<0.05$) more P in their bones than the Bonsmara calves. This might be because less P was lost through their faeces as compared to the Bonsmara calves from 2 – 6 h even though the difference was significant ($P<0.05$) only at 5 h post-treatment (Table 11.). Friesian calves showed a significantly ($P<0.05$) higher bone P concentrations of 136.16 mg P g^{-1} at 3 h as compared to pre treatment value of 95.41 mg P g^{-1} at 0 h and post-treatment values of 116.89 mg P ^{-}g at 6 h and also 107.33 mg P g^{-1} at 24 h (Table 10.). Bonsmara calves showed a significantly ($P<0.05$) higher bone P concentration of 148.10 mg P g^{-1} at 3 h as compared to the pre treatment value of 95.14 mg P g^{-1} at 0 h, 91.06 mg P g^{-1} at 6 h and 98.13 mg P g^{-1} at 24 h post-treatment (dry weight) (Table 10.).

Cortical bone Ca of the Friesian calves was significantly ($P<0.05$) higher at 6 h and 24 h post-treatment as compared to the Bonsmara calves. Friesian calves showed significantly ($P<0.05$) lower bone Ca at 3 h as compared to pre treatment (0 h) and post-treatment concentrations at 6 and 24 h, whereas the cortical bone Ca of the Bonsmara calves remained significantly ($P<0.05$) lower than the pretreatment value at 0 h (Table 10.).

Cortical bone Mg of the Friesian calves did not differ through the experiment even though the Friesian calves showed less Mg in their faeces than that of the Bonsmara calves (Table 11.). At 6 h post-treatment the Ca:P ratio of the Friesian calves was significantly ($P<0.05$) lower than that of the Bonsmara calves. But it was reversed at 24 h where Friesian calves possessed a significantly ($P<0.05$) higher Ca:P ration than Bonsmara calves. The Ca:P ratio of the Friesian calves was significantly ($P<0.05$) lower at 0 h pre treatment than 3 h post-treatment but increased significantly ($P<0.05$) at 6 and 24 h (Table 10.).

4.2.4. Faecal minerals (dry weight)

The Friesian calves conserved more P from their faeces at 5 h post-treatment than the Bonsmara calves. The reverse was observed at 24 and 30 h post-treatment. Faeces taken from the same Friesian animals through time showed significantly ($P<0.05$) higher losses of P at 24 h (2.75 mg P g^{-1}) and 30 h (3.01 mg P g^{-1}) as compared to previous sampling periods (½, 1, 2, 3, 5 and 6 h post-treatment) and pre treatment (0 h). Faeces taken from the Bonsmara calves through time showed significantly ($P<0.05$) higher losses of P at 5 and 6 h post-treatment as compared to pre treatment (0 h) and post-treatment hours (½, 1, 2, 3, 24 and 30 h) (Table 11.).

Friesian calves significantly ($P<0.05$) reduced the loss of Ca throughout their faeces at 30 min as compared to the value observed at pre treatment hour (0 h). At 1 and 2 h post-treatment, Friesian calves continued to lose more Ca than the previous hour (Table 11.). At 3, 5 and 6 h post-treatment, Friesian calves lost more Ca compared to any other time in the experiment and were significantly ($P<0.05$) reduced at 24 and 30 h (Table 11.). Bonsmara calves showed significantly ($P<0.05$) more faecal Ca at 24 h and 30 h as compared to any other time during the experiment. As concentrations were compared between experimental hours, Bonsmara calves lost significantly ($P<0.05$) more Ca through their faeces at 24 and 30 h than at any other time during the experiment. This indicates that Bonsmara calves conserved Ca by preventing its loss throughout the faeces at ½ h (2.99 mg Ca g^{-1}) as compared to 0 h pre treatment (3.58 mg Ca g^{-1}) but then the concentrations elevated from 1, 2, 5 and 6 h post-treatment on a dry weight basis (Table 11.).

4.2.5. Bone minerals (ash weight)

At 3 h post-treatment, Friesian calves had significantly ($P<0.05$) less bone P (207.51 mg P g^{-1}) as compared to the Bonsmara calves (230.39 mg P g^{-1}) (Table

12.). However, at 6 h where the Friesian calves had significantly ($P<0.05$) more P in their bones than the Bonsmara calves (Ash weight). This might indicate that Bonsmara calves put more P in their bones 3 h post-treatment and Friesians 6 h post-treatment. At 3 h post-treatment, Friesian calves had significantly ($P<0.05$) more bone P than at 0 h pre treatment and 6 and 24 h post-treatment (Table 12.). This indicates that Friesian calves were storing more P in their bones at 3 h post-treatment than at 0 h pre treatment and 6 and 24 h post-treatment (Table 12.).

At 3, 6 and 24 h post-treatment, Friesian calves had significantly ($P<0.05$) more Ca in their bones as compared to Bonsmara calves. This indicates that Friesian calves were able to source more calcium from somewhere else in the body as the faecal Ca concentrations were also significantly ($P<0.05$) higher in the faeces (Table 13.) as compared to the Bonsmara calves.

There were no significant ($P<0.05$) differences in the cortical Bone Mg between Friesian and Bonsmara calves. However, as the bone sample of the same calves were compared across time, Friesian calves had reabsorbed significantly ($P< 0.05$) more Mg out of their bones at 3, 6 and 24 h post-treatment as compared to pre treatment (0 h). The same trend was observed with the Bonsmara calves. This indicates that Friesian and Bonsmara calves are able to reabsorb Mg from their bones after intravenous injection with tetracycline.

Cortical bone Ca:P ratio of the Friesian calves was significantly ($P<0.05$) higher than that of the Bonsmara at 24 h post-treatment. Cortical bone Ca:P ratio of the Friesian calves and Bonsmara calves were higher at 0 h pre treatment than at 3, 6, and 24 h post-treatment. Friesian calves had significantly ($P<0.05$) more overall Ca concentration as compared to the Bonsmara calves. All other cortical bone P, Mg, and Ca:P ratio remained the same.

4.2.6. Faecal minerals (ash weight)

Results in Table 13 show that faecal P content of the Friesian calves was significantly ($P<0.05$) lower than that of the Bonsmara calves at ½ hr, 1, and 5 hrs post-treatment. The reverse was observed at 2 and 24 h post-treatment (Table 13.). Friesian Calves had lost more P through their faeces at 24 h and at 30 h post-treatment as compared to 0 hr pre treatment as well as at previous sampling periods at ½, 1, 2, 3, 5, and 6 h post-treatment. Bonsmara calves lost more P at ½, 1, 5 and at 30 h post-treatment than at 0 h pretreatment and at 2, 3, 6 and 24 h on an ash weight basis (Table 13.). This however signifies that the breeds lost P at different times of the experiment.

The results given in Table 13 also show significant ($P<0.05$) differences in concentrations of faecal Ca between Friesian and Bonsmara calves. These differences were in any case larger in the faeces of Friesian as compared to that of the Bonsmara calves. Faecal Mg was however significantly ($P<0.05$) lower in the Friesian calves than in the Bonsmara calves at ½, 1, 2, 3, 5 and 6 h but more at 24 and 30 h post-treatment ash weight (Table 13.)

4.2.7. Blood minerals (mg/100 ml serum)

Values for blood P measured as mg/100 ml serum (Table 14.) were not different between Friesian and Bonsmara calves throughout the experiment. Blood P of the Friesian calves through time also showed no significant differences between pre treatment values at 0 h pretreatment and post-treatment values at ½, 1, 2, 3, 5, 6, 24 and 30 h. However, Bonsmara calves showed significantly ($P<0.05$) higher blood P at 0 h as compared to half an hour after treatment with tetracycline and 1, 2, 3, 5, 6, 24, and 30 h post-treatment.

Blood Ca concentrations of Friesian calves were significantly ($P<0.05$) more than those observed in Bonsmara calves throughout the experiment. Peak blood Ca

concentration was observed at 5 h after injection with tetracycline in Friesian calves which was higher than concentrations at 0 h pre treatment and at ½, 1, 2, 3, 6, 24 and 30 h post-treatment. Peak blood Ca in the Bonsmara calves was observed 0 h before treatment but then reduced at half an hour, 1, 2, 3, 5, 6, 24 and 30 h after treatment with tetracycline. Blood Mg concentrations of the Friesian calves were significantly ($P<0.05$) higher throughout the experiment (Table 14.) compared with the Bonsmara calves. Peak Mg concentration of the Friesian calves were observed half an hour after treatment and 30 h after treatment in the Bonsmara calves.

4.3. Sex Differences through time.

4.3.1. Bone minerals (fresh weight)

At 6 h post-treatment, male animals had significantly ($P<0.05$) less bone P than female calves, the overall means also showed that female animals had significantly ($P<0.05$) more cortical bone P (106.16 mg Pg^{-1}) compared with the males (90.83 mg Pg^{-1}). Male and female calves had significantly ($P<0.05$) more bone P at 3 h as compared to 0 h pre treatment, and also at 6 and 24 h post-treatment.

There were no significant ($P<0.05$) differences in the cortical bone Ca of the male and female calves at 3, 6, and 24 h. But as the comparisons were made on the calves through time, the cortical bone Ca of both male and female calves was significantly ($P<0.05$) less throughout the experiment compared with pre treatment values at 0 h (Table 15.). Male and female calves lost Mg from their bones as concentrations at 0 h pre treatment are significantly ($P<0.05$) higher than those observed at 3, 6 and 24 h post-treatment (Table 15.).

Male calves had a significantly ($P<0.05$) higher Ca:P ratio at 6 and 24 h after the IV injection with tetracycline than female calves. The overall Ca:P ratio of males (2.20) was significantly ($P<0.05$) greater than that of females (1.76) (Table 15.).

There were no significant (P<0.05) differences in bone thickness (mm) between male and female calves at 0, 3, 6 and 24 h. Through time, both male and female calves had significant (P<0.05) increases in bone thickness at 3, 6 and 24 h as compared to 0 h pre treatment. This indicates that both male and female calves are able to increase bone thickness post-treatment with tetracycline as compared to pre treatment values (Table 15.).

4.3.2. Faecal minerals (fresh weight)

Male calves showed significantly (P<0.05) less P in their faeces as compared to female calves at every sampling period from ½ h post treatmet to 30 h post-treatment including the overall mean. This indicates that female calves lose more P throughout their faeces as compared to male calves (Table 16.). Male calves lost more P through their faeces 30 h after treatment as compared to all other sampling periods, whereas female calves lost more P at all sampling periods after treatment compared to hour 0 before treatment with tetracycline (Table 16.).

Male calves lost significantly (P<0.05) more Ca through their faeces throughout the experiment as compared to the female calves (Table 16.). Male calves lost significantly (P<0.05) more Ca at 3, 5 and 6 h as compared to all other sampling periods of the experiment, whereas female calves significantly (P<0.05) lost more Ca at 24 h and 30 h post-treatment compared to all other sampling periods on a fresh weight basis.

Male calves lost significantly (P<0.05) more Mg through their faeces half an hour after treatment with tetracycline and at 1, 2 and 5 h post-treatment as compared to female calves. Male calves lost significantly (P<0.05) more Mg through the faeces at ½, 1, 5, 6, 24 and 30 h compared with pre treatment values at 0 h, and female calves lost significantly (P<0.05) more Ca in the faeces at 1, 2, 5, 24 and 30 h than was lost in the faeces at 0 h pretreatment (Table 16.).

4.3.3. Bone minerals (dry weight)

Male calves had significantly ($P<0.05$) less bone P at 6 h as compared to the female calves and when the overall means were compared, the female calves had significantly ($P<0.05$) more cortical bone P (119.34 mg P g^{-1}) compared to the cortical bone P found in the male calves (102.71 mg P g^{-1}) (Table 17.). Male and female calves had significantly ($P<0.05$) more P at 3 h than at any other time in the experiment (i.e. 0, 6 and 24 h). No significant differences were observed between cortical bone Ca concentrations of male as compared to female calves (Table 17.). However, in both groups of calves, the cortical bone concentrations of Ca were significantly ($P<0.05$) lower at all sampling periods compared to the pre treatment values.

As with Ca, the injection of tetracyclines reduced the concentration of Mg in cortical bone because the concentration of Mg in cortical bone was significantly ($P<0.05$) lower at all sampling periods post-treatment in both male and female animals compared with pre treatment values (Table 17.).

Bone Ca:P ratio was significantly ($P<0.05$) higher at 6 and 24 h post-treatment in male calves as compared to female calves. A lower male P to female P should have had a contributing factor to a higher male Ca:P ratio as compared to female calves (Table 17.).

4.3.4. Faecal minerals (dry weight)

Males tended to conserve more P in their body by reducing the amount of P that was lost in the faeces as compared to female calves. As observed in Table 18., male calves had significantly ($P<0.05$) less P in their faeces as compared to the female calves half an hour after treatment with tetracycline and at 1, 2, 3, 5, 6 and 24 h post-treatment. As comparisons were made between experimental times, male calves significantly ($P<0.05$) lost more P through their faeces at 30 h as

compared to the pretreatment value the opposite was true at 30 min, 1, 2, 3, 6 and 24 h post-treatment (Table 18.). Female calves had more P in their faeces at all sampling periods from 1h to 30 h post-treatment compared with the pre treatment value and the 30 minute value on a dry weight.

Male calves had significantly ($P<0.05$) more Ca in their faeces as compared to female calves throughout the experiment. This condition was observed even during the pre treatment hour at 0 h pre treatment. In males, peak Ca losses in the faeces on a dry weight basis were observed at 3, 5 and 6 h after treatment with tetracycline than at any other time in the experiment whereas in female calves, significant ($P<0.05$) peak losses were observed at 24 and 30 h after the treatment.

Male calves significantly ($P<0.05$) lost more Mg half an hour post-treatment and at 1, 2 and 5 h as compared to female calves. Looking at the overall means, male calves had significantly ($P<0.05$) less faecal P, but significantly ($P<0.05$) more faecal Ca and Mg compared with female calves on a dry weight basis (Table 18.). Table 21 shows that male calves had more blood P but less blood Ca and Mg compared with female calves. This indicated that male calves were reabsorbing the P from the faeces and keeping it in the blood, but the females were not reabsorbing the Ca and Mg from the faeces but rather losing it from the body in the faeces compared with the males (Table 18, Table 21.).

4.3.5. Bone minerals (ash weight)

Female calves had significantly ($P<0.05$) more P in their bones 6 h after treatment as compared to male calves. The samples taken from the calves were tested through time and male calves had significantly ($P<0.05$) more P in their bones at 3 h than at 0 h (pre treatment), 6 h and 24 h post-treatment as a result of the P conserved at 30 min, 1, 2 and 3 h from the faeces (Table 20.). Female calves also reflected higher P values in their bones at 3 h for the same reasons as in male calves above (Table 20.) on the ash weight basis.

Values for bone Ca based on mg Ca g^{-1} (Table 19.) were not significantly ($P<0.05$) different between male and female calves. Male calves had significantly ($P<0.05$) less Ca in their bones at 3 and 6 h as compared to pre treatment hour (0 h) and at 24 h post-treatment. Whereas, female calves had significantly ($P<0.05$) less Ca 3, 6 and 24 h after tetracycline was injected (Table 19.).

Male calves had more bone Mg 3 h after treatment with tetracycline than female calves on an ash weight basis. The bone Mg concentrations at 0 h pre treatment for both male and female calves were significantly ($P<0.05$) higher than values observed at 3, 6 and 24 h post-treatment. This would indicate that the animals were losing bone Mg as a result of the injection of tetracycline.

The Ca:P ratio was significantly ($P<0.05$) higher in male calves at 24 h as compared of female calves. This might have led to a significantly ($P<0.05$) higher bone thickness of males as compared to that of female calves at 6 and 24 h post-treatment (Table 19.). Male calves had a significantly ($P<0.05$) lower Ca:P ratio at 3 h than at 0 h pre treatment and at 6 and 24 h post-treatment. This however was influenced by a higher bone P level at 3 h as compared to other hours of the experiment and a lower Ca level at 3 h than anywhere else in the experiment. This explains why Ca can influence lower Ca:P ratio (Table 19.) on the ash weight basis. When P levels are higher and Ca levels are lower, the Ca:P levels will be lower and vice versa as shown in Table 19..

4.3.6. Faecal minerals (ash weight)

It was only at 2 h post-treatment that male calves had significantly ($P<0.05$) more P in their faeces than female calves. Half an hour after treatment with tetracycline male calves retained more P in their body by reducing the P lost throughout their faeces than did female calves. This was also observed at 1, 6, 24 and 30 h post-treatment (Table 20.). Male calves had significantly ($P<0.05$) less P in their faeces 3 h after treatment with tetracycline and 6 and 24 h compared to pre treatment

value at 0 h and also had significantly (P<0.05) less P in their faeces compared to half an hour after treatment and 1, 2, 5, and 30 h post-treatment (ash weight).

It was only at 30 h post-treatment where male calves had significant (P<0.05) less Ca and Mg in their faeces than anywhere else in the experiment as compared to female calves. Male calves lost significantly (P<0.05) more Ca and Mg through their faeces at 30 min, 1, 2, 3, 5, 6 and 24 h than female calves. These results agree with the previous findings related to mineral homeostasis in which faecal P, Ca and Mg responded differently to various influences. In this regard, the IV injection of tetracycline led to more P being spared than Ca and Mg at 30 min, 1, 6, 24 and 30 h whereas Ca and Mg were lost at the same time except at 30 h post-treatment (Table 20.). At 30 h post-treatment the body's homeostatic mechanisms must have been able to adjust to the injection of the tetracycline and the body was again able to reduce the loss of Ca and Mg through the faeces among the males compared with the females. Further research needs to be done to explain why this happened.

4.3.7. Blood minerals (mg/100 ml serum)

The mean blood P of male calves were significantly (P<0.05) higher than that of female calves at 30 min, 1 h and 24 h post-treatment. The P concentration was lower at 30 min, 2, 3, 5, 6, 24 and 30 h post-treatment as compared to 0 h pre treatment but was significantly (P<0.05) higher at 1 h post-treatment compared with 0 h pre treatment. Significantly lower (P<0.05) P values were observed at all sampling periods except 30 h post-treatment in female calves as compared to 0 h pre treatment (Table 21.).

Blood Ca concentrations of the male animals were significantly (P<0.05) lower than those of the female calves at 30 min, 1, 24 and 30 h post-treatment. It was only at 5 h post-treatment that the blood Ca concentration of male calves was significantly (P<0.05) higher than that of the female calves. The lowest blood Ca

concentration of male calves was observed at 30 h post-treatment. The highest blood Ca concentration was observed 5 h post-treatment as compared to anywhere else in the experiment. Blood Ca concentration of female calves was the highest at 0 h pre treatment but was also high at 24 h post-treatment (Table 21.).

Blood Mg concentration of male calves was significantly lower than that of the female calves at 24 and 30 h post-treatment. The opposite was true at 2, 3, and 5 h post-treatment where the male calves had significantly ($P<0.05$) more blood Mg compared to female calves (Table 21.).

The highest blood Mg concentration of male animals was observed at 5 h post-treatment and the lowest at 30 h post treatment. The highest blood Mg concentration of female animals was observed at 24 h post-treatment and the lowest was observed at 3 hours of the experiment (Table 21.).

4.4. Discussion (time effect)

4.4.1. Experimental treatment group (time effect)

4.4.1.1. Bone (fresh, dry and ash weight, Ca:P ratio, bone thickness)

Calves in the treated group had significantly higher bone P at 6 h than those in the control group on a fresh, dry and ash weight basis and at the same time, higher cortical bone thickness but no significant differences between bone Ca:P ratio were observed (Tables 1, 2, 3). Phosphorus helps build bone density and maintains bone mass in conjunction with other minerals, such as calcium (Karn, 2001). No significant ($P<0.05$) findings were found in bone Ca and Mg concentrations between the treated and the control animals through time on a fresh and dry weight basis (Table 1. and 3). On the ash weight basis, treated calves resorbed Ca out of the bones at 3 h post-treatment. The reverse was observed at 6 h post-treatment with tetracycline while bone thickness was also higher in the treated calves than the control calves at 6 h (Table 5.). These results

would indicate that in response to the chelation of the blood Ca by the tetracycline (Vernillo and Rifkin, 1998), the body pulled Ca out of the bone in the first 3 hours, because there was significantly less bone Ca at 3 h in treated calves compared with control calves. But at 6 h post-treatment, there was significantly ($P<0.05$) more bone Ca in treated calves compared with control calves indicating that the body homeostatic mechanisms were able to respond within the next 3 hours and replace the Ca taken out of the bone (Table 5.). This is supported by significantly ($P<0.05$) lower blood Ca in treated calves compared with control calves at 1, 2 and 3 h and significantly higher blood Ca in treated calves compared with control calves at 6 h (Table 7.). Further support for this is observed in significantly higher faecal Ca in the treated calves compared with control calves at 1 and 3 h but significantly ($P<0.05$) lower faecal Ca in the treated calves compared with control calves at 6 h (Table 2.). In response to the chelation of Ca in the blood by the tetracycline, the calves were pulling Ca out of the bone at 3 h and putting it in the blood but the blood levels were being depleted by loss of Ca in the faeces at 1 to 3 h. But in only 3 h, the body was able to respond to this disruption in Ca homeostasis so that by 6 hr, the cortical bone Ca concentrations were significantly ($P<0.05$) higher in treated calves compared to control calves as a result of a significantly ($P<0.05$) decreased loss of Ca in the faeces at 6 h and a significantly ($P<0.05$) increased blood Ca concentration at 6 h.

4.4.1.2. Faeces (fresh, dry and ash weight)

It is evident from this research that animals are able to respond quickly to disturbances in mineral homeostatic mechanisms by absorbing minerals from the colon back into the blood or by secreting minerals into the colon from the blood. Male calves were able to decrease the loss of Ca in the faeces within 30 minutes of the IV injection of tetracycline. Faecal Ca was significantly ($P<0.05$) lower at 30 minutes after treatment compared with pre treatment values (Table 18.). At the same time, faecal Mg was significantly ($P<0.05$) higher 30 minutes after treatment compared with pre treatment values among male animals (Table 18.). Friesian

calves were able to significantly (P<0.05) reduce the amount of P lost in the faeces at 30 minutes after treatment with IV tetracycline compared with pre treatment values (Table 13.). All three results indicate that the animals were able to adjust the amount of minerals lost in the faeces within 30 minutes, something that could only happen if the exchange of minerals between gut and blood occurred at the level of the colon. Further research needs to be done to investigate the ability of animals to regulate loss of mineral by way of absorption and excretion via the colon.

4.4.1.3. Blood (mg/100 ml serum)

Blood P of the treated calves was significantly (P<0.05) lower compared to the controls at 24 and 30 h post-treatment with tetracycline while blood Ca was higher. Similar results were reported by Pugh *et al* (1994) where increase in serum P concentrations were accompanied by a decrease in serum Ca concentrations at parturition in cows fed broiler litter which is said to suppress blood Ca. Riviere *et al.* (1999) reported the ability of tetracyclines to chelate Ca in the blood thereby leading to lower concentrations of Serum Ca. This was also found in this study where blood Ca concentrations were significantly (P<0.05) lower at 1, 2 and 3 h at the initial stages of the experiment as compared to the controls (Table 7.). The animal's homeostatic mechanism was triggered to respond to the chelation of Ca by tetracycline and therefore bone was used as a source of Ca to replace chelated Ca in the blood (Table 5.). Tetracycline seems to affect bone resorption of Ca, and based on the serum and bone concentrations, it is assumed that tetracycline causes an increase in bone resorption of Ca, rather than deposition.

4.4.2. Experimental breed groups (time effect)

4.4.2.1. Bone (fresh, dry, and ash weights, Ca:P ratio, bone thickness)

Friesian calves had significantly (P<0.05) higher overall means for P, Ca and cortical bone thickness compared with Bonsmara calves. Cortical bone Mg was

however not significantly ($P<0.05$) higher. These results agree with those of Beighle *et al.* (1994) where more P was found in dairy breeds than in beef and mixed breeds.

4.4.2.2. Faeces (fresh, dry, and ash weights)

As revealed in this research, animals were able to regulate very quickly the amount of P, Ca and Mg lost or conserved through the faeces at different times of the experiment. This has important implications for assisting the animal to conserve the loss of minerals from the body through the faeces especially in deficiency situations such as in times of drought or any time that the dietary minerals are low. However the Friesian calves were losing more Ca from the body through faeces as reflected by significantly ($P<0.05$) higher faecal Ca concentrations at 30 min until 30 h post-treatment as compared to the Bonsmaras. This clearly indicated that Friesians were getting rid of excess Ca by losing it through the faeces and at the same time, conserving Mg by absorbing it from the gut into the body due to the tetracycline injection.

4.4.2.3. Blood (mg/100 ml serum)

No significant findings were observed in blood P between the Friesian and Bonsmara calves but Ca and Mg were significantly ($P<0.05$) higher throughout the experiment in Friesian calves compared with the Bonsmara calves (Table 14.). In the current study, blood P concentrations were not influenced by the biological type of cattle through time, however blood Ca and Mg were affected (Table 14.). Bonsmaras had significantly less blood Ca during the entire experiment compared with the Friesians, and the Bonsmaras had a significantly lower overall mean blood Mg concentration for the entire experiment (Table 14.). The effect of tetracycline chelation on calcium must have suppressed more calcium in the Bonsmara calves than in the Friesian Calves (Table 14.).

4.4.3. Experimental sex group (time effect)

4.4.3.1. Bone (fresh, dry and ash weight, Ca:P ratio and bone thickness)

Male animals had significantly less bone P but tended to have more bone Ca and Mg than female animals throughout the experiment as shown by the overall means. Similarly, Beighle *et al.* (1994) found the same significant results when they compared the bone P concentration between male and female animals. Similarly, the overall mean for bone Ca:P ratio of male animals was higher than that of females with no differences between bone thickness although the males tended to have thicker bones. From the results of this research it would appear that the injection of tetracycline iv caused a significant decrease in both cortical bone Ca and Mg based on significantly ($P<0.05$) less cortical bone Ca and Mg at all sampling periods in both male and female animals at post-treatment compared with pre treatment values at hour 0 (Tables 15, 17, 19). The same was not true for cortical bone P.

The results of several investigations have indicated that the injection of tetracycline IV ties up blood Ca and can have serious effects on levels of circulating blood Ca. (Newman and Frank, 1976; White and Pearce, 1982; Martin, 1985; Lambs *et al.*, 1988, Riviere *et al.*, 1999). Results from this research indicate that this drop in available Ca in the blood caused the body homeostatic mechanisms to pull Ca out of the bone to make it available in the blood. In addition, this research indicate that the tetracycline had the same effect on Mg homeostasis. If this is true, the concurrent injection of tetracycline in animals with hypomagnesemic tetany could have beneficial effects by pulling Mg out of bone and putting it in the blood.

Further research needs to be conducted in this area, for instance, by tying up the Mg in the rumen experimentally thereby producing a hypomagnesemia, and then treating with IV tetracyclines instead of IV magnesium.

4.4.3.2. Faeces (fresh, dry and ash weight)

On a fresh weight basis the mean faecal concentration of P was significantly ($P<0.05$) greater in female calves compared with male calves at each sampling period except at 30 h, and the overall mean faecal P was significantly ($P<0.05$) higher for the female calves compared to the male calves on a fresh weight basis (5.73 vs 4.65 mg P g^{-1}), on a dry weight basis (6.21 vs 4.95 mg P g^{-1}) and on an ash weight basis (36.02 vs 30.69 mg P g^{-1}).

On a fresh, dry and ash weight basis, the males had significantly ($P<0.05$) more Ca in their faeces compared with the females at every sampling period after the iv injection of tetracycline and for the overall mean (12.41 vs 6.61 mg Pg^{-1}).

The overall mean for the faecal Mg was significantly ($P<0.05$) higher in the males (2.11mg Mg g^{-1}) compared with the females (1.25 mg Mg g^{-1}) and the faecal Mg was consistently higher among males compared to the females at each sampling period.

Results reported inTables 9, 11, 13 show conclusive evidence of the ability of the homeostatic mechanisms in the colon and lower gut to move P, Ca and Mg into and out of the gut in response to IV injection of tetracyclines. Based on previous reports that the iv injection of tetracycline causes the chelation of Ca by the tetracycline and an abrupt reduction of available Ca in the blood (Lambs *et al.*, 1988; Riviere *et al.*, 1999), it is supposed that the injection of tetracyclines in this experiment caused the same abrupt reduction of available Ca in the blood. Based on these results, the response of the body's homeostatic mechanisms to the drop in blood Ca was different for males and females and was different for P, Ca and Mg (Tables 16, 18, 20). It would be expected that a drop in blood Ca would stimulate Ca homeostatic mechanisms but it is less expected to see the P and Mg homeostatic mechanisms respond with significant differences in faecal P and Mg

concentrations in the faeces. This is a clear indication of the interaction of Ca, P and Mg in the overall effort of the body to maintain homeostasis.

A significant ($P<0.05$) decrease in faecal P of male as compared to female animals throughout the experiment indicates a P sparing effect of the tetracyclines in the male animals. Male animals tended to spare more P by reducing P lost in their faeces through time and throughout the experiment compared with female calves on a fresh, dry and ash weight basis. The opposite was true of faecal Ca and Mg. This however explains why males had more blood P half an hour and 1 h after treatment with tetracycline than females (Table 21.) because the males had significantly ($P<0.05$) less faecal P compared to the females at half an hour and 1 h after treatment (Table 16, 18 and 20).

One would have expected that there would be an increase in bone P among the male animals as a result of the significant ($P<0.05$) decrease in faecal P and significant ($P<0.05$) increase in blood P at one half and 1 h post-treatment. But the opposite was true with significantly ($P<0.05$) less bone P among males (86.34 mg P g^{-1}) compared with females (121.61 mg P g^{-1}) at 6 h post-treatment on a dry weight basis (Table 17.). It was observed that the effect of the IV tetracycline on the body homeostatic mechanisms was so great that the body was unable to move P into the bone but rather had to pull P from both the bone and faeces to maintain a higher blood P in response to those mechanisms. It is noteworthy that at the same time, blood Ca was significantly ($P<0.05$) lower among males at one half and 12 h post-treatment (7.52 and 6.56 mg Ca g^{-1}) compared with females (8.67 and 8.10 mg Ca g^{-1}). This is in agreement with Beighle *et al.*, (1994 and 1997) who found that the homeostatic mechanisms for Ca and P are different so that when P is being incorporated into bone, Ca is being resorbed and when Ca is being absorbed into bone, P is being resorbed.

In this research, blood P was significantly ($P<0.05$) higher in males compared to females but blood Ca was significantly ($P<0.05$) lower in males compared with

females (Table 21.). Faecal P was significantly (P<0.05) lower in males compared with females but faecal Ca was significantly (P<0.05) higher in males compared with females (Tables 16, 18, 20.). In addition, the overall means for cortical bone P was significantly (P<0.05) lower in males compared with females but the overall means for cortical bone Ca were not significantly different (Table 17.).

4.4.3.3. Blood (mg/100 ml serum)

In general, blood P was consistent in being significantly (P<0.05) higher in males compared with females at various stages of the experiment, but blood Ca was inconsistent with significantly (P<0.05) lower blood values among males at 30 min and 1 h but significantly (P<0.05) higher values among males at 5 h compared with females and then significantly (P<0.05) lower values among males compared with females again at 24 and 30 h. This was also true for Mg with male calves having significantly (P<0.05) higher blood Mg at 3 and 5 h post-treatment compared with female calves but at 2, 24 and 30 h blood Mg was significantly (P<0.05) lower in male calves compared with female calves (Table 18.).

4.5. Interaction of minerals

4.5.1. Introduction

Correlation is a measure of the relationship between two or more variables. Correlation coefficients can range from -1.00 to +1.00. The value of -1.00 represents a perfect negative correlation while a value of +1.00 represents a perfect positive correlation and a mid value of 0.00 represents a lack of correlation.

Correlation determines the extent to which values of the two variables are "proportional" to each other. Proportion means linearly related. In this research, correlation was employed to compare the relationship between sample variables of the main factors (e.g. bone minerals vs blood minerals vs faecal minerals) and also within variables of the same sample of the main factors (e.g. Bone P vs bone Ca

vs bone Mg) of the experimental groups. Bone and faecal concentrations are expressed in a dry weight basis.

This has helped the researcher to identify the pattern in which the sample minerals are interacting in proportion due to the IV injection of tetracycline given to calves and also whether an increase in one variable leads to a decrease in the other variable or vice-versa. This will add to the available literature on the animal homeostatic mechanism. Dias *et al.* (2006) showed that P and Ca metabolism are closely related as evidenced by the ratio of these minerals in the bidirectional flow between plasma and bone and between plasma and tissues. The revised model provided an improved prediction of the P and Ca metabolism that can then be used to assess mineral requirements and to estimate losses to the environment (Dias *et al.* 2006).

4.5.2. Treated group

4.5.2.1. Between sample interactions

4.5.2.1.1. Phosphorus

Mean bone and blood P of the treated calves acted in divergence with faecal P as they were decreasing as faecal P was increasing (Fig 1.). A Correlation coefficient of $r = -0.76$, $P<0.05$ between bone P and faecal P and $r = -0.47$, $P<0.05$ between blood P and faecal P indicate the strong negative correlation between these variables. This means that an increase in one variable leads to a decrease in the other variable. This is the reason why researchers emphasise the importance of these minerals in bones and blood to obtain a correct picture of the mineral concentrations of the entire body (Beighle *et al.* 1994, Mosel *et al.* 1993).

Blood P and bone P showed a weak positive correlation ($r = 0.12$, $P<0.05$) indicating a relationship between these two variables in this research (Fig 1). Results from this research agree with the results of Beighle (1999) that P was removed from the bone as a result of the anionic excess and resulted in higher

concentration of serum P and faecal P due to the anionic diets as compared to cationic diets.

4.5.2.1.2. Calcium

Bone and blood Ca followed each other as faecal Ca deflected from the two. A correlation of r= -0.89, (P<0.05) for bone related to faecal Ca and r= -0.99, P<0.05 for blood compared to faecal Ca is a clear indication that a decrease in faecal Ca will lead to an increase bone and blood Ca (Fig 2). A strong positive correlation of r= 0.83, P<0.05 between blood and bone Ca shows that both variables are strongly related as an increase in the concentration of blood leads to an increase in the bone concentrations (Fig 1). In this instance, animals in the treated group lost more Ca throughout their faeces 3 hr after the IV injection with tetracycline, but then recovered the lost Ca at 6hr post-treatment as the faecal concentrations decreased and those of blood and bone increased (Fig 2). Desmecht *et al.* (1995) reported a triphasic pattern of calcium decay with an initial fast drop then a plateau and lastly, a relatively fast drop again which agrees with the results found in this research.

4.5.2.1.3. Magnesium

There was a strong negative correlation of r= -0.90, P<0.05 between bone and faecal Mg and a correlation of r= - 0.33, P<0.05 between bone and blood Mg. But a positive correlation (r= 0.67, P<0.05) was observed as faecal and blood Mg were compared. It has been observed in this study that the concentration of blood Mg remained constant, and could be the affinity of tetracycline to chelate calcium ion. The same results were also observed by Jorgersen *et al.* (1999) while using Na_2EDTA to chelate calcium ions in the blood.

4.5.2.2. Between mineral interactions

4.5.2.2.1. Bone minerals

Figure 4 seems to indicate a complete dissimilarity between bone P and bone Ca when animals are given an IV injection of tetracycline as a treatment. When bone P increases at 3 h, bone Ca reduces and when bone P decreases at 6 h, bone Ca increases. This however indicates the ability of the animal body to regulate these minerals in the bones so the Ca:P ratio could be maintained. Bone Mg showed a reducing trend (Fig 4). Bone mineral content is the result of the balance between rates of bone accretion and rates of bone resorption. A correlation of r= -0.61, $P<0.05$ between bone P and Ca indicate a strong negative relationship between these variables, meaning that the increase in one variable strongly affected the reduction of the other variable on the treated group (Fig 4). Beighle, (2000) indicated that P resorption from bone is independent of Ca and Mg resorption and the results from this research strongly support this observation. This negative correlation supports the reports in the literature of the independent movement of Ca and P in and out of bone (Beighle *et. al.* 1995 and 1999).

4.5.2.2.2. Faecal minerals

As in bone samples at 3 and 6 h (Fig 4), it is an important finding that faecal P and Ca also acted in dissimilarity. When faecal P is depressed, faecal Ca is increased at 6 and 24 h (Fig 5). This however supports the highest bone minerals especially P that is increased at the times where faecal P is decreased. This indicates the ability of the animal body to put more P into the body by absorbing P from the large intestine through blood and store it in the bone. When bone was significantly ($P<0.05$) higher in treatment compared with control animals at 6 hr, the faecal P was significantly ($P<0.05$) less in treatment animals compared with controls at 6 h. However, Ca was lost more than P throughout the faeces (Fig 5). A strong negative correlation coefficient of r= -0.87, $P<0.05$ is an indication that a decrease in faecal P was coupled with an increase in faecal Ca of calves treated with an IV

injection of tetracycline. Faecal Mg and P displayed a weak positive correlation (r= 0.33, P<0.05) and a weak negative correlation (r= -0.17, P<0.05) as compared to faecal Ca. Less has been said about the relationship between Mg, Ca and P in faeces as it is observed in the current study that Mg tends to increase or decrease as Ca or P do the same (Fig 5).

4.5.2.2.3. Blood minerals

Interaction of bone and faecal Ca and P tend not to affect blood P and Ca as shown by a weak negative correlation of r= -0.08, P<0.05 where the correlations for bone, faecal and blood are exactly the same. This is somehow not surprising as P and Ca can be resorbed and absorbed from the bone and pass through blood to the faeces or vice versa. Blood P and blood Mg showed a weak positive correlation of r= 0.21, P>0.05 that was not significantly different. The same situation is observed between blood Mg and blood Ca with a correlation of r= 0.35, P<0.05 (Fig 6). Mg plays an important role in the animal body that includes being part of a large number of enzymes for example ATPase, kinases and phosphatases required for enzyme activation. Mg metabolism is not under the control of hormonal regulation (Martins and Schweigel, 2000). That might be the reason for the abrupt and unpredictable Mg increases and decreases in blood and bone samples in this study while P and Ca increase or decrease.

It is clearly demonstrated that both Ca and P decreased at 3 h post-treatment with tetracycline but then, blood P showed much less variation during the trial compared to Ca (Fig 6). The chelating of the blood Ca must have been responsible for the decrease in blood Ca but did not have the same effect on blood P. Then at 5 hr a feedback mechanism was probably responsible for the significantly (P<0.05) increase in blood Ca. A further investigation is needed between faecal, blood and bone values at 48 and 72 h or longer to see the long-term effect of IV injection of tetracycline (Fig 6).

4.5.3. Friesian group

4.5.3.1. Between sample interactions

4.5.3.1.1. Phosphorus

Bone and blood P of the Friesian calves on a dry weight seems to be related. This is indicated by the positive correlation (r=0.41, P<0.05). The opposite was true when bone and faecal P were correlated (r=-0.38, P<0.05) and also between faecal P and blood P with a correlation of r= -0.54, P<0.05 (Fig 7). This however indicates that the P found in the blood is incorporated into the bone for bone formation in the Friesian calves. Hence, absorption of P from the colon resulted in a negative relationship between blood and bone P and faecal P (Fig 7). There was a suspicion on the ability of blood P concentration to accurately portray P status of the animal and highlighted the ability of the animal body to maintain blood concentration of P from its stores in the bone (Cohen, 1973).

4.5.3.1.2. Calcium

Mean bone Ca and blood Ca of the Friesian calves showed the same trend throughout the experiment. This is pointed out by the strong positive correlation (r= 0.72, P<0.05) between bone and blood Ca. This indicates the ability of Friesian calves to regulate both blood and bone Ca in these 2 variables as an increase in blood Ca leads to an increase in bone Ca. However, faecal Ca acted in divergence as compared to both the blood and bone Ca and supported by a strong negative correlation of r= -0.43, P<0.05 compared to bone Ca and r= -0.58, P<0.05 as compared to blood Ca.

It is important to note that at 3 h post-treatment with tetracycline, Friesian calves tended to resorb Ca from the bones through the blood and secreted into the colon for elimination throughout the faeces (Fig 8). This was however reversed at 6 h as the animals increased the Ca in the blood and hence into the bone with a decrease in faecal Ca concentration (Fig 8).

4.5.3.1.3. Magnesium

Bone Mg of the Friesian Calves showed a strong negative correlation (r= -0.84) when compared to the faecal Mg and a weak negative correlation (r= -0.11, P>0.05) as compared to blood Mg. But as faecal Mg was compared to the blood Mg, a positive relationship was observed (r= 0.53, P<0.05). This might indicate the ability of Friesian Calves to increase blood and faecal Mg with a reduction in bone Mg and vice versa when animals are injected with an IV injection of tetracycline (Fig 9).

4.5.3.2. Between mineral interactions

4.5.3.2.1. Bone minerals

Bone P and Ca of the Friesian calves acted in divergence as an increase in Ca lead to a decrease in P (Fig 10). This is verified by a strong and significant negative correlation possessed (r= - 0.80, P<0.05) that was significant. Bone P and Mg also showed a weak but positive correlation of r= 0.11, P<0.05, an indication that they might somehow be related. This relationship between bone P, Ca and Mg has been investigated by different scientists and they have pointed out that bone P and Ca act in divergence due to the ability of animals to respond to external influences as diets and supplements (Block, 1984), ammonium chlorides (Beighle, 2000), Ammonium sulphate (Motsei and Beighle, 2006) and tetracycline infusion in this study.

Friesian Calves were able to increase bone P at 3 h with a decrease in bone Ca. The opposite was true at 6 h post-treatment as bone P reduced with an increase in bone Ca (Fig 10).

4.5.3.2.2. Faecal minerals

Compared to faecal Ca, faecal P and faecal Mg acted in divergence with a strong negative correlation coefficient of r= -0.83, P<0.05 for P versus Ca and r= - 0.89, P<0.05 for faecal Ca versus Mg (Fig 11). Faecal P and Mg showed a strong positive relationship (r= 0.96, P<0.05). Faecal P increased from 0 h until at 1 h and began to reduce until at 3 h, the opposite was true with faecal Ca. The faecal P decreased at 3 h and 5 h but increased at 5, 6 and 24 h of the experiment (Fig 11). The opposite was also true with faecal Ca. Faecal Mg acted like faecal P in this experiment.

4.5.3.2.3. Blood minerals

Blood Ca and P indicated a weak negative correlation that explained a divergence between the two experimental variables of the Friesian calves (r= - 0.25, P<0.05). Blood P and Mg showed a weak positive correlation (r= 0.34, P<0.05). This might mean that the two variables were related even though the relationship was weak. Blood Ca and Mg showed a stronger relationship (r= 0.61, P>0.05). Blood P showed a stable flow from 0 h pretreatment until 24 h post-treatment, but blood Ca and Mg showed a reducing trend until 2 h, increased until 5 h and reduced again at 6 with an increase at 24 h (Fig 12). Blood minerals are considered to be a non-reliable indicator of mineral homeostasis due to fluctuations caused by many external and internal factors (Beighle *et al.,* 1994; Uysal,1998).

4.5.4. Bonsmara group

4.5.4.1. Between sample interactions

4.5.4.1.1. Phosphorus

Bone P of the Bonsmara calves was positively related to blood P (r= 0.41, P<0.05) but negatively correlated to faecal P (r= -0.88, P<0.05). Blood P was also negatively related to faecal P (r= -0.84, P<0.05). Bone P increased from 0 h pre

treatment to 3 h post-treatment and decreased thereafter at 6 h (Fig 13). Faecal P increased from 0 h to 6 h post-treatment but decreased at 24 h. Bone P was resorbed after 3 h and up until 24 h post-treatment when compared with the concentration at 3 h (Fig 13).

4.5.4.1.2. Calcium

Bone Ca of the Bonsmara calves decreased from 0 h until at 24 h post-treatment, while faecal Ca decreased until 3 h post-treatment and then increased at 6 h and 24 h post-treatment, while blood Ca showed a tendency to decline throughout the experiment (Fig 14). Bone and faecal Ca were not related as shown by a significant negative correlation of r= -0.65, P<0.05. Bone Ca and blood Ca were strongly positively correlated (r= 0.95, P<0.05). Faecal Ca and blood Ca were negatively correlated (r= -0.341, P<0.05). Bonsmara calves lost more Ca throughout their faeces beginning at 6 h after the onset of the experiment compared with previous sampling periods (Table 9.). The Ca lost in the faeces could have come from the Ca resorbed from bone as bone concentrations of Ca were reducing throughout the experiment dry weight basis (Fig 14).

4.5.4.1.3. Magnesium

Bone and blood Mg were reducing from 0 h until at 3 h post-treatment. But this trend changed after 3 h when bone Mg continued to reduce while blood Mg increased until 24 h. Mg was spared from being lost through the faeces from 0 h until at 3 h and increased steadily until 6 h to 24 h post-treatment (Fig 15). Bone and faecal Mg were not correlated (r= -0.47, P<0.05). The same results were also observed when bone and blood Mg were compared (r= -0.68, P<0.05). The results here indicate that Mg was resorbed from bone of Bonsmara calves and lost through faeces 6 h after treatment with tetracycline (Fig 15).

4.5.4.2. Between mineral interactions

4.5.4.2.1. Bone minerals

Bone Ca of the Bonsmara calves reduced with reduction in bone Mg. But bone P increased until at 3 h but then declined until at 6 h. It continued to increase again at 24 h post-treatment (Fig 16). Bone P and Ca of the Bonsmara calves were weakly correlated (r =0.06, P>0.05) and the relationship was also not significant. This means that the two variables are not related. The same results were also observed as bone P was compared with bone Mg (r= 0.01, P>0.05). But as bone Ca was compared with bone Mg, it was observed that the two variables were strongly positively correlated (r= 0.98, P<0.05). This indicates that bone Ca reduces with reduction in bone Mg and increases with increase in bone Mg. The opposite was true for bone P versus bone Ca and Mg (Fig 16). In the treatment of hypomagnesemic tetany and milk fever, blood Ca and Mg levels could be increased by treatment with tetracycline. It was observed in this experiment that tetracycline was able to pull Ca and Mg out of the bone at the same time (Fig 16). Motsei and Beighle (2006) also found the concentration of magnesium (Mg) and calcium (Ca) in bone responding in the same direction at a lower concentration of ammonium sulphate fed as a feed supplement. Further research is needed to investigate the interactions of these bone minerals.

4.5.4.2.2. Faecal minerals

Faecal Ca of Bonsmara calves was lower at ½ h and increased at 2 h. It remained stable until at 5 h and steadily increased until at 24 h post-treatment. Faecal Mg declined until at 2 h and increased until at 24 h. Faecal P remained stable until at 3 h and increased at 5 h and decreased until at 24 h post-treatment (Fig 17). Faecal P and Ca had a negative correlation (r= -0.54, P<0.05) and there was a strong negative correlation when faecal P was compared to faecal Mg (r= - 0.70, P<0.05). But as faecal Ca was compared to faecal Mg, it was observed that the two variables were strongly positively correlated (r= 0.79, P<0.05). Fig 16 shows

that bone Ca and bone Mg had a strong positive correlation (r= 0.98, P<0.05) with faecal Ca and Mg showing the same trend. It might be concluded that Ca and Mg that was resorbed from bone was the Ca and Mg that was lost in the faeces (see Fig 16 and 17).

4.5.4.2.3. Blood minerals

Blood P of Bonsmara calves were significantly (P<0.05) lower than pre treatment values throughout the experiment, but there were no significant differences in blood P values at any of the sampling periods compared with pre treatment values among the Friesian calves (Table 14.). Blood Ca remained stable until at 24 h in the Bonsmara calves. Blood Mg fluctuated throughout the experiment (Fig 18). Blood P and Ca were shown to be strongly related (r= 0.71, P<0.05) but as compared to blood Mg, blood P showed a negative correlation (r= -0.46, P<0.05). Blood Ca was not correlated with blood Mg (r= 0.15, P<0.05).

These results indicate that an increase in blood P leads to an increase in blood Ca but not as the two are compared to blood Mg. It is therefore concluded that Ca in the blood was lost in the faeces at 5 h until at 24 h (Fig 17) when faecal P reduced at that time.

4.5.5. Male group

4.5.5.1. Between sample interactions

4.5.5.1.1. Phosphorus

Bone P of male calves increased from 0 h pre treatment with tetracycline until 3 h but then reduced again at 6 h and then slightly increased at 24 h (Fig 19). Faecal P showed to slightly reduce from 0 until at 3 h and slightly increased at 6h and then reduce again at 24 h post-treatment (see Fig 19).

Bone P showed a negative correlation (r= -0.42, P<0.05) as compared with faecal P. As bone P and blood P were compared, it was noted that the two were weakly correlated (r= 0.04, P>0.05). As blood P was compared to faecal P it was observed that they were positively correlated (r= 0.29, P>0.05) even though the relationship was weak and not significant. The results indicate that when bone P concentration increases, faecal P reduces in male calves based on the significantly (P<0.05) negative correlation stated above (Fig 19).

4.5.5.1.2. Calcium

A sharp drop in bone Ca concentrations of male calves was observed from 0 h pretreatment with tetracycline followed by an increase at 6 h and decreased at 24 h (Fig 20). Faecal Ca increased at 3 h and decreased at 6 h and 24 h post-treatment while blood Ca decreased at 3 h, increased at 6 h and decreased at 24 h post-treatment (Fig 20). No relationship (r= 0.01, P>0.05) was observed between bone and faecal Ca concentrations but a weak positive correlation between faecal and blood Ca concentrations was observed (r=0.37, P<0.05). Bone and blood Ca showed a strong positive and significant correlation (r= 0.85, P<0.05) that was significant. When the bone Ca dropped, blood Ca also dropped. The Ca resorbed from bone was also not in the blood as blood Ca also dropped, but faecal Ca went up. Results suggest that Ca resorbed from the bone might have been lost in the faeces as there was a strong positive correlation between the blood and faecal Ca (Fig 20). Calcium is tightly regulated in mammals because of the critical role of calcium ion concentrations in many physiological functions, for instance the skeletal outflow of calcium during lactation influenced by the high demand in dairy cows (Howard and Smith, 1999).

4.5.5.1.3. Magnesium

Bone Mg of male calves showed a decreasing trend from the onset of the experiment until at 24 h post-treatment. Faecal Mg was stable from 0 h pre

treatment until at 3 h post-treatment but increased at 6 h and 24 h post-treatment with tetracycline. Blood Mg increased from 0 h until at 3 h and reduced at 6 h and then slightly increased at 24 h post-treatment (Fig 21).

Bone Mg diverged with faecal Mg supported by a strong negative and significant correlation (r= -0.95, P<0.05). Bone and blood Mg had a positive correlation of r= 0.41, P<0.05. Faecal and blood Mg were not correlated (r= -0.48, P>0.05). A decrease in bone Mg led to an increase in faecal Mg (Fig 21).

4.5.5.2. Between mineral interactions

4.5.5.2.1. Bone minerals

Male calves managed to spare P by increasing the concentrations in their bone from 0 h until at 3 h post-treatment. Phosphorus was mobilised from bone 6 h after treatment but regained again 24 h post-treatment (Fig 22). Bone P and bone Ca of male calves showed a strong negative correlation (r= -0.67, P<0.05) while bone P and bone Mg showed a positive correlation (r= 0.42, P<0.05). Bone P expressed as dry weight, showed very much the same picture as observed in the Bonsmara and Friesian calves, with an overall diversion with bone Ca during the trial. The results signify the differences with which the animal body homeostatic mechanism works as the P moves into the bone, Ca moves out, in this case in response to the IV injection of tetracycline (Fig 22). This is in agreement with Beighle *et al.* (1994).

4.5.5.2.2. Faecal minerals

Faecal P of male calves on a dry weight basis fluctuated from 0 h until at 24 h with peaks at 0, 2 and 5 h and the lower points of P concentrations at 30 min, 3 and 24 h post-treatment. Faecal Ca drastically reduced from 0 h to 30 min and increased until 5h and then decreased until the end of the experiment. Faecal Mg increased

up to 1 h then decreased up to 3 h and then increased again to the end of the experiment (Fig 23).

Faecal P and Ca of male calves showed a positive relationship (r = 0.52, P<0.05). Both variables showed a negative correlation when compared to faecal Mg which were r= -0.60, P<0.05 compared to faecal P and r= -0.84, P<0.05 when compared to faecal Ca. This indicates that faecal P and Ca were increasing and decreasing at more or less the same time but diverged with faecal Mg (Fig 23). Ca that peaked in faeces should be the one resorbed from bone as it was reducing in bone (Fig 20) at the same time (3, 5 and 6 h) where it was increasing in faeces (Fig 23) and in blood (Fig 24).

4.5.5.2.3. Blood minerals

There was an inconsistent response to the IV injection of tetracycline by blood Ca and P of male calves. They responded in the same direction at ½ hour, 3 and 30, but when blood Ca went up blood P was going down at 5 and 6 h and when blood Ca was going down blood P went up at 1 and 24 h. Blood Ca differed with blood Mg until at 2 h post-treatment with tetracycline, and both acted in concurrence from 3h until at 24 h post-treatment (Fig 24). It is however surprising to see the relationship between blood Ca and Mg being a strong negative correlation (r= -0.60, P<0.05). Blood P also showed weak relationships with blood Mg (r= 0.05, P<0.05) and blood Ca (r= 0.14, P<0.05).

4.5.6. Female group

4.5.6.1. Between sample interactions

4.5.6.1.1. Phosphorus

Female calves conserved P in their body by putting more P in their bones at 3 h but resorbed P from the same bones at 6 and 24 h post-treatment. When that happened, an increase in faecal P was also observed until at 6 h and depressed at

24 h after treatment. P in the blood was depressed until at 6 h but increased at 24 h post-treatment (Fig 25).

It was evident that there was no relationship between blood and bone P of female calves (r= 0.01, P<0.05) and a strong negative correlation between faecal P and blood P (r= -0.85, P<0.05). However, there was a positive correlation between bone P and faecal P (r= 0.50, P<0.05). This would indicate the ability of female calves to increase and decreased bone P at the same time as faecal P concentrations were decreasing and increasing. The opposite was observed with male calves (Fig 19). However, bone and blood P of both female and male calves showed a very strong positive correlation (r= 0.85, P<0.05) for female and r= 0.86, P<0.05 for males. There were no differences in the way in which both male and female calves react towards external stimuli on mineral homeostatic mechanisms. Female calves mobilised P to and from bone at the same proportion as male calves.

4.5.6.1.2. Calcium

Female calves resorbed Ca out of their bones 3 h post-treatment with tetracycline but regained it again at 6 h and the reverse was true at 24 h post-treatment. Faecal Ca was depressed 3 h after treatment but then increased until 24 h while blood Ca was depressed at 3 h and remained stable at 6 h and increased at 24 h (Fig 26).

Bone Ca was not related to faecal Ca as shown by a weak negative insignificant relationship between these variables (r= -0.25, P>0.05). It appeared that bone Ca of female calves was positively related with Ca in the blood (r= 0.51, P<0.05). Faecal Ca was related to blood Ca even though the relationship was weak (r= 0.29, P<0.05) but significant.

4.5.6.1.3. Magnesium

Bone Mg of female calves tended to reduce from 0 h until 3 h post-treatment, recovered at 6 h and continued to be resorbed from bone at 24 h post-treatment. Both blood and faecal Mg were suppressed at 3 h but increased until at 24 h post-treatment (Fig 27). Bone Mg was negatively related to faecal Mg (r= - 0.42, P<0.05) and blood (r= -0.24, P<0.05). Faecal and blood Mg were positively related (r= 0.77, P<0.05). Blood, bone and faecal Mg all responded to IV tetracycline in the same way at 3 and 6 h but at 24 h, the bone Mg decreased while blood blood and faecal Mg increased indicating that Mg pulled out of the bone was going into the blood and then out with the faeces near the end of the trial (Fig 27, Table 21.).

4.5.6.2. Between mineral interactions

4.5.6.2.1. Bone minerals

It shows that bone P acted in divergence with bone Ca of female calves. When bone P increased bone Ca reduced 3 h after treatment and the opposite was true 6 h after the treatment with tetracycline. However at 24 h both variables decreased slightly. Bone Mg followed bone Ca and not bone P. The above statement is supported by a strong negative correlation between bone P and bone Ca (r= -0.65, P<0.05) and bone Mg (r= -0.48, P<0.05), while bone Ca and bone Mg were strongly related (r= 0.95, P<0.05). The above results confirm the findings of Beighle *et al.* (1994).

4.5.6.2.2. Faecal minerals

Faecal P of female calves increased until at 1 h post-treatment but reduced slightly until 3 h, increased until 6 h and suppressed at 24 h post-treatment. Faecal Ca reduced 30 min post-treatment and 1 h thereafter, then drastically increased at 6 h and 24 h respectively. Faecal Mg was suppressed until at 2 h but then increased

at 3h, suppressed again at 5 h and increased at 6 and 24 h post-treatment (Fig 29).

As in male calves, female calves showed a positive relationship between faecal P and faecal Ca (r= 0.32, P<0.05) and no relationship when compared to faecal Mg (r= 0.05, P<0.05). Faecal Ca and faecal Mg showed a strong positive and significant relationship (r= 0.96, P<0.05).

4.5.6.2.3. Blood minerals

As in male calves, female calves tended to suppress Ca and Mg in their blood from 0 h pre treatment until at 3 h post-treatment. The concentrations were increased at 5 h but it reduced at 6 h and then increased at 24 h post-treatment. Blood P remained more or less stable within the 7 – 8 mg/100 ml serum (Fig 30).

Blood P was weakly related with blood Ca (r= 0.37, P<0.05) but there seem to be no relationship when it was compared to blood Mg (r= 0.00, P<0.05). In support of the statement above, it was discovered that blood Ca and blood Mg were strongly related with strong correlation of r=0.92, P<0.05 (Fig 30).

4.6 Results (Overall)

4.6.1. Experimental treatment groups (overall)

4.6.1.1. Fresh weight

4.6.1.1.1. Bone

The overall means for P were 106.12mg P g^{-1} for the treated group and 99.20 mg P g^{-1} for the control group. Ca contents were 178.50 mg Ca g^{-1} for the treatment and 185.90 mg Ca g^{-1} for the control group. Mg contents were 3.18 mg Mg g^{-1} for the treated group and 3.26 mg Mg g^{-1} for the control. Ca:P ratio was 1.72 for the treated group and 1.87 for the control group and bone thickness was 3.28 mm for the treated group and 2.75 mm for the control group (Table 1.).

The two groups did not differ in the overall mean cortical bone P, Ca, Mg concentrations and even cortical bone Ca:P ratio and cortical bone thickness except for cortical bone P where the treated animals had significantly ($P<0.05$) more bone P (182.01 mgP g^{-1}) compared to the control animals (164.63 mgP g^{-1}) on an ash weight basis. Bone loss occurs when resorption exceeds bone formation. In this instance the bone minerals did not differ with that of the control group, except for cortical bone P on an ash weight basis leading to a conclusion that tetracycline did not affect the overall mean bone minerals on a fresh weight basis. Although no relationship was greater, it appears that in the cortical bone, all measures were more predictive on the individual experimental times (0, 3, 6 and 24 h) than of total or overall bone minerals.

4.6.1.1.2. Faeces

The overall means for faecal P fresh weight were 5.08 mg P g^{-1} for the treated group and 5.39 mg P g^{-1} for the control group. Ca content was 9.77 mg Ca g^{-1} for the treated and 9.29 mg Ca g^{-1} for the control group. Mg content was 1.73 mg Mg g^{-1} for the treated group and 1.93 mg Mg g^{-1} for the control (Table 2.).

Overall values showed that the treated calves significantly ($P<0.05$) conserved P (5.08 mg P g^{-1}) by reducing the concentrations from their faeces as compared to the control calves (5.39 mg P g^{-1}). Overall faecal Ca and Mg showed no significant differences between the treated calves and control calves on a fresh weight basis (Table 2.).

4.6.1.2. Dry weight

4.6.1.2.1. Bone

The overall means for cortical bone P dry weight were 120.12 mg P g^{-1} for the treatment group and 112.43 mg P g^{-1} for the control group. Ca content was 199.01 mg Ca g^{-1} for the treatment and 202.03 mg Ca g^{-1} for the control group. Mg

content was 3.58 mg Mg g^{-1} for the treated group and 3.56 mg Mg g^{-1} for the control. Ca:P ratio was 1.69 for the treated group and 1.85 for the control group and bone thickness was 3.28 mm for the treated group and 2.75 mm for the control group (Table 3.).

The IV administration of tetracycline did not have the overall effect on the concentrations of cortical bone P, Ca and Mg which in turn did not affect the overall cortical Ca:P ratio and cortical bone thickness. This is also supported by the results observed on Table 4. where there were no significant differences between the faecal P, Ca and Mg on a dry weight basis.

4.6.1.2.2. Faeces

The overall means for faecal dry weight were 5.44 mg P g^{-1} for the treated group and 5.81 mg P g^{-1} for the control group. Ca content was 10.44 mg Ca g^{-1} for the treated and 10.00 mg Ca g^{-1} for the control group. Mg content was 1.88 mg Mg g^{-1} for the treated group and 2.09 mg Mg g^{-1} for the control (Table 4.). Overall faecal P, Ca and Mg of the treated group and the control group were not significantly different (Table 4.).

4.6.1.3. Ash weight

4.6.1.3.1. Bone

The overall means for cortical bone P ash weight were 186.34 mg P g^{-1} for the treatment group and 164.63 mg P g^{-1} for the control group. Ca content was 302.08 mg P g^{-1} for the treatment and 301.95 mg P g^{-1} for the control group. Mg content was 5.46 mg Mg g^{-1} for the treated group and 5.38 mg Mg g^{-1} for the control. Ca:P ratio was 1.68 for the treated group and 1.75 for the control group and bone thickness was 3.28 mm for the treated group and 2.75 mm for the control group (Table 5.).

4.6.1.3.2. Faeces

The overall means for faecal P ash weight were 31.30 mg P g^{-1} for the treatment group and 35.55 mg P g^{-1} for the control group. Ca content was 60.47 mg Ca g^{-1} for the treated and 61.78 mg Ca $g^{-1}$for the control group. Mg content was 10.99 mg Mg g^{-1} for the treated group and13.03 mg Mg g^{-1} for the control (Table 6.).

Unlike faecal Ca, faecal P and Mg had significantly ($P<0.05$) less concentrations than the control calves. This was probably a result of the storing effect by the body of the P and Mg that was conserved in the faeces. Overall faecal P and Mg concentrations were significantly ($P<0.05$) lower in the treated calves than in the control calves on the ash weight basis (Table 6.).

4.6.1.4. Blood minerals

The overall mean for P was 7.54 mg/100 ml serum for treated calves and 7.85 mg/100 ml serum for the control group. Ca content was 7.86 mg/100 ml serum for the treatment group and 8.06 mg/100 ml serum for the control group. Mg content was 1.54 mg/100 ml serum for the treated calves and 1.37 mg/100 ml serum for the control (Table 7.). Results from this experiment are within the range reported by Beighle *et al.* (1995) who reported a weekly sampling concentration of 7.052 mg/dl on blood collected from the jugular and 7.170 mg/dL from weekly samples collected from the tail. Beighle *et al.* (1995) also reported values of samples collected on a monthly basis to be 7.466 mg/dL from the jugular blood and 7.950 mg/dL from coccygeal blood.

Overall mean blood Mg showed significantly ($P<0.05$) higher values on the treated calves as compared to the controls. Blood P and Ca yielded no significant differences ($P>0.05$) between the treatment group and the control group (Table 7.). Serum P is closely related to serum Ca because both form the hydroxyapatite

lattice structure of bone. Because serum Ca did not differ within treatment groups in this experiment, serum P was also expected to respond likewise.

4.6.2. Experimental breed groups (overall)

4.6.2.1. Fresh weight

4.6.2.1.1. Bone

The breed effect proved to have a significant ($P< 0.05$) effect on the overall level of bone P, Ca, Ca:P ratio and bone thickness. Overall means for P were 106.81 mg P g^{-1} for the Friesian calves and 98.54 mg P g^{-1} for the Bonsmara calves (Table 8.). Ca content was 199.02 mg Ca g^{-1} for the Friesian calves and 164.54 mg Ca g^{-1} for the Bonsmara calves. Mg content was 3.35 mg Mg g^{-1} for the Friesian calves and 3.09 mg Mg g^{-1} for the Bonsmara calves. Ca:P ratio was 1.89 for the Friesian calves and 1.74 for the Bonsmara calves and bone thickness was 3.27 mm for the Friesian calves and 2.77 mm for the Bonsmara calves (Table 8.).

When the overall mean differences were compared between the two experimental groups, it was observed that Friesian calves had significantly ($P<0.05$) more bone P, Ca and Ca:P ratio and bone thickness as compared to the Bonsmara calves. Bone Mg did not differ significantly ($P>0.05$) (Table 8.).

4.6.2.1.2. Faeces

The overall means for P were 2.08 mg P g^{-1} for the Friesian calves and 2.06 mg P g^{-1} for the Bonsmara calves. Ca content was 6.48 mg Ca g^{-1} for the Friesian calves and 3.16 mg Ca g^{-1} for the Bonsmara calves. Mg content was 0.58 mg Mg g^{-1} for the Friesian calves and 0.77 mg Mg g^{-1} for the Bonsmara calves (Table 9.).

Friesian calves had significantly ($P<0.05$) more overall faecal Ca and less Mg in their faeces than the Bonsmara animals on a fresh weight basis. It is therefore important to note that the overall mean results indicate that Friesian calves

significantly (P<0.05) lost Ca and resorbed Mg by putting more Ca into their faeces and resorbed Mg from the gastrointestinal tract than did the Bonsmara calves on a Fresh weight basis (Table 9.). Under physiological conditions, Ca^{2+} ions are absorbed mainly in the small intestine, where about 90% of the overall Ca^{2+} is absorbed (Wasserman, 2004). Small amounts of Ca and probably P, and Mg are absorbed from the stomach and large intestine, the colon accounts for less than 10% of the total Ca^{2+} absorption. These results indicate that more Ca depletion in the bone occurred at 3 h and led to the excretion of Ca through the faeces. This is observed by the significant (P<0.05) differences in the mean faecal Ca at all sampling periods of the experiment even at 30 minutes after the injection of the tetracycline which would not give time for the ingesta to move from the small intestines.

The significant (P<0.05) difference in faecal Ca at 30 minutes after the injection of tetracycline would indicate that active absorption of Ca took place in the colon instead of the small intestine because the contents of the intestine would not have had time to move from the small intestine to the rectum in 30 minutes. The Bonsmara calves also had a significant (P<0.05) difference in the faecal P concentration at 30 minutes compared with pre treatment value. With significantly (P<0.05) more P in the faeces at 30 minutes compared with 0 h, it would indicate that P was being pulled out from the blood and placed in the faeces in the colon. Further research is needed to investigate this important finding.

4.6.2.2. Dry weight

4.6.2.2.1. Bone

The overall means for P were 120.12 mg P g^{-1} for the Friesian calves and 112.43 mg P g^{-1} for the Bonsmara calves. Ca content was 216.59 mg Ca g^{-1} for the Friesian calves and 184.45 mg Ca g^{-1} for the Bonsmara calves. Mg content was 3.66 mg Mg g^{-1} for the Friesian calves and 3.48 mg Mg g^{-1} for the Bonsmara calves. Ca:P ratio was 1.83 for the Friesian calves and 1.71 for the Bonsmara

calves and bone thickness was 3.27 mm for the Friesian calves and 2.77 mm for the Bonsmara calves (Table 10.).

There was a significantly ($P<0.05$) higher overall cortical bone P and Ca concentration and bone thickness among the Friesian calves compared with the Bonsmara calves on a dry weight basis (Table 10.). When tested, the overall mean of bone Mg and Ca:P ratio did not show any difference between the Friesian and Bonsmara calves.

4.6.2.2.2. Faeces

The overall means for P were 2.29 mg P g^{-1} for the Friesian calves and 2.22 mg P g^{-1} for the Bonsmara calves. Ca content was 6.90 mg Ca g^{-1} for the Friesian calves and 3.41 mg Ca g^{-1} for the Bonsmara calves. Mg content was 0.62 mg Mg g^{-1} for the Friesian calves and 0.83 mg Mg g^{-1} for the Bonsmara calves (Table 11.).

The analysis of the faeces on a dry weight basis produced similar results as in fresh weight basis, that indicated significantly ($P<0.05$) higher concentrations of Ca and lower faecal Mg from the Friesian calves as compared to the Bonsmara calves.

4.6.2.3. Ash weight

4.6.2.3.1. Bone

The overall means for P were 183.46 mg P g^{-1} for the Friesian calves and 178.86 mg P g^{-1} for the Bonsmara calves. Ca content was 330.76 mg Ca g^{-1} for the Friesian calves and 273.28 mg Ca g^{-1} for the Bonsmara calves. Mg content was 5.56 mg Mg g^{-1} for the Friesian calves and 5.27 mg Mg g^{-1} for the Bonsmara calves. Ca:P ratio was 1.84 for the Friesian calves and 1.59 for the Bonsmara calves and bone thickness was 3.26 mm for the Friesian calves and 2.77 mm for the Bonsmara calves (Table 12.).

Levels of P, Mg and Ca:P ratio, and bone thickness of the cortical rib bones showed no significant differences (P>0.05) between the Friesian and the Bonsmara calves when expressed in mg/g ash weight. When Ca levels were analysed, the Friesian calves had significantly higher cortical bone Ca than the Bonsmara animals (Table 12.).

4.6.2.3.2. Faeces

The overall means for P were 13.41 mg P g^{-1} for the Friesian calves and 13.34 mg P g^{-1} for the Bonsmara calves. Ca content was 38.26 mg Ca g^{-1} for the Friesian calves and 21.23 mg Ca g^{-1} for the Bonsmara calves. Magnesium content was 3.55 mg Mg g^{-1} for the Friesian calves and 5.13 mg Mg g^{-1} for the Bonsmara calves (Table 13.).

When data was analysed to allow the overall comparison between breeds, there were huge (P=0.0001) differences observed between Ca and Mg concentrations of faeces collected from the Friesian calves as compared to the Bonsmara calves. Friesian calves lost more faecal Ca and at the same time preserved more Mg than the Bonsmara calves (Table 13.) by absorbing more Mg from their gut and in turn losing more Ca throughout their faeces on an ash weight basis (Table 13.).

4.6.2.4. Blood minerals

When measured in mg/100 ml serum, the mean blood P concentration of the Friesian calves as compared to that of the Bonsmara calves did not produce any significant (P>0.05) differences. In divergence with blood P, the breed of animals sampled was responsible for significant (P<0.05) differences in blood Ca in Friesian calves (11.06 mg/100 ml serum), as compared to the Bonsmara calves (4.86 mg/100 ml serum) and blood Mg in Friesian (1.92 mg/100 ml serum) as compared to Bonsmara calves (0.99 mg/100 ml serum).

4.6.3. Experimental sex groups (overall)

4.6.3.1. Fresh weight

4.6.3.1.1. Bone

The overall means for P were 94.30 mg P $g^{-1}$for the male calves and 111.05 mg P g^{-1} for the female calves. Ca content was 185.13 mg Ca g^{-1} for the male calves and 178.43 mg Ca g^{-1} for the female calves. Mg content was 3.28 mg Mg g^{-1} for the male calves and 3.16 mg Mg g^{-1} for the female calves. Ca:P ratio was 2.06 for the male calves and 1.63 for the female calves and bone thickness was 3.14 mm for the male calves and 2.90 mm for the female calves (Table 15.).

Overall observations showed a significantly ($P<0.05$) lower bone P in male calves as compared to the female calves, and as a result this led to a significantly ($P<0.05$) higher cortical bone Ca:P ratio among the male calves compared with female calves (Table 15.). The sex effect between male and female calves did not affect the cortical bone Ca, Mg and bone thickness significantly (Table 15.).

4.6.3.1.2. Faeces

The overall means for P were 4.63 mg P g^{-1} for the male calves and 5.85 mg P g^{-1} for the female calves. Ca content was 12.48 mg Ca g^{-1} for the male calves and 6.61 mg Ca g^{-1} for the female calves. Mg content was 2.16 mg Mg g^{-1} for the male calves and 1.50 mg Mg g^{-1} for the female calves (Table 16.).

Overall mean faecal P of male calves was significantly ($P<0.05$) lower than that of female calves. Faecal Ca and Mg concentration reflected more Ca and Mg loss through the faeces of male calves than female calves (Table 16.). The observation in the current study reflects strong relationship between P and Ca measures of a research conducted by Motsei and Beighle (2006), that when P increases, Ca decreases and vice versa.

4.6.3.2. Dry weight

4.6.3.2.1. Bone

The overall means for P were 107.01 mg P g^{-1} for the male calves and 125.54 mg P g^{-1} for the female. Ca content was 204.31 mg Ca g^{-1} for the male calves and 196.74 mg Ca g^{-1} for the female calves. Mg content was 3.65 mg Mg g^{-1} for the male calves and 3.49 mg Mg g^{-1} for the female calves. Ca:P ratio was 2.00 for the male calves and 1.79 for the female calves and bone thickness was 3.14 mm for the male calves and 2.90 mm for the female calves (Table 17.).

Results here show that there are overall significant ($P<0.05$) differences in the concentration of P and Ca:P ratio in the cortical bone. Bone P of male calves were significantly ($P<0.05$) less than that of female calves on a dry weight basis as presented in 13.3.1.1 above (Table 17.).

4.6.3.2.2. Faeces

The overall means for P were 4.92 mg P g^{-1} for the male calves and 6.34 mg P g^{-1} for the female calves. Ca content was 13.30 mg Ca g^{-1} for the male calves and 7.15 mg Ca g^{-1} for the female calves. Mg content was 2.33 mg Mg g^{-1} for the male calves and 1.63 mg Mg g^{-1} for the female calves (Table 18.).

Male calves had significantly ($P<0.05$) lower overall faecal P (4.92 mg P g^{-1}) as compared to female calves (6.34 mg P g^{-1}) but the overall faecal Ca and Mg of male calves were significantly ($P<0.05$) higher than those of female calves.

4.6.3.3. Ash weight

4.6.3.3.1. Bone

The overall means for P were 173.85 mg P g^{-1} for the male calves and 188.46 mg P g^{-1} for the female calves. Ca content was 307.63 mg Ca g^{-1} for the male calves

and 296.40 mg Ca g^{-1} for the female calves. Mg content was 5.59 mg Mg g^{-1} for the male calves and 5.24 mg Mg g^{-1} for the female calves. Ca:P ratio was 1.86 for the male calves and 1.59 for the female calves and bone thickness was 3.16 mm for the male calves and 2.90 mm for the female calves (Table 19.).

No overall relationship was significantly ($P<0.05$) different between male and female calves. Total bone Ca, P, Mg and Ca:P ratio showed no overall significance, meaning that the influence of intravenous injection of tetracycline had not caused any overall differences between the bone mineral contents of male and female calves on an ash weight basis (Table 19.).

4.6.3.3.2. Faeces

The overall means for P were 30.21 mg P g^{-1} for the male calves and 36.65 mg P g^{-1} for the female calves. Ca content was 78.55 mg Ca g^{-1} for the male calves and 44.95 mg Ca g^{-1} for the female calves. Mg content was 14.44 mg Mg g^{-1} for the male calves and 9.59 mg Mg g^{-1} for the female calves (Table 20.). Overall mean differences showed significantly ($P<0.05$) lower P lost throughout faeces of male calves as compared to female calves. Faecal Ca and Mg were significantly ($P<0.05$) higher in the faeces of male calves than female calves on an ash weight basis (Table 20.). Giving an IV injection of tetracycline reduced the loss of P through the faeces on male calves compared with female calves. Female calves conserved more Ca and Mg but not P on the ash weight basis (Table 20.).

4.6.3.3. Blood minerals

The overall pattern of blood Ca levels of male calves showed to be significantly ($P<0.05$) lower (7.58 mg/100 ml serum) as compared to female calves (8.35 mg/100 ml serum). On average, males had no different levels of blood P and Mg as compared to fernale calves (Table 21.).

4.7. Discussion (overall)

4.7.1. Experimental treatment groups (overall)

Introduction

This section discusses the overall effect of tetracycline on mineral homeostasis of calves. Any statistical differences observed between the two groups will indicate the effect of tetracycline on the overall mineral content of calves. In general only modest changes were observed in this experiment.

4.7.1.1. Bone (fresh, dry and ash weight, Ca:P ratio, bone thickness)

No overall significant differences in bone minerals, Ca:P ratio and bone thickness were observed on a fresh and dry weight basis, but the opposite was true on bone P concentration on an ash weight basis between the treated and the control calves (Table 5.). When a control multiparous cow and 5 untreated primiparous cows were used to evaluate the effect of parity on the variables that were measured, Taylor *et al.* (2008) found no effect of parity on Ca, PTH or 25-OH concentrations. Younger animals had greater osteocalcin in their blood than second lactating and older cows. Higher osteocalcin is an indication that younger animals were forming more bones than older ones.

4.7.1.2. Faeces (fresh, dry and ash weight)

Overall P was depressed in the faeces of the treated calves than on the control while Ca and Mg yielded no significant differences on both fresh and dry weight basis whereas on ash weight, both P and Mg were depressed in the treated calves than in the control calves (Table 2, 4, 6).

4.7.1.3. Blood (mg/100 ml serum)

Overall mean blood P and Ca yielded no significant differences, but Mg showed a significantly higher value for treated calves than the control calves (Table 7.). These results disagree with the findings of Taylor *et al.* (2008) that serum P and Ca were different over time and were within normal range for adult ruminants (8 – 10 mg/dL and 4 – 8 mg/dL respectively). In this experiment, it was understandable when these calves did not yield any significant differences as they were still at their initial growth phase. Bone serves as a metabolic reservoir that supplies Ca and P and other minerals where they are needed most, in this case the blood were the Ca chelating effect of tetracycline took effect.

4.7.2. Experimental breed groups (overall means)

Introduction

This section discusses the effect of tetracycline on mineral homeostasis in Friesian and Bonsmara calves as measured by the overall mean. This will highlight the breed effect on mineral homeostasis as a result of the IV injection of tetracycline.

4.7.2.1. Bone (fresh, dry and ash weight, Ca:P ratio, bone thickness)

On a fresh weight basis, Friesian calves had a siginificantly ($P<0.05$) higher overall mean P, Ca, Ca:P ratio and bone thickness than the Bonsmara calves but not bone Mg (Table 8.). On the dry weight, Friesian calves had a higher overall mean P, Ca and bone thickness than Bonsmara calves while other variables were not significantly ($P>0.05$) different for both breeds of calves (Table 10.).On the ash weight, Ca seems to be the only bone variable that differed significantly ($P<0.05$) between Friesian and Bonsmara calves (Table 12).

4.7.2.2. Faeces (fresh, dry, ash)

Friesian calves seemed to have lost more Ca than the Bonsmara calves as the overall means of Ca concentrations were significantly ($P<0.05$) higher in faeces of the former than the latter on a fresh, dry and ash weight basis. The opposite was true for faecal Mg.

4.7.2.3. Blood (mg/100 ml serum)

Friesian calves possessed higher overall mean of Ca and Mg in their blood than the Bonsmara calves. There was no significant difference in the mean blood P between breeds.

4.7.3. Experimental sex groups (overall means)

Introduction

This section discusses the overall effect of sex on mineral homeostasis of calves treated with an IV tetracycline. Statistical differences observed between these two groups will give a clear picture of how different sex groups react to changes occurring in the body, in this case to the IV injection with tetracycline which chelates Ca in blood.

4.7.3.1. Bone (fresh, dry and ash weight, Ca:P ratio, bone thickness)

All variables showed no significant differences in the overall means on a fresh, dry and ash weight basis except for the overall mean of P that was significantly ($P<0.05$) lower in male calves as compared to female calves (Table 15.). How the body's homeostatic mechanisms managed to cause an increase or decrease in bone, blood and faecal P remains unclear. However, the results of this research indicate that the accretion and resorption process occurred on a very acute basis,

as observed in the significant ($P<0.05$) difference in faecal P, Ca and Mg at 30 minutes after treatment with the tetracyclines (Table 9.)

4.7.3.2. Faeces (fresh, dry and ash weight)

There were significant ($P<0.05$) differences in the overall means of faecal P, Ca and Mg when male and female animals were compared. Male animals had significantly ($P<0.05$) less faecal P whereas female calves had significantly ($P<0.05$) less Ca and Mg (Table 18.). These results indicate the ability of the animals to conserve P, Ca and Mg by reducing the amount of minerals lost in the faeces. It is interesting that the males were able to conserve P whereas the females were able to conserve Ca and Mg. Further research is needed to investigate why this occurs.

4.7.3.3. Blood (mg/100 ml serum)

The overall mean showed that there was significantly ($P<0.05$) less Ca in the blood of male animals compared with female calves but P and Mg were not significantly ($P>0.05$) different between these two sex groups (Table 21.).

CHAPTER FIVE

5. CONCLUSION

When the mineral content of calves especially P, Ca and Mg are evaluated, it is important for researchers to take into consideration age, sex, breed and time. In the current study for instance, there were significant differences found between P, Ca and Mg between treated and control animals (Table 1 –7) between breeds (Tables 8 – 14) and between sexes (Table 15 - 21) and lastly, significant differences were also found when time was considered within treated groups, breed types and sex due to the external influence of IV tetracycline (all Tables).

There were minor differences in the mineral concentrations measured as fresh and dry weight basis. The ash weight seemed to be the most sensitive measure of faecal and bone samples as there were more significant differences ($P<0.05$) found on the ash weight basis than on the dry and fresh weight bases (all tables). It is not clear how the animal's body managed to increase bone and reduced faecal P. Phosphorus that was absorbed from the intestine preferably the small intestine was carried by the blood and stored in the bone which together with Ca, forms the hydroxyappetite which is the main mineral of bone (Fig 1). The same happened to Ca (Fig 2) and Mg (Fig 3) throughout the experiment. Bone P and Ca acted in divergence in most of the experimental times (Fig 4, 10, 22 and 28) regardless of the sex and breed types. This divergence might suggest that bone mineralization may have competed with soft tissues for the limited minerals available through the diet. Animals were not supplemented but fed *ad lib* roughage containing 50% *Medicargo sartiva* and 50% *Cencrus ciliaris.* Bone minerals were within the normal range on treated groups with P concentrations ranging from 92 mg P g^{-1} - 145.18 mg P g^{-1}, Ca from 191.93 mg Ca g^{-1} - 231.87 mg Ca g^{-1} and Mg from 3.35 - 4.35 mg Mg g^{-1} all on dry weight basis (Table 3.). Comparison of results of this study with those reported by Beighle *et al.* (1994) mineral levels in the rib bone samples were found to be higher in P and Mg than

those reported by Gonul *et al.* (2009) (132.76±17.4 and 2.38 ± 0.35 mg g^{-1}, respectively) and lower for Calcium 231.87 mg Ca g^{-1}in this study vs 333.23 mg Ca g^{-1} Gonul *et al.* (2009). In this study, values agree with those of Beighle *et al.* (1994) on a dry weight basis. This research agrees with previous research findings related to bone mineral homeostasis (Motsei and Beighle, 2006; Beighle *et al*, 1994; Beighle, 2000) in which bone Ca and bone P respond differently to various influences. Here the intravenous injection of tetracycline caused the bone P to significantly increase in treated animals compared to control animals especially at 3 h post-treatment (Table 1.) but the same IV tetracycline caused the bone Ca to significantly ($P<0.05$) decrease in treated animals compared to the control animals at 3 h after treatment (Table 1.). Faecal minerals (P, Ca and Mg) were also in the normal range from 4.87 - 6.29 mg P g^{-1}, 8.07 - 12.54 mg Ca g^{-1}, and 1.36 - 2.88 mg Mg g^{-1} regardless of the sex or breed or treatment groups on a dry weight basis. Blood minerals (P, Ca and Mg) were within the normal range from 6.71 – 8.49 mg/100ml serum, 6.26 – 10.76 mg/100ml serum [1] and 1.26 – 1.71 mg/100ml serum respectively. In the study conducted by Mellau *et al.* (2001), the average Ca^{2+} concentration at recumbency was 0.43 mmol/l with a range of 0.39 – 0.52 mmol/l, 0.53 – 0.61 mmol/l (Wang and Beede, 1990,1992), 0.45 – 0.48 mmol/l (Jorgensen *et al.* 1999) and 0.48 ± 0.11 mmol/l (Desmecht *et al.* 1995). Blood mineral values in this study were more than the values above and no calves showed signs of recumbency or mineral deficiency.

LIST OF TABLES

TABLE 1. Mean bone P, Ca and Mg, Ca:P ratio and bone thickness totals between treatment groups of all breeds and sex (mg g^{-1} fresh weight)

TIME (H)	*0*	*3*	*6*	*24*	*OVERALL MEAN*
Cortical Bone P (mg P g^{-1})					
TREATMENT	89.38^{ax} ± 7.79	128.35^{ay} ± 10.71	101.02^{ax} ± 8.96	88.99^{ax} ± 8.11	**106.12^{a} ± 9.26**
CONTROL	82.51^{a} ± 7.03	124.07^{a} ± 10.26	81.06^{b} ± 6.79	92.53^{a} ± 7.65	**99.20^{a} ± 8.23**
Cortical Bone Ca (mg Ca g^{-1})					
TREATMENT	211.70^{ay} ± 14.78	167.60^{ax} ± 9.69	193.05^{ay} ± 10.96	174.86^{axy} ± 14.29	**178.50^{a} ± 11.65**
CONTROL	195.71^{a} ± 9.31	191.58^{b} ± 18.40	187.63^{a} ± 31.03	178.50^{a} ± 11.23	**185.90^{a} ± 20.22**
Cortical Bone Mg (mg Mg g^{-1})					
TREATMENT	3.95^{az} ± 0.32	3.32^{ay} ± 0.18	3.27^{ay} ± 0.22	2.97^{ax} ± 0.33	**3.18^{a} ± 0.24**
CONTROL	3.71^{a} ± 0.19	3.48^{a} ± 0.22	3.33^{a} ± 0.52	2.96^{a} ± 0.16	**3.26^{a} ± 0.30**
Cortical Bone Ca:P Ratio					
TREATMENT	2.37^{az} ± 0.3	1.31^{ax} ± 0.07	1.89^{ay} ± 0.04	1.96^{ay} ± 0.03	**1.72^{a} ± 0.05**
CONTROL	2.37^{a} ± 0.22	1.54^{a} ± 0.065	2.13^{a} ± 0.25	1.93^{a} ± 0.07	**1.87^{a} ± 0.12**
Cortical Bone Thickness (mm)					
TREATMENT	2.43^{ax}	3.22^{ay}	3.45^{az}	3.16^{ay}	**3.28^{a}**
CONTROL	2.72^{a}	3.00^{a}	2.60^{b}	2.66^{b}	**2.75^{a}**

$^{a, b}$ means with different letters in a column are significantly different between treated groups ($p<0.05$)

$^{x, y, z}$ means with different letters in a row are significantly different within treatment groups ($p<0.05$)

TABLE 2. Mean faecal P, Ca and Mg totals between treatment groups of all breeds and sex (mg g^{-1} fresh weight)

TIME (H)	*0*	*30 min*	*1*	*2*	*3*	*5*	*6*	*24*	*30*	*OVERALL MEAN*
Faecal P (mg P g^{-1})										
TREATMENT	5.01^{ay} ± 0.99	5.00^{ay} ± 0.65	5.46^{az} ± 0.44	4.58^{ax} ± 0.38	4.52^{ax} ± 0.20	5.44^{az} ± 0.36	5.16^{ayz} ± 0.51	4.89^{ax} ± 0.46	5.57^{az} ± 0.57	**5.08^{a} ± 0.45**
CONTROL	4.59^{a} ± 1.09	5.16^{a} ± 0.50	4.88^{b} ± 2.22	5.69^{b} ± 0.60	5.55^{b} ± 0.41	5.72^{a} ± 0.49	5.79^{a} ± 0.73	4.87^{a} ± 0.36	5.46^{a} ± 0.55	**5.39^{b} ± 0.73**
Faecal Ca (mg Ca g^{-1})										
TREATMENT	8.91^{ax} ± 0.88	8.15^{ax} ± 0.69	9.59^{ay} ± 1.06	9.33^{ay} ± 0.75	11.25^{az} ± 1.88	10.07^{ayz} ± 0.82	9.58^{ay} ± 0.63	10.01^{ayz} ± 0.57	10.20^{ayz} ± 0.75	**9.77^{a} ± 0.89**
CONTROL	9.57^{b} ± 0.87	8.55^{a} ± 0.77	7.77^{b} ± 0.74	8.51^{a} ± 0.58	8.52^{b} ± 0.61	9.68^{a} ± 0.51	11.58^{b} ± 0.99	10.22^{a} ± 1.06	9.41^{a} ± 0.92	**9.28^{a} ± 0.77**
Faecal Mg (mg Mg g^{-1})										
TREATMENT	1.46^{ax} ± 0.14	1.72^{ax} ± 0.10	1.67^{ax} ± 0.12	1.19^{ax} ± 0.05	1.25^{ax} ± 0.05	1.41^{ax} ± 0.19	1.67^{ax} ± 0.12	2.39^{ay} ± 0.44	2.57^{ay} ± 0.52	**1.73^{a} ± 0.19**
CONTROL	1.90^{a} ± 0.14	1.71^{a} ± 0.19	1.54^{a} ± 0.10	1.33^{a} ± 0.12	1.75^{a} ± 0.16	1.63^{a} ± 0.08	2.15^{b} ± 0.19	2.64^{a} ± 0.23	2.68^{a} ± 0.25	**1.93^{a} ± 0.16**

$^{a, b}$ means with different letters in a column are significantly different between treated groups ($p<0.05$)

$^{x, y,z}$ means with different letters in a row are significantly different within treatment groups ($p<0.05$)

TABLE 3. Mean bone P, Ca, Mg, Ca:P ratio and bone thickness totals between treatment groups of all breeds and sex (mg g^{-1} dry weight)

TIME (H)	*0*	*3*	*6*	*24*	*OVERALL MEAN*
Cortical Bone P (mg P g^{-1})					
TREATMENT	98.15^{ax} ± 8.20	145.18^{ay} ± 12.53	114.32^{ax} ± 9.82	100.87^{ax} ± 8.51	**120.12^{a}** ± 10.28
CONTROL	92.40^{a} ± 7.48	139.08^{a} ± 11.30	93.63^{b} ± 7.33	104.59^{a} ± 7.67	**112.43^{a}** ± 8.77
Cortical bone Ca (mg Ca g^{-1})					
TREATMENT	231.87^{ay} ± 15.13	191.93^{ax} ± 9.16	209.73^{ay} ± 11.74	195.38^{axy} ± 14.59	**199.01^{a}** ± 11.83
CONTROL	208.90^{a} ± 13.47	199.28^{a} ± 22.54	206.16^{a} ± 34.40	200.64^{a} ± 14.26	**202.03^{a}** ± 23.73
Cortical bone Mg (mg Mg g^{-1})					
TREATMENT	3.91^{az} ± 0.31	3.78^{ay} ± 0.19	3.60^{ay} ± 0.24	3.35^{ax} ± 0.33	**3.58^{a}** ± 0.25
CONTROL	4.35^{a} ± 0.29	3.68^{a} ± 0.26	3.66^{a} ± 0.57	3.35^{a} ± 0.24	**3.56^{a}** ± 0.35
Cortical bone Ca:P Ratio					
TREATMENT	2.36^{az} ± 0.045	1.32^{ax} ± 0.02	1.83^{ay} ± 0.02	1.94^{ay} ± 0.021	**1.69^{a}** ± 0.02
CONTROL	2.26^{a} ± 0.04	1.43^{a} ± 0.032	2.20^{a} ± 0.02	1.92^{a} ± 0.03	**1.85^{a}** ± 0.02
Cortical Bone Thickness (mm)					
TREATMENT	2.43^{ax}	3.22^{ay}	3.45^{az}	3.16^{ay}	**3.28^{a}**
CONTROL	2.72^{a}	3.00^{a}	2.60^{b}	2.66^{b}	**2.75^{a}**

$^{a, b}$ means with different letters in a column are significantly different between treated groups ($p<0.05$)

$^{x, y, z}$ means with different letters in a row are significantly different within treatment groups ($p<0.05$)

TABLE 4. Mean faecal P, Ca and Mg totals between treatment groups of all breeds and sex (mg g^{-1} dry weight)

TIME (H)	*0*	*30 min*	*1*	*2*	*3*	*5*	*6*	*24*	*30*	*OVERALL MEAN*
Faecal P (mg P g^{-1})										
TREATMENT	5.40^{ax} ± 0.60	5.00^{ax} ± 0.85	5.87^{ay} ± 0.45	4.91^{ax} ± 0.40	4.87^{ax} ± 0.21	5.89^{ay} ± 0.40	5.56^{ax} ± 0.31	5.28^{ax} ± 0.50	6.11^{ay} ± 0.63	**5.44^{a}** ± 0.47
CONTROL	4.96^{a} ± 0.69	5.58^{b} ± 0.54	5.27^{b} ± 0.62	6.23^{b} ± 1.22	5.85^{b} ± 0.31	6.18^{a} ± 0.31	6.29^{b} ± 0.80	5.26^{a} ± 0.35	5.88^{a} ± 0.60	**5.81^{a}** ± 0.59
Faecal Ca (mg Ca g^{-1})										
TREATMENT	9.57^{ax} ± 0.93	8.07^{ax} ± 0.87	10.25^{ax} ± 0.75	10.08^{ax} ± 0.81	12.06^{ay} ± 0.49	10.87^{ax} ± 0.89	10.29^{ax} ± 0.53	10.80^{ax} ± 0.73	11.135^{axy} ± 0.80	**10.44^{a}** ± 0.73
CONTROL	11.53^{a} ± 1.13	9.21^{a} ± 0.82	8.36^{b} ± 0.92	9.20^{a} ± 0.63	9.11^{b} ± 0.79	10.42^{a} ± 0.55	12.54^{b} ± 1.08	11.06^{a} ± 0.97	10.10^{a} ± 0.96	**10.00^{a}** ± 0.84
Faecal Mg (mg Mg g^{-1})										
TREATMENT	1.59^{ax} ± 0.16	1.71^{ax} ± 0.21	1.79^{ax} ± 0.13	1.44^{ax} ± 0.05	1.36^{ax} ± 0.05	1.53^{ax} ± 0.19	1.80^{ax} ± 0.11	2.57^{ay} ± 0.47	2.80^{ay} ± 0.58	**1.88^{a}** ± 0.22
CONTROL	2.08^{a} ± 0.12	1.86^{a} ± 0.17	1.68^{a} ± 0.04	1.45^{a} ± 0.14	1.89^{a} ± 0.18	1.77^{a} ± 0.09	2.34^{b} ± 0.21	2.85^{a} ± 0.24	2.88^{a} ± 0.28	**2.09^{a}** ± 0.16

$^{a, b}$ means with different letters in a column are significantly different between treated groups ($p<0.05$)

$^{x, y,z}$ means with different letters in a row are significantly different within treatment groups ($p<0.05$)

TABLE 5. Mean bone P, Ca and Mg, Ca:P ratio and bone thickness totals between treatment groups of all breeds and sex (mg g^{-1} ash weight)

TIME (H)	*0*	*3*	*6*	*24*	*OVERALL MEAN*
Cortical Bone P (mg P g^{-1})					
TREATMENT	168.99^{ax} ± 13.61	225.44^{ay} ± 17.26	181.49^{ax} ± 13.69	152.10^{ax} ± 11.67	**186.34^{a}** ± 14.21
CONTROL	130.60^{b} ± 8.09	212.46^{a} ± 14.98	150.78^{b} ± 10.58	164.67^{a} ± 12.29	**164.63^{b}** ± 12.62
Cortical Bone Ca (mg Ca g^{-1})					
TREATMENT	324.45^{ay} ± 13.10	269.50^{ax} ± 23.55	325.28^{ay} ± 21.41	311.46^{ay} ± 23.71	**302.08^{a}** ± 22.89
CONTROL	325.82^{a} ± 14.36	300.71^{b} ± 38.51	288.77^{b} ± 36.15	316.38^{a} ± 19.69	**301.95^{a}** ± 31.45
Cortical Bone Mg (mg Mg g^{-1})					
TREATMENT	6.48^{ay} ± 0.5	5.42^{ax} ± 0.31	5.60^{ax} ± 0.39	5.36^{ax} ± 0.54	**5.46^{a}** ± 0.41
CONTROL	5.92^{a} ± 0.42	5.69^{a} ± 0.38	5.18^{a} ± 0.65	5.28^{a} ± 0.35	**5.38^{a}** ± 0.46
Cortical bone Ca:P ratio					
TREATMENT	1.92^{ay} ± 0.022	1.20^{ax} ± 0.12	1.79^{ay} ± 0.011	2.05^{ay} ± 0.013	**1.68^{a}** ± 0.048
CONTROL	2.49^{a} ± 0.015	1.42^{a} ± 0.0012	1.92^{a} ± 0.011	1.92^{a} ± 0.01	**1.75^{a}** ± 0.0074
Cortical Bone Thickness (mm)					
TREATMENT	2.43^{ax}	3.22^{ay}	3.45^{ay}	3.16^{ay}	**3.27^{a}**
CONTROL	2.72^{a}	3.00^{a}	2.60^{b}	2.66^{b}	**2.75^{a}**

$^{a, b}$ means with different letters in a column are significantly different between treated groups ($p<0.05$)

$^{x, y, z}$ means with different letters in a row are significantly different within treatment groups ($p<0.05$)

Table 6. Mean faecal P, Ca and Mg totals between treatment groups of all breeds and sex (mg g^{-1} ash weight)

TIME (H)	*0*	*30 min*	*1*	*2*	*3*	*5*	*6*	*24*	*30*	*OVERALL MEAN*
Faecal P (mg P g^{-1})										
TREATMENT	31.77^{ay} ± 2.36	33.45^{ay} ± 5.33	35.37^{az} ± 4.36	27.36^{axy} ± 2.78	23.67^{ax} ± 2.21	32.41^{ay} ± 2.85	28.07^{ay} ± 3.07	31.77^{ay} ± 4.28	38.29^{az} ± 4.83	**31.30^{a} ± 3.71**
CONTROL	33.75^{a} ± 5.11	40.88^{a} ± 8.49	37.27^{a} ± 6.94	27.18^{a} ± 4.92	35.11^{b} ± 0.93	36.49^{a} ± 3.71	40.08^{b} ± 4.38	30.20^{a} ± 2.10	37.23^{a} ± 6.67	**35.55^{b} ± 4.77**
Faecal Ca (mg Ca g^{-1})										
TREATMENT	57.46^{axy} ± 4.78	53.37^{ax} ± 5.78	60.24^{ay} ± 4.56	57.04^{axy} ± 6.15	61.31^{ay} ± 7.23	62.90^{ay} ± 4.45	55.20^{ax} ± 3.70	66.22^{az} ± 7.49	67.45^{az} ± 5.96	**60.47^{a} ± 5.67**
CONTROL	67.03^{b} ± 6.05	64.26^{b} ± 10.89	56.21^{b} ± 7.24	59.25^{a} ± 4.43	51.45^{b} ± 2.71	63.69^{a} ± 6.14	76.75^{b} ± 7.76	64.69^{a} ± 6.79	57.98^{b} ± 7.23	**61.78^{a} ± 6.64**
Faecal Mg (mg Mg g^{-1})										
TREATMENT	9.78^{axy} ± 1.19	11.88^{ay} ± 0.98	11.17^{ay} ± 1.14	8.24^{axy} ± 0.58	6.23^{ax} ± 0.46	8.45^{axy} ± 0.79	9.30^{axy} ± 0.90	15.48^{ayz} ± 3.20	17.19^{az} ± 3.19	**10.99^{a} ± 1.41**
CONTROL	13.84^{b} ± 0.95	14.19^{b} ± 1.72	12.18^{a} ± 3.98	10.16^{a} ± 0.98	7.17^{a} ± 0.77	12.22^{b} ± 0.92	14.85^{b} ± 1.36	16.53^{a} ± 1.88	16.99^{a} ± 2.62	**13.03^{b} ± 1.78**

$^{a, b}$ means with different letters in a column are significantly different between treated groups ($p<0.05$)

$^{x, y,z}$ means with different letters in a row are significantly different within treatment groups ($p<0.05$)

TABLE 7. Mean blood P, Ca and Mg totals between treatment groups of all breeds and sex (mg/100 ml serum)

TIME (H)	0	30 min	1	2	3	5	6	24	30	*OVERALL MEAN*
Blood P (mg/100 ml serum)										
TREATMENT	8.49^{ax} ± 0.25	7.88^{ax} ± 0.22	7.87^{ax} ± 0.21	7.76^{ax} ± 0.32	8.09^{ax} ± 0.38	7.54^{ax} ± 0.32	6.71^{ax} ± 0.19	7.17^{ax} ± 0.21	7.35^{ax} ± 0.30	**7.54^{a} ± 0.27**
CONTROL	8.29^{a} ± 0.32	7.76^{a} ± 0.27	7.79^{a} ± 0.21	7.81^{a} ± 0.20	7.83^{a} ± 0.33	7.59^{a} ± 0.34	7.70^{a} ± 0.58	8.18^{b} ± 0.24	8.15^{b} ± 0.26	**7.85^{a} ± 0.30**
Blood Ca (mg/100 ml serum)										
TREATMENT	9.74^{az} ± 0.32	8.00^{ay} ± 0.70	6.73^{ax} ± 0.70	6.26^{ax} ± 1.67	6.52^{ax} ± 0.99	10.76^{az} ± 0.66	9.00^{ay} ± 0.40	8.46^{ay} ± 0.33	7.18^{ay} ± 0.24	**7.86^{a} ± 0.71**
CONTROL	9.25^{a} ± 0.57	8.19^{a} ± 0.27	7.81^{b} ± 0.21	7.92^{b} ± 0.20	8.21^{b} ± 0.32	10.53^{a} ± 0.34	7.21^{b} ± 0.58	7.98^{a} ± 0.24	6.68^{a} ± 0.27	**8.06^{a} ± 0.30**
Blood Mg (mg/100 ml serum)										
TREATMENT	1.54^{ax} ± 0.10	1.56^{ax} ± 0.05	1.52^{ax} ± 0.07	1.38^{ax} ± 0.03	1.50^{ax} ± 0.05	1.71^{ax} ± 0.06	1.49^{ax} ± 0.06	1.56^{ax} ± 0.04	1.63^{ax} ± 0.10	**1.54^{a} ± 0.06**
CONTROL	1.51^{a} ± 0.04	1.35^{b} ± 0.04	1.31^{b} ± 0.04	1.31^{a} ± 0.04	1.44^{a} ± 0.06	1.48^{b} ± 0.06	1.26^{b} ± 0.06	1.51^{a} ± 0.05	1.31^{b} ± 0.08	**1.37^{b} ± 0.05**

$^{a, b}$ means with different letters in a column are significantly different between treated groups ($p<0.05$)

$^{x, y, z}$ means with different letters in a row are significantly different within treatment groups ($p<0.05$)

TABLE 8. Mean bone P, Ca and Mg, Ca:P ratio and bone thickness totals between breeds (mg g^{-1} fresh weight)

TIME (H)	*0*	*3*	*6*	*24*	*OVERALL MEAN*
Cortical Bone P (mg P g^{-1})					
FRIESIAN	84.69^{ax} ± 8.15	121.14^{ay} ± 11.19	104.76^{ax} ± 10.36	94.53^{ax} ± 9.39	**106.81^{a} ± 10.31**
BONSMARA	87.20^{ax} ± 6.68	131.29^{ay} ± 9.78	77.32^{bx} ± 5.39	87.00^{ax} ± 6.36	**98.54^{b} ± 7.17**
Cortical Bone Ca (mg Ca g^{-1})					
FRIESIAN	207.35^{ay} ± 11.62	183.46^{ax} ± 18.99	210.26^{ay} ± 15.54	203.34^{ay} ± 12.78	**199.02^{a} ± 15.77**
BONSMARA	200.07^{ay} ± 12.45	175.72^{axy} ± 9.10	167.87^{bxy} ± 26.44	150.02^{bx} ± 12.74	**164.54^{b} ±16.09**
Cortical Bone Mg (mg Mg g^{-1})					
FRIESIAN	3.74^{az} ± 0.22	3.48^{ay} ± 0.20	3.49^{ay} ± 0.27	3.07^{ax} ± 0.27	**3.35^{a} ± 0.25**
BONSMARA	3.92^{az} ± 0.28	3.32^{ay} ± 0.19	3.11^{ax} ± 0.46	2.86^{ax} ± 0.22	**3.09^{a} ± 0.29**
Cortical Bone Ca:P Ratio					
FRIESIAN	2.45^{az} ± 0.21	1.51^{ax} ± 0.03	2.01^{ay} ± 0.02	2.15^{ay} ± 0.02	**1.89^{a} ± 0.02**
BONSMARA	2.29^{az} ± 0.22	1.34^{ax} ± 0.021	2.17^{bz} ± 0.012	1.72^{by} ± 0.012	**1.74^{b} ± 0.01**
Cortical Bone Thickness (mm)					
FRIESIAN	2.76^{ax}	3.38^{ay}	3.32^{ay}	3.10^{ay}	**3.27^{a}**
BONSMARA	2.39^{ax}	2.85^{ay}	2.74^{by}	2.73^{by}	**2.77^{b}**

$^{a, b}$ means with different letters in a column are significantly different between treated groups ($p<0.05$)

$^{x, y,z}$ means with different letters in a row are significantly different within treatment groups ($p<0.05$)

TABLE 9. Mean faecal P, Ca and Mg totals between breeds (mg g^{-1} fresh weight)

TIME (H)	*0*	*30 min*	*1*	*2*	*3*	*5*	*6*	*24*	*30*	*OVERALL MEAN*
Faecal P (mg P g^{-1})										
FRIESIAN	1.92^{ax} ± 0.75	2.06^{ax} ± 0.32	2.15^{ax} ± 0.31	1.96^{ax} ± 0.11	1.92^{ax} ± 0.10	1.80^{ax} ± 0.22	2.02^{ax} ± 0.22	2.57^{ay} ± 0.27	2.18^{ax} ± 0.45	**2.08^{a}** ± 0.25
BONSMARA	1.92^{aw} ± 0.34	2.03^{ax} ± 0.83	2.05^{ax} ± 0.51	2.08^{ax} ± 0.53	2.04^{ax} ± 0.21	2.34^{by} ± 0.43	2.23^{ay} ± 0.12	1.79^{bw} ± 0.23	2.05^{bx} ± 0.25	**2.06^{a}** ± 0.82
Faecal Ca (mg Ca g^{-1})										
FRIESIAN	5.28^{ax} ± 0.44	5.03^{ax} ± 0.49	6.03^{ax} ± 0.69	5.90^{ax} ± 0.35	8.11^{ay} ± 1.91	8.30^{ay} ± 0.67	7.74^{ay} ± 0.73	5.18^{ax} ± 0.61	5.59^{ax} ± 0.50	**6.48^{a}** ± 0.74
BONSMARA	3.30^{bx} ± 0.32	2.92^{bw} ± 0.07	2.83^{bw} ± 0.11	2.98^{bw} ± 0.25	2.92^{bw} ± 0.29	2.86^{bw} ± 0.26	3.35^{bx} ± 0.23	3.77^{by} ± 0.22	3.50^{by} ± 0.06	**3.16^{b}** ± 0.02
Faecal Mg (mg Mg g^{-1})										
FRIESIAN	0.38^{ax} ± 0.05	0.24^{ax} ± 0.13	0.27^{ax} ± 0.03	0.21^{ax} ± 0.04	0.25^{ax} ± 0.03	0.31^{ax} ± 0.07	0.29^{ax} ± 0.07	1.40^{ay} ± 0.44	1.69^{ay} ± 0.54	**0.58^{a}** ± 0.17
BONSMARA	0.75^{by} ± 0.03	0.80^{by} ± 0.06	0.74^{by} ± 0.01	0.58^{bw} ± 0.01	0.69^{bx} ± 0.03	0.68^{bx} ± 0.03	0.88^{bz} ± 0.04	0.91^{bz} ± 0.04	0.89^{bz} ± 0.03	**0.77^{b}** ± 0.01

$^{a, b}$ means with different letters in a column are significantly different between treated groups ($p<0.05$)

$^{x, y, z}$ means with different letters in a row are significantly different within treatment groups ($p<0.05$)

TABLE 10. Mean bone P, Ca and Mg, Ca:P ratio and bone thickness totals between breeds (mg g^{-1} dry weight)

TIME (H)	*0*	*3*	*6*	*24*	*OVERALL MEAN*
Cortical Bone P (mg P g^{-1})					
FRIESIAN	95.41^{ax} ± 8.78	136.16^{ay} ± 12.96	116.89^{ax} ± 10.33	107.33^{ax} ± 9.07	**120.12^{a} ± 10.79**
BONSMARA	95.14^{ax} ± 6.90	148.10^{ay} ± 10.87	$91.06a^{bx}$ ± 6.18	98.13^{ax} ± 7.11	**112.43^{b} ± 8.05**
Cortical Bone Ca (mg Ca g^{-1})					
FRIESIAN	227.12^{ay} ± 11.93	197.32^{ax} ± 21.53	230.06^{ay} ± 16.90	222.39^{ay} ± 13.78	**216.59^{a} ± 17.40**
BONSMARA	213.65^{axy} ± 16.67	193.90^{ax} ± 10.17	185.82^{bx} ± 29.23	173.64^{bx} ± 15.07	**184.45^{b} ± 13.13**
Cortical Bone Mg (mg Mg g^{-1})					
FRIESIAN	4.07^{ax} ± 0.22	3.78^{ax} ± 0.24	3.83^{ax} ± 0.30	3.36^{ax} ± 0.29	**3.86^{a} ± 0.27**
BONSMARA	4.19^{az} ± 0.39	3.67^{axy} ± 0.21	3.44^{ax} ± 0.51	3.34^{ax} ± 0.28	**3.48^{a} ± 0.33**
Cortical Bone Ca:P Ratio					
FRIESIAN	2.38^{az} ± 0.015	1.45^{ax} ± 0.0013	1.97^{ay} ± 0.001	2.07^{ay} ± 0.02	**1.83^{a} ± 0.007**
BONSMARA	2.25^{az} ± 0.011	1.31^{ax} ± 0.003	2.04^{by} ± 0.03	1.77^{by} ± 0.002	**1.71^{a} ± 0.011**
Cortical Bone Thickness (mm)					
FRIESIAN	2.76^{ax}	3.38^{ay}	3.32^{ay}	3.10^{ay}	**3.27^{a}**
BONSMARA	2.39^{ax}	2.85^{ay}	2.74^{by}	2.73^{by}	**2.77^{b}**

$^{a, b}$ means with different letters in a column are significantly different between treated groups ($p<0.05$)

$^{x, y, z}$ means with different letters in a row are significantly different within treatment groups ($p<0.05$)

TABLE 11. Mean faecal P, Ca and Mg totals between all breeds (mg g^{-1} dry weight)

TIME (H)	*0*	*30 min*	*1*	*2*	*3*	*5*	*6*	*24*	*30*	*OVERALL MEAN*
Faecal P (mg P g^{-1})										
FRIESIAN	2.04^{ax} ± 0.35	2.18^{ax} ± 0.34	2.25^{ax} ± 0.33	2.12^{ax} ± 0.64	1.90^{ax} ± 0.09	1.92^{ax} ± 0.24	2.16^{ax} ± 0.23	2.75^{ay} ± 0.29	3.01^{ay} ± 0.48	**2.29^{a} ± 0.33**
BONSMARA	2.08^{aw} ± 0.38	2.10^{aw} ± 0.05	2.22^{aw} ± 0.06	2.26^{aw} ± 0.01	2.21^{aw} ± 0.03	2.54^{bw} ± 0.03	2.42^{aw} ± 0.04	1.95^{bw} ± 0.02	2.24^{bw} ± 0.06	**2.22^{a} ± 0.07**
Faecal Ca (mg Ca g^{-1})										
FRIESIAN	6.79^{ay} ± 0.66	5.33^{ax} ± 0.53	6.31^{ay} ± 0.72	6.36^{ay} ± 0.37	8.58^{az} ± 0.62	8.87^{az} ± 0.72	8.26^{az} ± 0.78	5.54^{ax} ± 0.60	5.98^{ax} ± 0.46	**6.90^{a} ± 0.60**
BONSMARA	3.58^{bx} ± 0.40	2.99^{bw} ± 0.16	3.07^{bw} ± 0.15	3.23^{bw} ± 0.05	3.14^{bw} ± 0.26	3.11^{bw} ± 0.22	3.64^{bw} ± 0.20	4.08^{bx} ± 0.11	3.82^{bx} ± 0.30	**3.41^{b} ± 0.02**
Faecal Mg (mg Mg g^{-1})										
FRIESIAN	0.43^{ax} ± 0.06	0.26^{ax} ±. 0.11	0.28^{ax} ± 0.03	0.23^{ax} ± 0.04	0.26^{ax} ± 0.03	0.34^{ax} ± 0.06	0.32^{ax} ± 0.06	1.50^{ay} ± 0.05	1.81^{ay} ± 0.58	**0.62^{a} ± 0.12**
BONSMARA	0.81^{by} ± 0.02	0.83^{by} ± 0.02	0.80^{by} ± 0.01	0.66^{bw} ± 0.01	0.74^{bx} ± 0.02	0.74^{bx} ± 0.03	0.96^{bz} ± 0.03	0.98^{bz} ± 0.025	0.97^{bz} ± 0.028	**0.83^{b} ± 0.022**

$^{a, b}$ means with different letters in a column are significantly different between treated groups ($p<0.05$)

$^{x, y,z}$ means with different letters in a row are significantly different within treatment groups ($p<0.05$)

TABLE 12. Mean bone P, Ca and Mg, Ca:P ratio and bone thickness totals between breeds (mg g^{-1} ash weight)

TIME (H)	*0*	*3*	*6*	*24*	*OVERALL MEAN*
Cortical Bone P (mg P g^{-1})					
FRIESIAN	145.07^{ax} ± 11.07	207.51^{az} ± 17.81	184.11^{ay} ± 13.07	158.75^{ax} ± 12.59	**183.46^{a} ± 14.49**
BONSMARA	154.53^{ax} ± 10.62	230.39^{by} ± 14.43	148.16^{bx} ± 11.19	158.02^{ax} ± 11.37	**178.86^{a} ± 12.33**
Cortical Bone Ca (mg Ca g^{-1})					
FRIESIAN	314.96^{ax} ± 13.95	302.34^{ax} ± 33.48	334.74^{ax} ± 11.48	355.20^{ax} ± 23.24	**330.76^{a} ± 22.73**
BONSMARA	308.31^{az} ± 13.52	267.88^{bx} ± 28.58	279.32^{bx} ± 46.08	272.64^{bx} ± 20.16	**273.28^{b} ± 31.61**
Cortical Bone Mg (mg Mg g^{-1})					
FRIESIAN	6.19^{ay} ± 0.31	5.73^{ax} ± 0.35	5.60^{ax} ± 0.24	5.36^{ax} ± 0.48	**5.56^{a} ± 0.35**
BONSMARA	6.21^{ay} ± 0.61	5.38^{ax} ± 0.34	5.18^{ax} ± 0.79	5.27^{ax} ± 0.41	**5.27^{a} ± 0.51**
Cortical Bone Ca:P Ratio					
FRIESIAN	2.36^{ay} ± 0.21	1.46^{ax} ± 0.05	1.82^{ax} ± 0.043	2.24^{ay} ± 0.018	**1.84^{a} ± 0.037**
BONSMARA	2.00^{az} ± 0.22	1.16^{ax} ± 0.056	1.89^{ay} ± 0.06	1.73^{by} ± 0.037	**1.59^{a} ± 0.051**
Cortical Bone Thickness (mm)					
FRIESIAN	2.76^{ax}	3.38^{ay}	3.32^{ay}	3.10^{ay}	**3.26^{a}**
BONSMARA	2.39^{ax}	2.85^{ay}	2.74^{by}	2.73^{by}	**2.77^{b}**

$^{a, b}$ means with different letters in a column are significantly different between treated groups ($p<0.05$)

$^{x, y, z}$ means with different letters in a row are significantly different within treatment groups ($p<0.05$)

Table 13. Mean faecal P, Ca and Mg totals between breeds (mg g^{-1} ash weight)

TIME (H)	*0*	*30 min*	*1*	*2*	*3*	*5*	*6*	*24*	*30*	*OVERALL MEAN*
Faecal P (mg P g^{-1})										
FRIESIAN	12.72^{ay} ± 2.07	11.98^{ax} ± 2.43	12.57^{ay} ± 2.20	13.95^{ay} ± 0.74	12.04^{ay} ± 0.83	11.61^{ax} ± 1.48	13.62^{ay} ± 1.66	15.71^{az} ± 1.25	15.83^{az} ± 2.40	**13.41^{a} ± 1.62**
BONSMARA	13.20^{ay} ± 2.41	15.60^{bz} ± 3.51	15.02^{bz} ± 3.08	10.15^{bw} ± 2.01	11.69^{ax} ± 2.30	14.32^{bz} ± 3.08	13.63^{ay} ± 3.58	11.57^{bx} ± 2.14	14.93^{az} ± 3.10	**13.34^{a} ± 2.13**
Faecal Ca (mg Ca g^{-1})										
FRIESIAN	32.13^{ax} ± 3.54	27.16^{ax} ± 4.88	34.20^{ax} ± 3.99	35.82^{ax} ± 2.33	44.89^{ay} ± 5.52	50.24^{ay} ± 5.92	47.88^{ay} ± 4.88	33.34^{ax} ± 4.62	32.57^{ax} ± 5.01	**38.26^{a} ± 4.64**
BONSMARA	23.09^{bz} ± 4.28	22.62^{by} ± 3.80	20.56^{by} ± 3.18	20.12^{by} ± 2.06	16.97^{bx} ± 3.45	19.09^{by} ± 4.23	21.02^{by} ± 5.08	24.39^{bz} ± 3.67	23.22^{bz} ± 5.19	**21.23^{b} ± 4.60**
Faecal Mg (mg Mg g^{-1})										
FRIESIAN	2.64^{ay} ± 0.45	1.41^{ax} ± 0.32	1.65^{ax} ± 0.32	1.44^{ax} ± 0.16	1.63^{ax} ± 0.24	2.22^{ay} ± 0.49	2.11^{ay} ± 0.45	8.55^{az} ± 2.59	9.39^{az} ± 2.90	**3.55^{a} ± 0.93**
BONSMARA	5.25^{by} ± 1.06	6.16^{bz} ± 1.38	5.42^{by} ± 1.80	4.24^{bx} ± 1.39	2.94^{bw} ± 0.65	4.61^{bx} ± 1.31	5.51^{by} ± 0.80	5.87^{by} ± 1.40	6.20^{bz} ± 0.71	**5.13^{b} ± 0.98**

$^{a, b}$ means with different letters in a column are significantly different between treated groups ($p<0.05$)

$^{x, y, z}$ means with different letters in a row are significantly different within treatment groups ($p<0.05$)

TABLE 14. Mean blood P, Ca and Mg totals between breeds (mg/100 ml serum)

TIME (H)	*0*	*30 min*	*1*	*2*	*3*	*5*	*6*	*24*	*30*	*OVERALL MEAN*
Blood P (mg/100 ml serum)										
FRIESIAN	7.74^{ax} ± 0.19	7.80^{ax} ± 0.20	7.65^{ax} ± 0.22	7.71^{ax} ± 0.22	8.11^{ax} ± 0.24	7.66^{ax} ± 0.22	7.18^{ax} ± 0.42	7.45^{ax} ± 0.16	7.37^{ax} ± 0.22	**7.61^{a} ± 0.23**
BONSMARA	9.04^{ay} ± 0.37	7.84^{ax} ± 0.29	8.01^{ax} ± 0.20	7.86^{ax} ± 0.31	7.81^{ax} ± 0.47	7.48^{ax} ± 0.43	7.23^{ax} ± 0.35	7.89^{ax} ± 0.29	8.12^{ax} ± 0.34	**7.78^{a} ± 0.33**
Blood Ca (mg/100 ml serum)										
FRIESIAN	13.71^{ay} ± 0.73	10.97^{axy} ± 2.33	9.43^{ax} ± 2.11	9.31^{ax} ± 3.38	9.92^{ax} ± 2.73	16.47^{az} ± 1.51	11.32^{axy} ± 0.53	11.82^{ay} ± 0.59	9.30^{ax} ± 0.47	**11.06^{a} ± 1.71**
BONSMARA	5.27^{bxy} ± 0.15	5.22^{bxy} ± 0.14	5.12^{bxy} ± 0.15	4.86^{bx} ± 0.11	4.82^{bx} ± 0.13	4.81^{bx} ± 0.09	4.89^{bx} ± 0.11	4.62^{bx} ± 0.09	4.55^{bx} ± 0.05	**4.86^{b} ± 0.11**
Blood Mg (mg/100 ml serum)										
FRIESIAN	2.10^{axy} ± 0.07	1.81^{ax} ± 0.05	1.78^{ax} ± 0.08	1.67^{ax} ± 0.05	1.91^{ax} ± 0.08	2.17^{az} ± 0.09	1.75^{ax} ± 0.07	2.07^{axy} ± 0.07	2.18^{az} ± 0.14	**1.92^{a} ± 0.07**
BONSMARA	0.95^{ax} ± 0.08	1.10^{bx} ± 0.04	1.06^{bx} ± 0.04	1.01^{bx} ± 0.02	1.03^{bx} ± 0.03	1.02^{bx} ± 0.02	1.00^{bx} ± 0.04	1.01^{bx} ± 0.01	0.76^{bx} ± 0.04	**1.00^{b} ± 0.03**

$^{a, b}$ means with different letters in a column are significantly different between treated groups ($p<0.05$)

$^{x, y,z}$ means with different letters in a row are significantly different within treatment groups ($p<0.05$)

TABLE 15. Mean bone P, Ca and Mg, Ca:P ratio and bone thickness totals between sex (mg g^{-1} fresh weight)

TIME (H)	*0*	*3*	*6*	*24*	*OVERALL MEAN*
Cortical Bone P (mg P g^{-1})					
MALE	80.41^{ax} ± 6.97	123.82^{ay} ± 10.18	75.82^{ax} ± 7.05	83.26^{ax} ± 7.40	**94.30^{a} ± 8.12**
FEMALE	91.48^{bx} ± 7.85	128.61^{ay} ± 10.78	106.27^{bx} ± 8.68	98.26^{ax} ± 8.36	**111.05^{b} ± 9.28**
Cortical Bone Ca (mg Ca g^{-1})					
MALE	210.39^{ay} ± 14.47	183.13^{ax} ± 16.70	191.72^{ax} ± 26.17	180.54^{ax} ± 18.49	**185.13^{a} ± 20.45**
FEMALE	197.02^{az} ± 9.63	176.06^{ax} ± 11.40	186.41^{ay} ± 15.81	172.82^{ax} ± 7.04	**178.43^{a} ± 11.42**
Cortical Bone Mg (mg Mg g^{-1})					
MALE	3.89^{az} ± 0.27	3.59^{ay} ± 0.19	3.27^{ay} ± 0.42	2.99^{ax} ± 0.26	**3.28^{a} ± 0.29**
FEMALE	3.78^{az} ± 0.23	3.21^{ay} ± 0.21	3.33^{ay} ± 0.31	2.94^{ax} ± 0.23	**3.16^{a} ± 0.25**
Cortical Bone Ca:P Ratio					
MALE	2.62^{az} ± 0.23	1.48^{ax} ± 0.20	2.53^{az} ± 0.51	2.17^{ay} ± 0.21	**2.06^{a} ± 0.30**
FEMALE	2.15^{az} ± 0.30	1.37^{ax} ± 0.12	1.75^{by} ± 0.31	1.76^{by} ± 0.29	**1.63^{b} ± 0.24**
Cortical Bone Thickness (mm)					
MALE	2.77^{ax}	3.25^{ay}	3.11^{ay}	3.05^{ay}	**3.14^{a}**
FEMALE	2.38^{ax}	2.98^{ay}	2.94^{ay}	2.78^{ay}	**2.90^{a}**

$^{a, b}$ means with different letters in a column are significantly different between treated groups ($p<0.05$)

$^{x, y, z}$ means with different letters in a row are significantly different within treatment groups ($p<0.05$)

TABLE 16. Mean faecal P, Ca and Mg totals between sex (mg g^{-1} fresh weight).

TIME (H)	*0*	*30 min*	*1*	*2*	*3*	*5*	*6*	*24*	*30*	*OVERALL MEAN*
Faecal P (mg P g^{-1})										
MALE	4.77^{ax} ± 0.74	4.77^{ax} ± 0.24	4.55^{ax} ± 2.00	4.72^{ax} ± 0.52	4.38^{ax} ± 0.22	4.88^{ax} ± 0.31	4.59^{ax} ± 0.61	3.98^{ax} ± 0.44	5.19^{ay} ± 0.72	**4.63^{a} ± 0.63**
FEMALE	4.83^{ax} ± 1.34	5.44^{by} ± 0.90	5.79^{by} ± 0.66	5.56^{by} ± 0.47	5.70^{by} ± 0.39	6.28^{bz} ± 0.55	6.39^{bz} ± 0.63	5.78^{by} ± 0.37	5.84^{ay} ± 0.39	**5.85^{b} ± 0.55**
Faecal Ca (mg Ca g^{-1})										
MALE	11.90^{axy} ± 0.95	11.76^{axy} ± 1.10	12.40^{ay} ± 1.02	12.54^{ay} ± 1.03	14.31^{az} ± 1.00	14.28^{az} ± 0.91	13.53^{az} ± 0.75	10.60^{ax} ± 0.82	10.41^{ax} ± 0.88	**12.48^{a} ± 0.93**
FEMALE	6.59^{bxy} ± 0.80	5.02^{bx} ± 0.36	4.96^{bx} ± 0.78	5.30^{bx} ± 0.30	5.46^{bx} ± 1.50	5.47^{bx} ± 0.43	7.64^{by} ± 0.87	9.65^{bz} ± 0.81	9.41^{bz} ± 0.80	**6.61^{b} ± 0.73**
Faecal Mg (mg Mg g^{-1})										
MALE	1.67^{ax} ± 0.14	2.12^{ay} ± 0.11	2.08^{ay} ± 0.05	1.66^{ax} ± 0.08	1.70^{ax} ± 0.12	2.04^{ay} ± 0.16	2.14^{ay} ± 0.08	2.62^{az} ± 0.24	2.93^{az} ± 0.33	**2.16^{a} ± 0.14**
FEMALE	1.70^{ay} ± 0.14	1.31^{bxy} ± 0.18	1.13^{bx} ± 0.17	0.87^{bx} ± 0.08	1.31^{axy} ± 0.10	1.00^{bx} ± 0.12	1.67^{ay} ± 0.23	2.40^{az} ± 0.43	2.32^{az} ± 0.45	**1.50^{b} ± 0.22**

$^{a, b}$ means with different letters in a column are significantly different between treated groups ($p<0.05$)

$^{x, y,z}$ means with different letters in a row are significantly different within treatment groups ($p<0.05$)

TABLE 17. Mean bone P, Ca and Mg, Ca:P ratio and bone thickness totals between sex (mg g^{-1} dry weight)

TIME (H)	*0*	*3*	*6*	*24*	*OVERALL MEAN*
Cortical Bone P (mg P g^{-1})					
MALE	89.83^{ax} ± 7.29	140.60^{ay} ± 11.88	86.34^{ax} ± 7.47	94.09^{ax} ± 7.89	**107.01^{a} ± 9.07**
FEMALE	100.72^{bx} ± 8.38	143.66^{ay} ± 11.95	121.61^{bx} ± 9.67	111.36^{ax} ± 8.32	**125.54^{b} ± 9.98**
Cortical Bone Ca (mg Ca g^{-1})					
MALE	222.07^{ay} ± 17.97	199.29^{ax} ± 18.13	209.34^{ax} ± 28.54	204.29^{ax} ± 21.12	**204.31^{a} ± 22.59**
FEMALE	218.70^{az} ± 10.63	191.92^{ax} ± 13.57	206.55^{ay} ± 17.60	191.74^{ax} ± 7.73	**196.74^{a} ± 12.96**
Cortical Bone Mg (mg Mg g^{-1})					
MALE	4.06^{az} ± 0.34	3.94^{ay} ± 0.21	3.57^{ay} ± 0.46	3.44^{ax} ± 0.33	**3.65^{a} ± 0.33**
FEMALE	4.20^{az} ± 0.27	3.51^{ay} ± 0.24	3.69^{ay} ± 0.34	3.26^{ax} ± 0.25	**3.49^{a} ± 0.27**
Cortical Bone Ca:P Ratio					
MALE	2.47^{az} ± 0.30	1.42^{ax} ± 0.20	2.42^{az} ± 0.23	2.17^{ay} ± 0.21	**2.00^{a} ± 0.21**
FEMALE	2.17^{az} ± 0.32	1.34^{ax} ± 0.12	1.70^{by} ± 0.07	1.72^{by} ± 0.05	**1.59^{b} ± 0.08**
Cortical Bone Thickness (mm)					
MALE	2.77^{ax}	3.25^{ay}	3.11^{ay}	3.05^{ay}	**3.14^{a}**
FEMALE	2.38^{ax}	2.98^{ay}	2.94^{ay}	2.78^{ay}	**2.90^{a}**

$^{a, b}$ means with different letters in a column are significantly different between treated groups ($p<0.05$)

$^{x, y, z}$ means with different letters in a row are significantly different within treatment groups ($p<0.05$)

TABLE 18. Mean faecal P, Ca and Mg totals between sex (mg g^{-1} dry weight)

TIME (H)	***0***	***30 min***	***1***	***2***	***3***	***5***	***6***	***24***	***30***	***OVERALL MEAN***
Faecal P (mg P g^{-1})										
MALE	5.18^{axy} ± 0.31	4.72^{ax} ± 0.42	4.90^{ax} ± 0.38	5.10^{ax} ± 1.10	4.54^{ax} ± 0.22	5.30^{axy} ± 0.34	4.95^{ax} ± 0.43	4.29^{ax} ± 0.47	5.60^{ay} ± 0.78	**4.92^{a} ± 0.52**
FEMALE	5.18^{ax} ± 0.98	5.87^{bxy} ± 0.97	6.32^{by} ± 0.71	6.04^{by} ± 0.53	6.18^{by} ± 0.31	6.77^{bz} ± 0.37	6.90^{bz} ± 0.69	6.25^{by} ± 0.41	6.40^{ay} ± 0.46	**6.34^{b} ± 0.55**
Faecal Ca (mg Ca g^{-1})										
MALE	14.05^{az} ± 1.22	11.88^{axy} ± 1.32	13.26^{ay} ± 1.08	13.52^{ay} ± 1.11	15.25^{az} ± 0.93	15.39^{az} ± 0.98	14.55^{az} ± 0.80	11.41^{axy} ± 0.82	11.14^{axy} ± 0.84	**13.30^{a} ± 0.99**
FEMALE	7.05^{bxy} ± 0.84	5.40^{bx} ± 0.36	5.34^{bx} ± 0.58	5.76^{bx} ± 0.33	5.92^{bx} ± 0.36	5.90^{bx} ± 0.46	8.29^{by} ± 0.80	10.46^{bz} ± 0.88	10.09^{bz} ± 0.92	**7.15^{b} ± 0.59**
Faecal Mg (mg Mg g^{-1})										
MALE	1.82^{ax} ± 0.11	2.15^{ay} ± 0.23	2.26^{ay} ± 0.05	1.95^{ax} ± 0.09	1.82^{ax} ± 0.13	2.22^{ay} ± 0.16	2.32^{ay} ± 0.07	2.82^{az} ± 0.25	3.14^{az} ± 0.35	**2.33^{a} ± 0.17**
FEMALE	1.84^{ay} ± 0.17	1.42^{bxy} ± 0.16	1.22^{bx} ± 0.12	0.94^{bx} ± 0.09	1.42^{axy} ± 0.11	1.08^{bx} ± 0.13	1.82^{ay} ± 0.25	2.61^{az} ± 0.47	2.55^{az} ± 0.49	**1.63^{b} ± 0.22**

a, b means with different letters in a column are significantly different between treated groups ($p<0.05$)

x, y,z means with different letters in a row are significantly different within treatment groups ($p<0.05$)

TABLE 19. Mean bone P, Ca and Mg, Ca:P ratio and bone thickness totals between sex (mg g^{-1} ash weight)

TIME (H)	*0*	*3*	*6*	*24*	*OVERALL MEAN*
Cortical Bone P (mg P g^{-1})					
MALE	153.59^{ax} ± 12.10	225.64^{ay} ± 19.46	143.22^{ax} ± 11.92	152.69^{ax} ± 12.79	**173.85^{a} ± 14.72**
FEMALE	146.00^{ax} ± 9.60	212.25^{ay} ± 12.78	189.05^{bxy} ± 12.34	164.08^{ax} ± 11.17	**188.46^{a} ± 12.09**
Cortical Bone Ca (mg Ca g^{-1})					
MALE	327.69^{ay} ± 11.65	287.84^{ax} ± 43.13	306.95^{ax} ± 29.63	328.10^{ay} ± 31.41	**307.63^{a} ± 34.80**
FEMALE	322.58^{ay} ± 15.82	282.37^{ax} ± 18.70	307.11^{ax} ± 27.93	299.73^{ax} ± 11.99	**296.40^{a} ± 19.54**
Cortical Bone Mg (mg Mg g^{-1})					
MALE	6.20^{az} ± 0.52	5.96^{ay} ± 0.33	5.29^{ax} ± 0.51	5.53^{axy} ± 0.47	**5.59^{a} ± 0.44**
FEMALE	6.20^{az} ± 0.40	5.15^{bx} ± 0.35	5.48^{ay} ± 0.53	5.10^{ax} ± 0.42	**5.24^{a} ± 0.43**
Cortical Bone Ca:P Ratio					
MALE	2.13^{ay} ± 0.23	1.28^{ax} ± 0.02	2.14^{ay} ± 0.33	2.15^{ay} ± 0.44	**1.86^{a} ± 0.26**
FEMALE	2.21^{az} ± 0.13	1.33^{ax} ± 0.024	1.62^{by} ± 0.03	1.83^{by} ± 0.02	**1.59^{a} ± 0.25**
Cortical Bone Thickness (mm)					
MALE	2.77^{ax}	3.25^{ay}	3.11^{ay}	3.05^{ay}	**3.16^{a}**
FEMALE	2.38^{ax}	2.98^{ay}	2.94^{ay}	2.78^{ay}	**2.90^{a}**

$^{a, b}$ means with different letters in a column are significantly different between treated groups ($p<0.05$)

$^{x, y, z}$ means with different letters in a row are significantly different within treatment groups ($p<0.05$)

Table 20. Mean faecal P, Ca and Mg totals between sex (mg g^{-1} ash weight)

TIME (H)	*0*	*30 min*	*1*	*2*	*3*	*5*	*6*	*24*	*30*	*OVERALL MEAN*
Faecal P (mg P g^{-1})										
MALE	34.47^{ay} ± 1.79	32.48^{ay} ± 1.60	31.57^{ay} ± 2.12	32.35^{ay} ± 3.05	26.22^{ax} ± 1.09	34.57^{ay} ± 1.33	27.95^{ax} ± 4.40	25.97^{ax} ± 2.58	30.60^{ay} ± 4.98	**30.21^{a} ± 5.64**
FEMALE	31.05^{ay} ± 5.69	41.86^{bz} ± 12.22	41.08^{bz} ± 9.17	22.19^{bx} ± 4.65	32.56^{ay} ± 2.05	34.33^{ay} ± 5.23	40.21^{bz} ± 3.03	36.01^{byz} ± 3.18	44.94^{bz} ± 6.52	**36.65^{b} ± 5.83**
Faecal Ca (mg Ca g^{-1})										
MALE	83.12^{ayz} ± 6.89	76.87^{ay} ± 8.89	81.86^{ayz} ± 6.47	81.63^{ayz} ± 7.81	82.79^{ayz} ± 6.99	95.08^{az} ± 5.39	82.19^{ayz} ± 6.02	70.05^{axy} ± 6.88	57.95^{ax} ± 6.93	**78.55^{a} ± 6.92**
FEMALE	41.37^{by} ± 3.93	40.75^{by} ± 7.78	34.59^{bx} ± 5.33	44.66^{by} ± 2.77	29.97^{bx} ± 2.98	31.51^{bx} ± 5.19	49.77^{by} ± 5.44	60.87^{bz} ± 7.41	67.48^{bz} ± 6.27	**44.95^{b} ± 5.39**
Faecal Mg (mg Mg g^{-1})										
MALE	12.81^{axy} ± 1.19	15.38^{ay} ± 0.81	15.15^{ay} ± 0.50	12.50^{axy} ± 0.77	10.79^{ax} ± 0.85	15.14^{ay} ± 0.67	13.16^{axy} ± 0.59	16.81^{az} ± 1.70	16.59^{az} ± 2.42	**14.44^{a} ± 1.04**
FEMALE	10.82^{ayz} ± 0.95	10.68^{byz} ± 1.88	8.20^{by} ± 1.09	5.91^{bxy} ± 0.78	2.61^{bx} ± 0.38	5.54^{bxy} ± 1.03	10.98^{byz} ± 1.68	15.20^{bz} ± 3.39	17.60^{bz} ± 3.39	**9.59^{b} ± 1.70**

$^{a, b}$ means with different letters in a column are significantly different between treated groups ($p<0.05$)

$^{x, y,z}$ means with different letters in a row are significantly different within treatment groups ($p<0.05$)

TABLE 21. Mean blood P, Ca and Mg totals between sex (mg/100 ml serum)

TIME (H)	*0*	*30 min*	*1*	*2*	*3*	*5*	*6*	*24*	*30*	*OVERALL MEAN*
Blood P (mg/100 ml serum)										
MALE	8.76^{ay} ± 0.29	8.05^{ax} ± 0.16	8.92^{ay} ± 0.16	7.78^{ax} ± 0.16	8.01^{ax} ± 0.28	7.50^{ax} ± 0.24	7.33^{ax} ± 0.18	7.98^{ax} ± 0.15	7.51^{ax} ± 0.16	**7.88^{a} ± 0.19**
FEMALE	8.02^{bz} ± 0.27	7.59^{by} ± 0.33	7.54^{by} ± 0.26	7.79^{ay} ± 0.37	7.91^{ay} ± 0.43	7.64^{ay} ± 0.41	7.08^{ax} ± 0.60	7.37^{bx} ± 0.30	7.98^{az} ± 0.41	**7.61^{a} ± 0.39**
Blood Ca (mg/100 ml serum)										
MALE	8.55^{ay} ± 0.27	7.52^{ayz} ± 0.31	6.56^{axy} ± 0.46	6.56^{axy} ± 1.72	7.01^{axy} ± 0.73	12.38^{az} ± 1.12	8.35^{ay} ± 0.28	6.89^{axy} ± 0.33	5.39^{ax} ± 0.35	**7.58^{a} ± 0.66**
FEMALE	10.43^{bz} ± 0.61	8.67^{byz} ± 2.16	8.00^{by} ± 1.80	7.61^{ax} ± 1.77	7.72^{ax} ± 2.11	8.90^{bz} ± 0.48	7.87^{ax} ± 0.37	9.55^{bz} ± 0.35	8.47^{byz} ± 0.17	**8.35^{b} ± 1.15**
Blood Mg (mg/100 ml serum)										
MALE	1.41^{ax} ± 0.05	1.45^{ax} ± 0.05	1.46^{ax} ± 0.06	1.42^{ax} ± 0.04	1.74^{ay} ± 0.08	1.75^{ay} ± 0.07	1.38^{ax} ± 0.07	1.43^{ax} ± 0.05	1.32^{ax} ± 0.11	**1.49^{a} ± 0.07**
FEMALE	1.64^{by} ± 0.10	1.46^{ax} ± 0.05	1.37^{ax} ± 0.05	1.27^{bx} ± 0.03	1.20^{bx} ± 0.03	1.44^{bx} ± 0.05	1.37^{ax} ± 0.05	1.64^{by} ± 0.05	1.62^{by} ± 0.06	**1.42^{a} ± 0.05**

$^{a, b}$ means with different letters in a column are significantly different between treated groups ($p<0.05$)

^{x,y,z} means with different letters in a row are significantly different within treatment groups ($p<0.05$

FIGURES

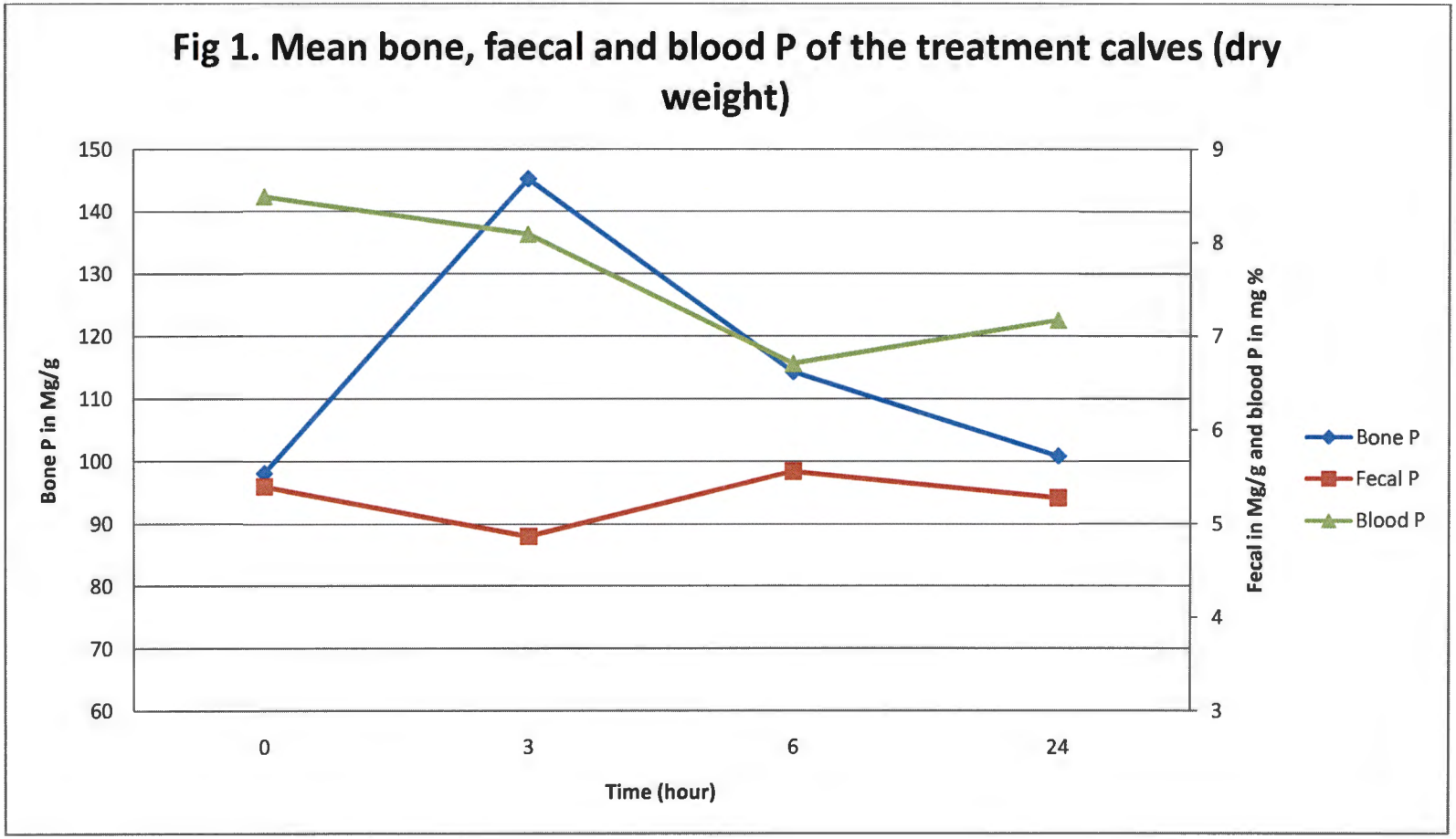


Fig 1. Mean bone, faecal and blood P of the treatment calves (dry weight)
Bone P in Mg/g
150
140
130
120
110
100
90
80
70
60
Fecal in Mg/g and blood P in mg %
9
8
7
6
5
4
3
0
3
6
24
Time (hour)
Bone P
Fecal P
Blood P

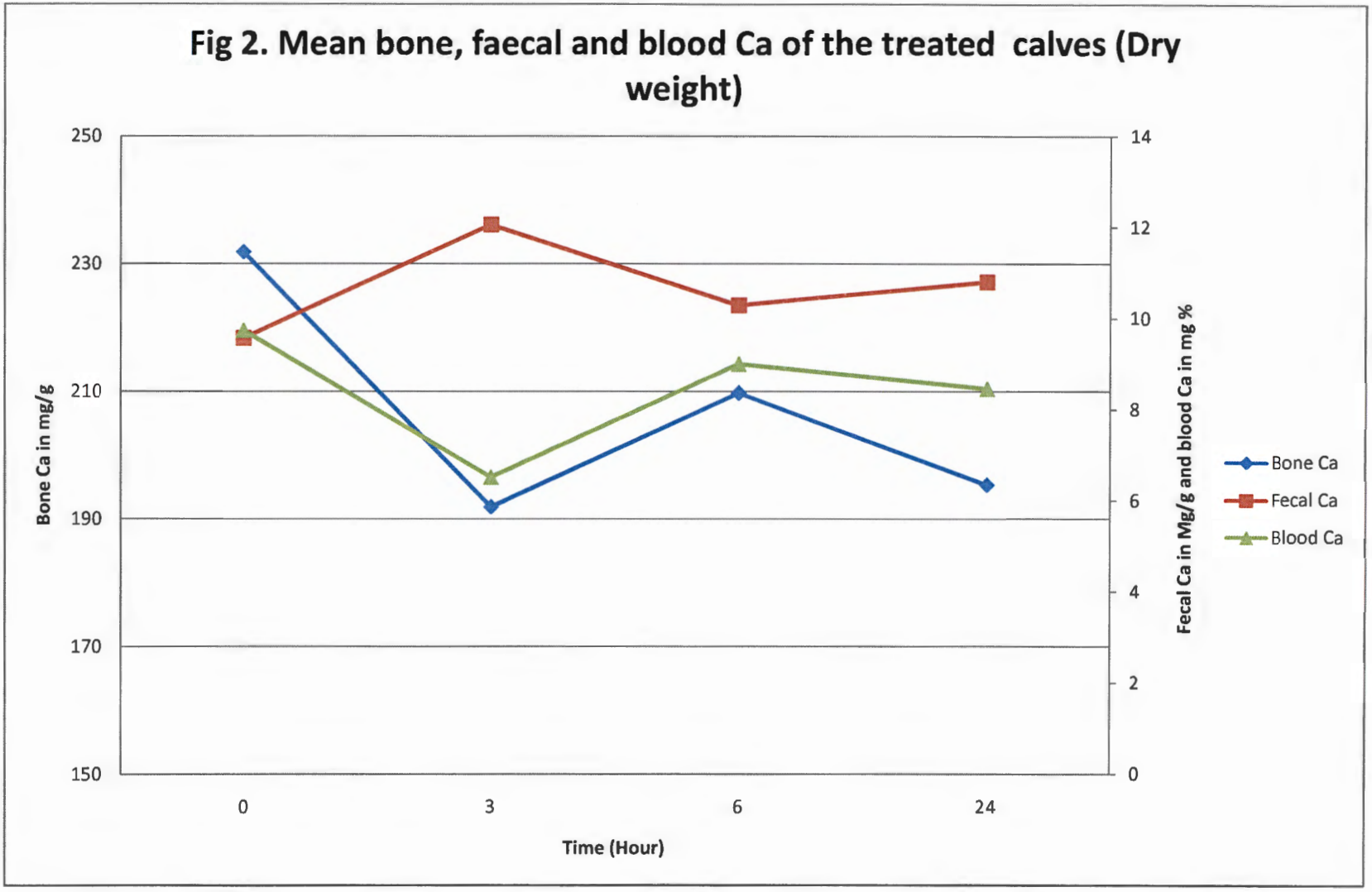


Fig 2. Mean bone, faecal and blood Ca of the treated calves (Dry weight)
250
230
210
190
170
150
Bone Ca in mg/g
14
12
10
8
6
4
2
0
Fecal Ca in Mg/g and blood Ca in mg %
0
3
6
24
Time (Hour)
Bone Ca
Fecal Ca
Blood Ca

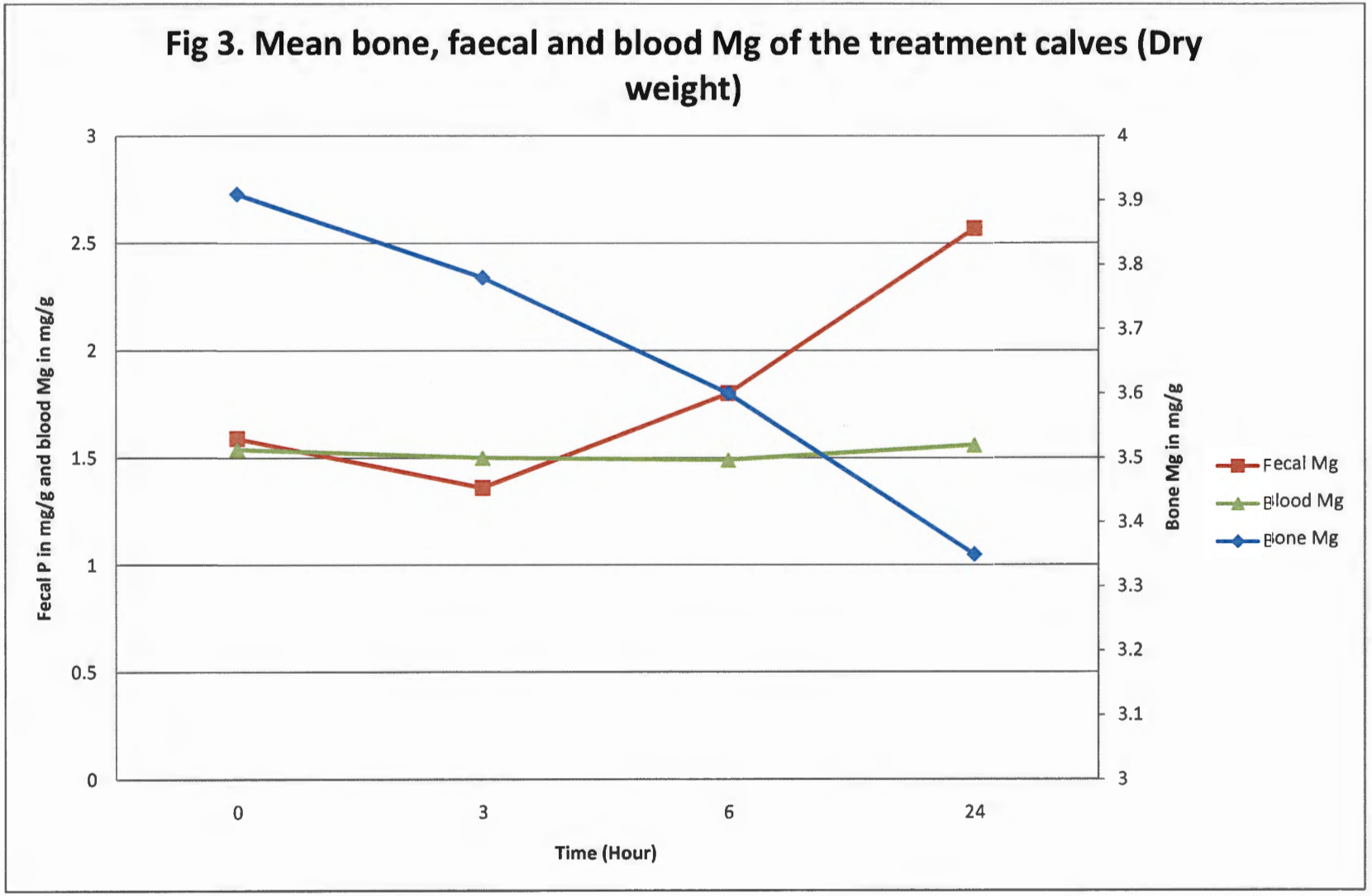


Fig 3. Mean bone, faecal and blood Mg of the treatment calves (Dry weight)
Fecal P in mg/g and blood Mg in mg/g
Bone Mg in mg/g
Time (Hour)
0
3
6
24
0
0.5
1
1.5
2
2.5
3
3
3.1
3.2
3.3
3.4
3.5
3.6
3.7
3.8
3.9
4
Fecal Mg
Blood Mg
Bone Mg

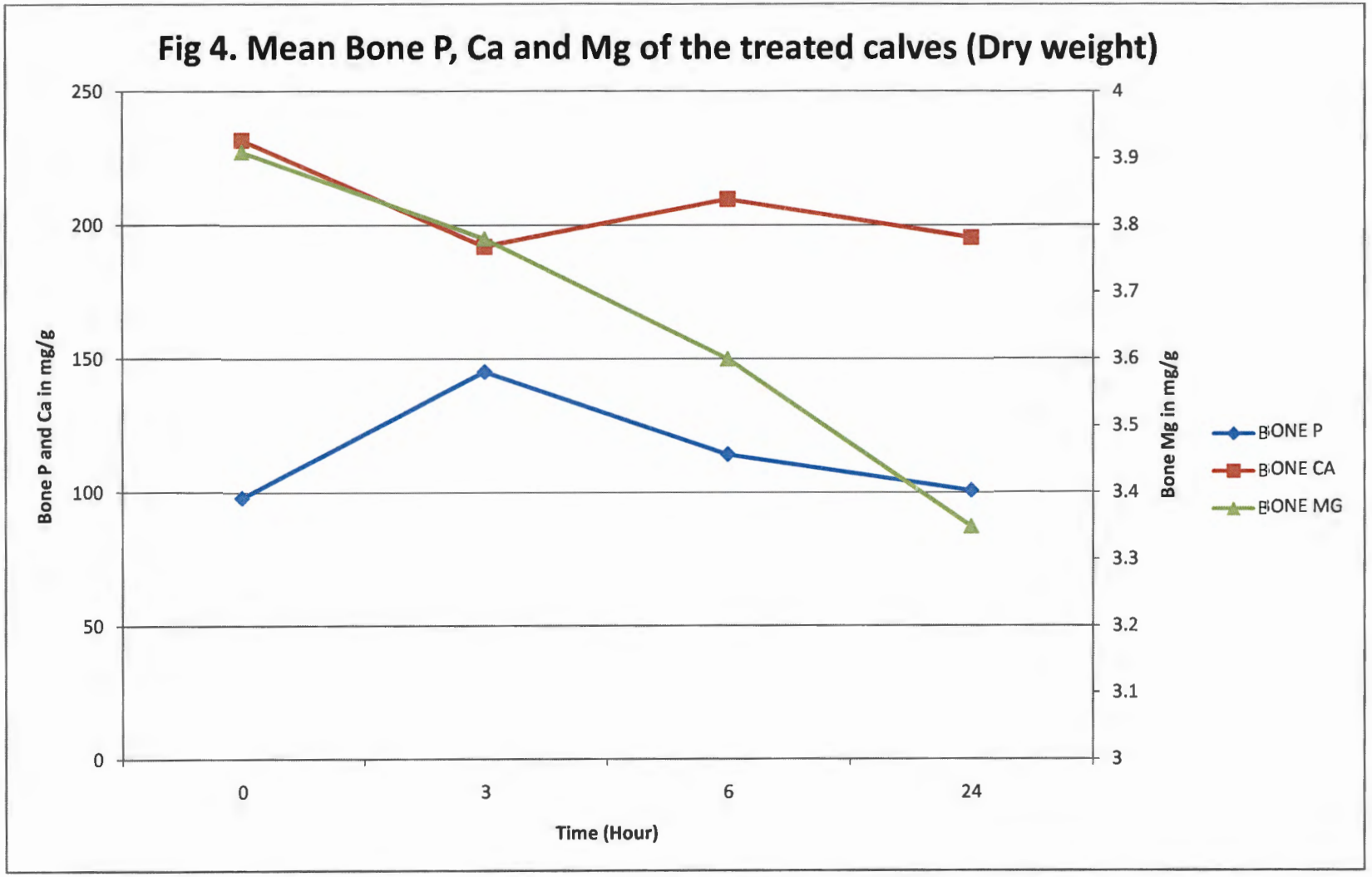


Fig 4. Mean Bone P, Ca and Mg of the treated calves (Dry weight)
250
200
150
100
50
0
Bone P and Ca in mg/g
4
3.9
3.8
3.7
3.6
3.5
3.4
3.3
3.2
3.1
3
Bone Mg in mg/g
0
3
6
24
Time (Hour)
BONE P
BONE CA
BONE MG

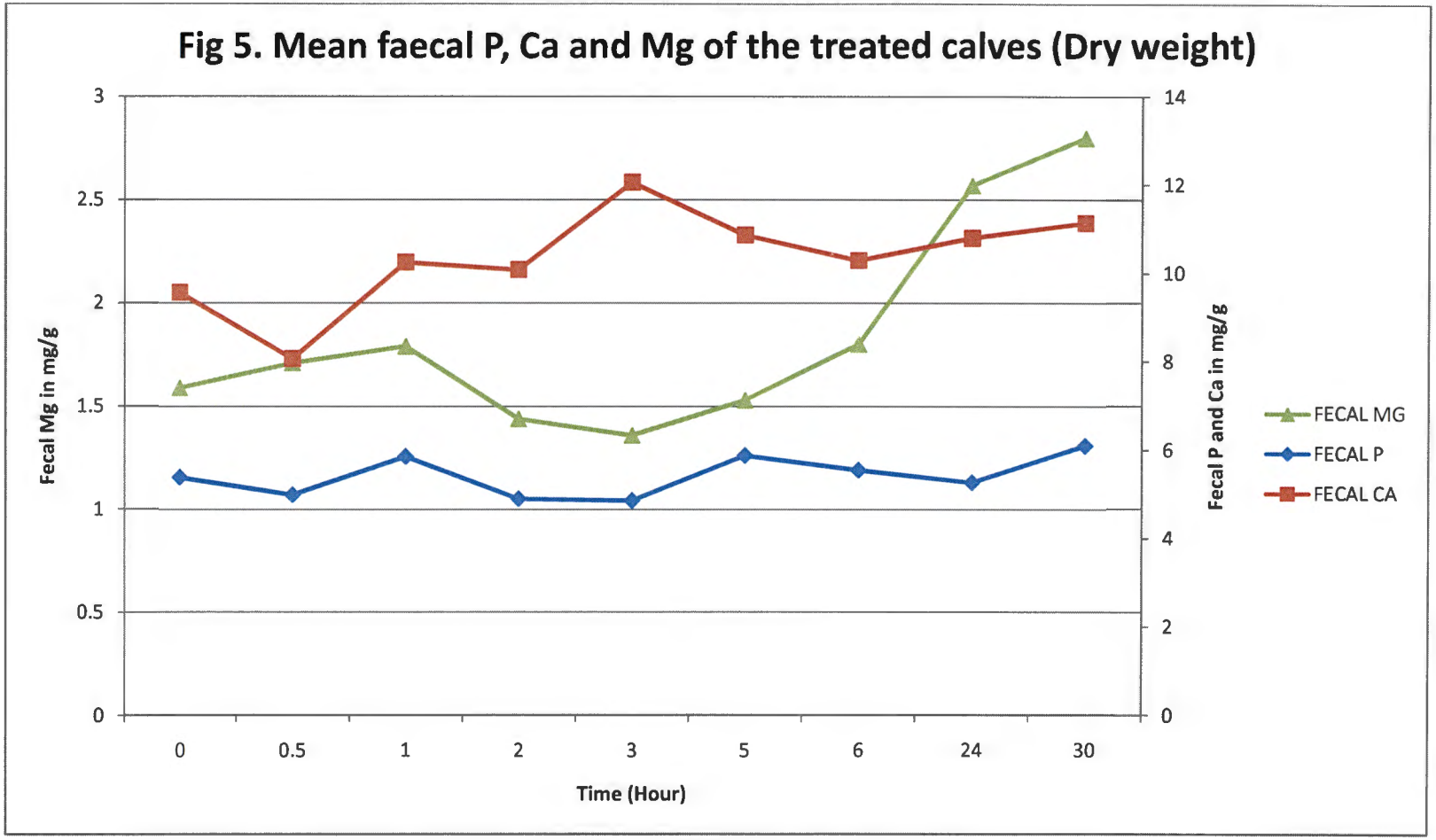


Fig 5. Mean faecal P, Ca and Mg of the treated calves (Dry weight)
Fecal Mg in mg/g
Fecal P and Ca in mg/g
Time (Hour)
0
0.5
1
2
3
5
6
24
30
FECAL MG
FECAL P
FECAL CA

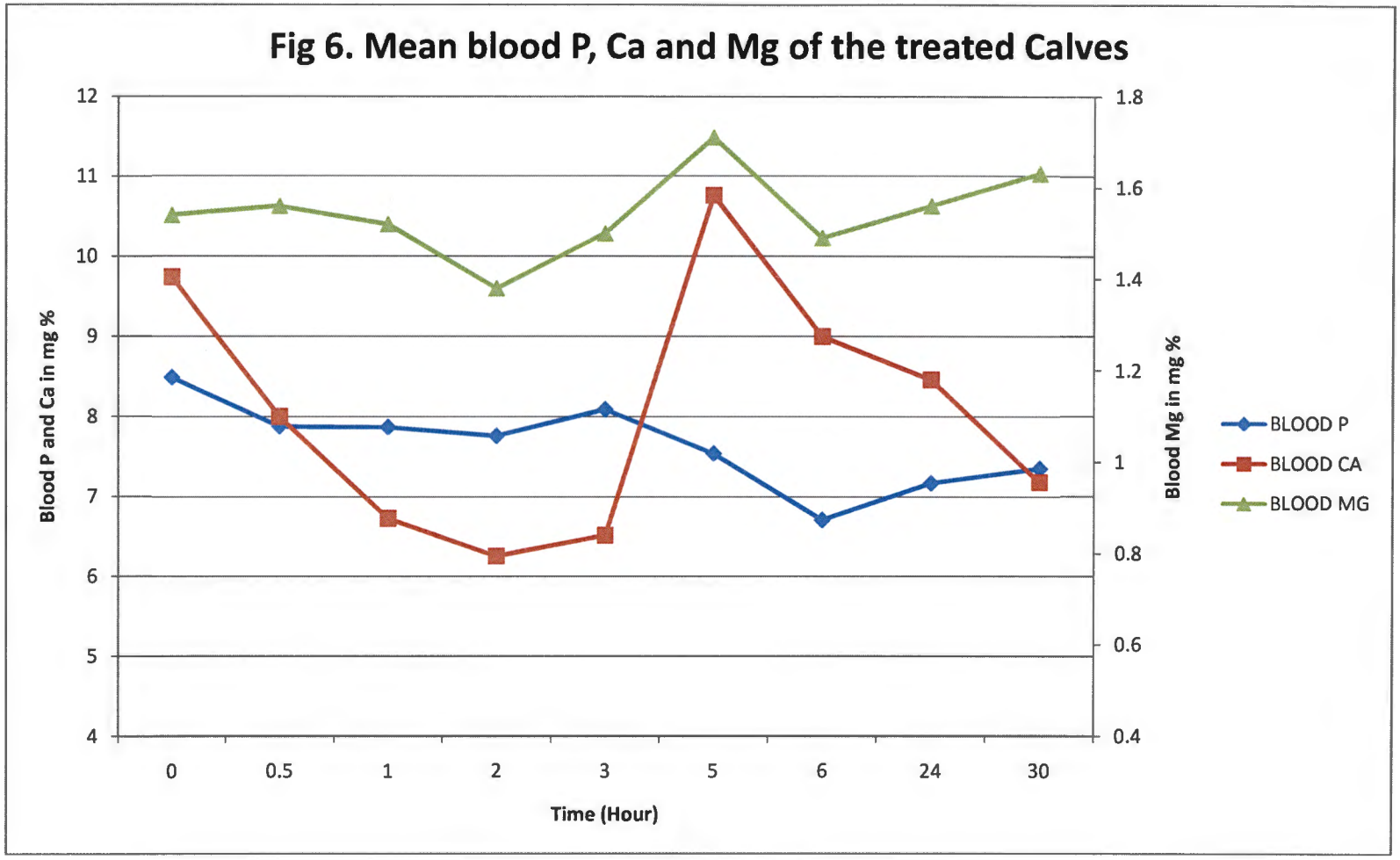


Fig 6. Mean blood P, Ca and Mg of the treated Calves
Blood P and Ca in mg %
Blood Mg in mg %
Time (Hour)
12
11
10
9
8
7
6
5
4
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0
0.5
1
2
3
5
6
24
30
BLOOD P
BLOOD CA
BLOOD MG

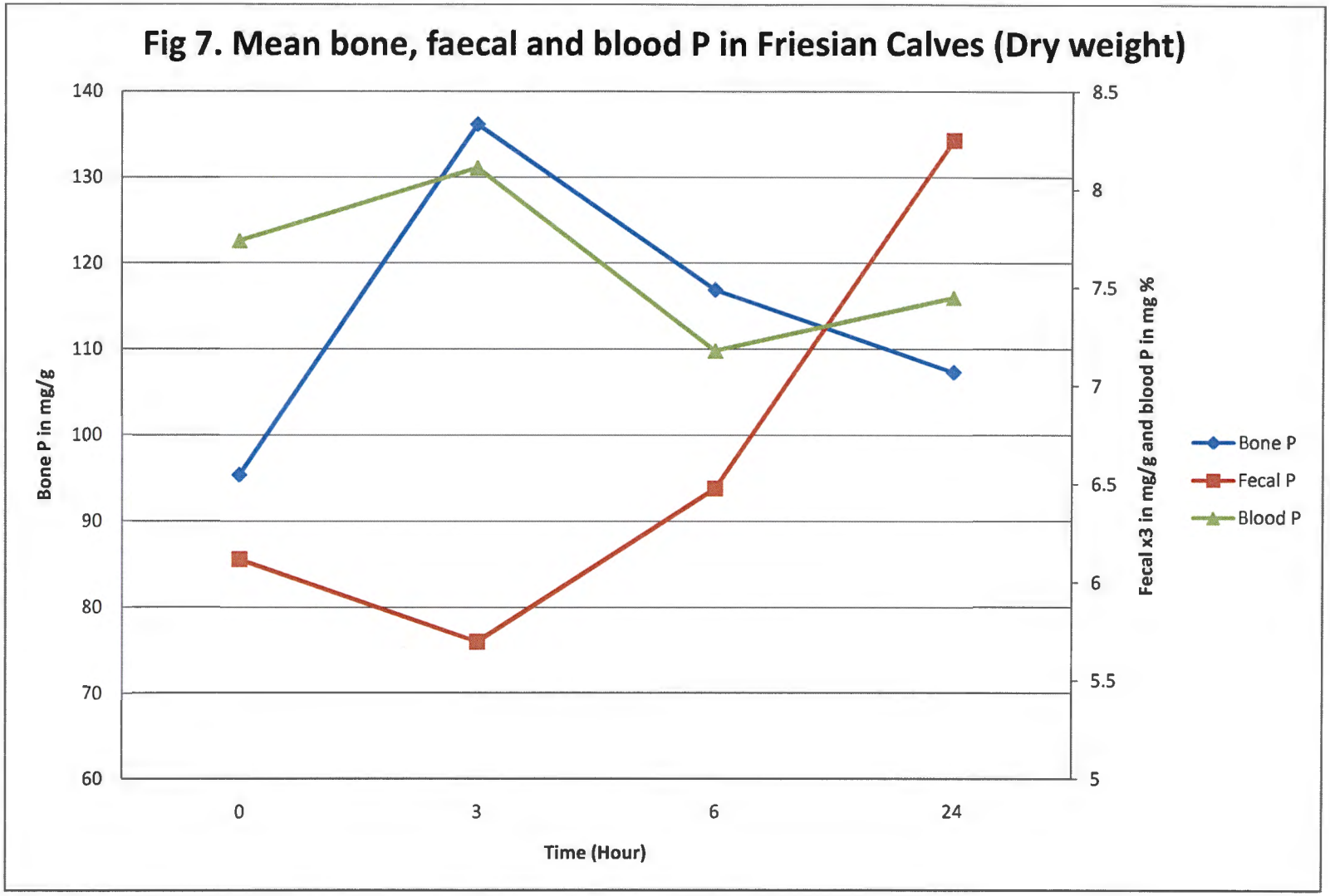


Fig 7. Mean bone, faecal and blood P in Friesian Calves (Dry weight)
Bone P in mg/g
Fecal x3 in mg/g and blood P in mg %
Time (Hour)
140
130
120
110
100
90
80
70
60
8.5
8
7.5
7
6.5
6
5.5
5
0
3
6
24
Bone P
Fecal P
Blood P

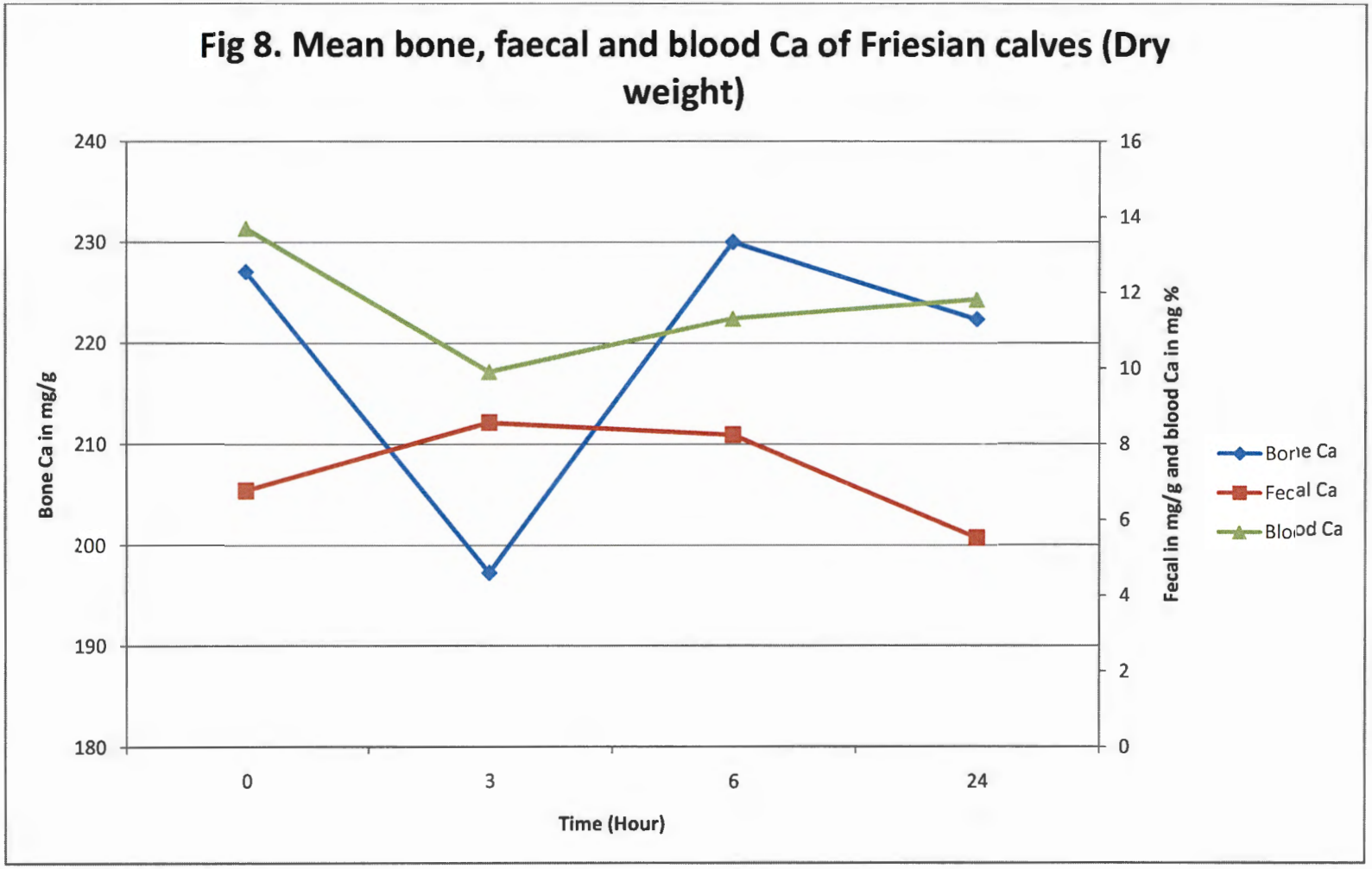


Fig 8. Mean bone, faecal and blood Ca of Friesian calves (Dry weight)
240
230
220
210
200
190
180
16
14
12
10
8
6
4
2
0
0
3
6
24
Time (Hour)
Bone Ca in mg/g
Fecal in mg/g and blood Ca in mg %
Bone Ca
Fecal Ca
Blood Ca

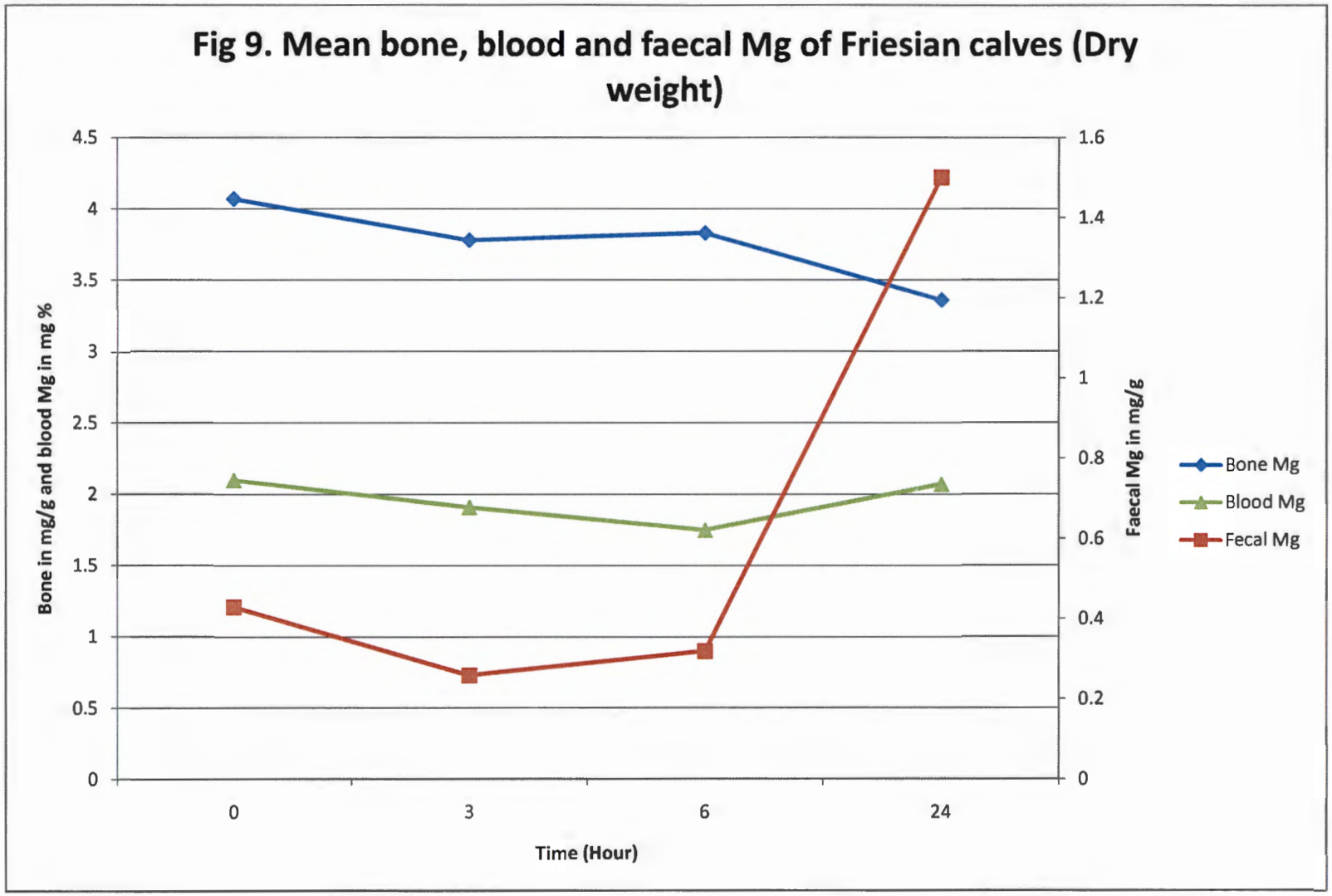


Fig 9. Mean bone, blood and faecal Mg of Friesian calves (Dry weight)
Bone in mg/g and blood Mg in mg %
Faecal Mg in mg/g
Time (Hour)
0
3
6
24
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Bone Mg
Blood Mg
Fecal Mg

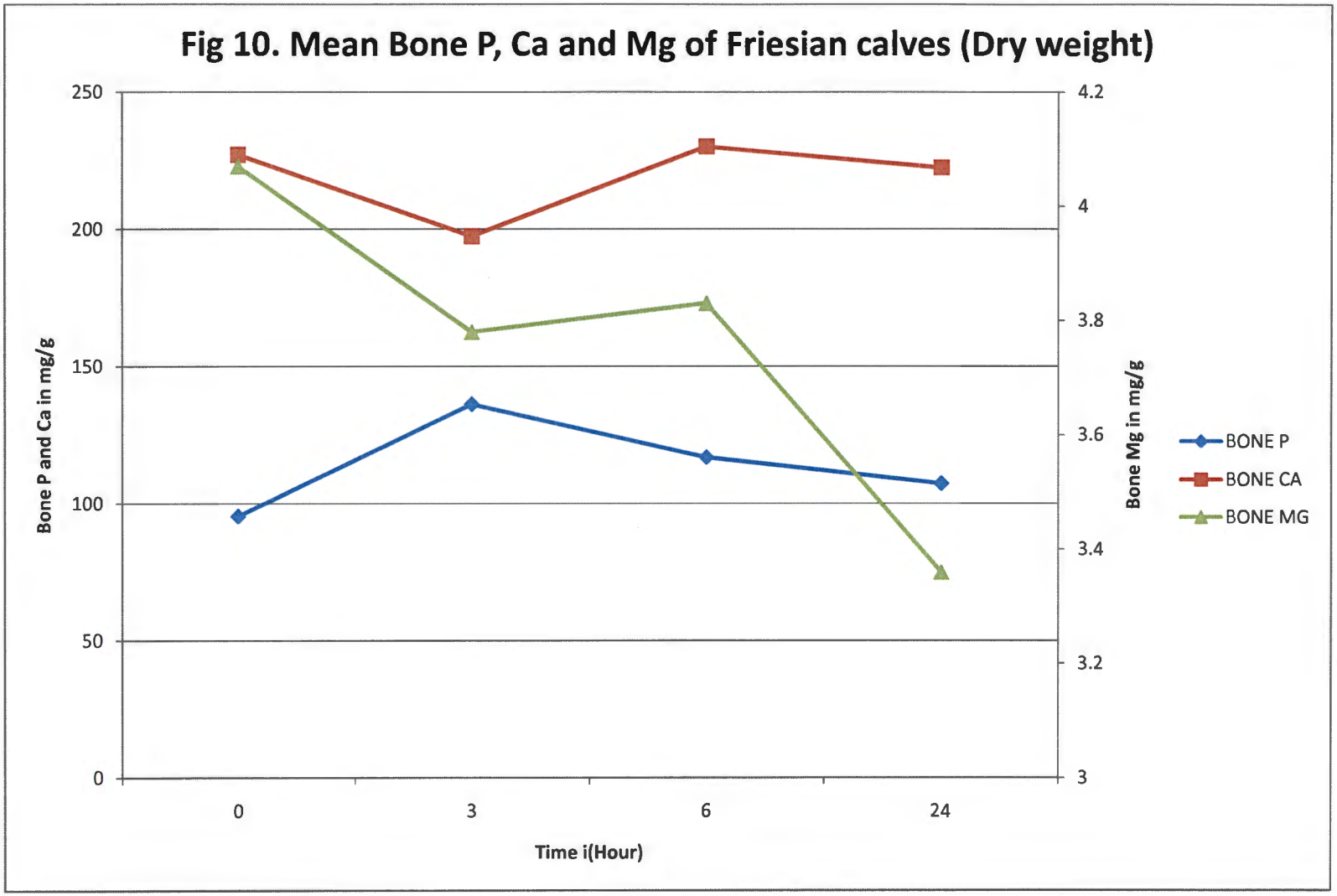


Fig 10. Mean Bone P, Ca and Mg of Friesian calves (Dry weight)
250
200
150
100
50
0
Bone P and Ca in mg/g
4.2
4
3.8
3.6
3.4
3.2
3
Bone Mg in mg/g
0
3
6
24
Time i(Hour)
BONE P
BONE CA
BONE MG

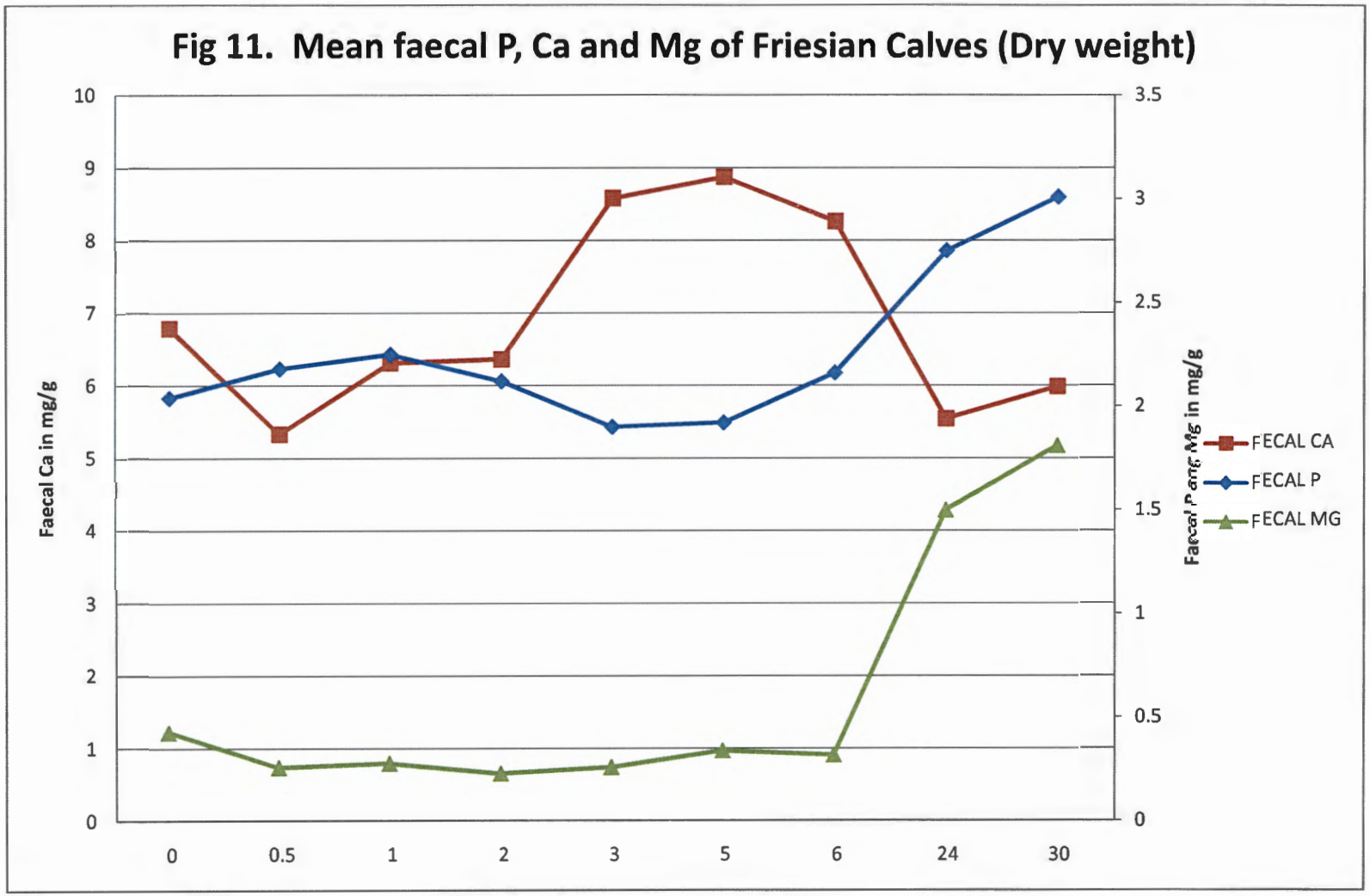


Fig 11. Mean faecal P, Ca and Mg of Friesian Calves (Dry weight)
Faecal Ca in mg/g
Faecal P and Mg in mg/g
10
9
8
7
6
5
4
3
2
1
0
3.5
3
2.5
2
1.5
1
0.5
0
0
0.5
1
2
3
5
6
24
30
FECAL CA
FECAL P
FECAL MG

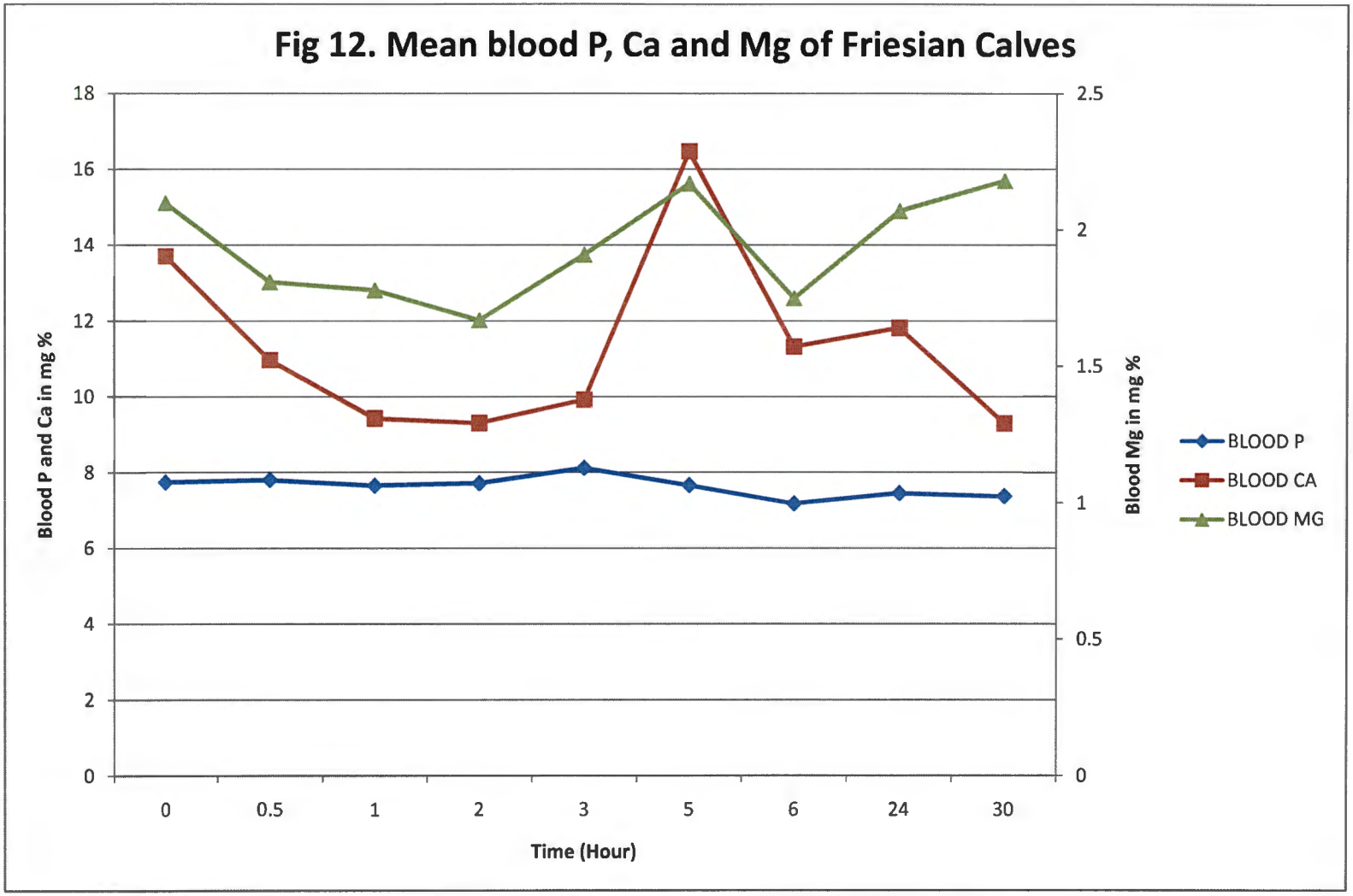


Fig 12. Mean blood P, Ca and Mg of Friesian Calves
Blood P and Ca in mg %
Blood Mg in mg %
Time (Hour)
0
0.5
1
2
3
5
6
24
30
BLOOD P
BLOOD CA
BLOOD MG

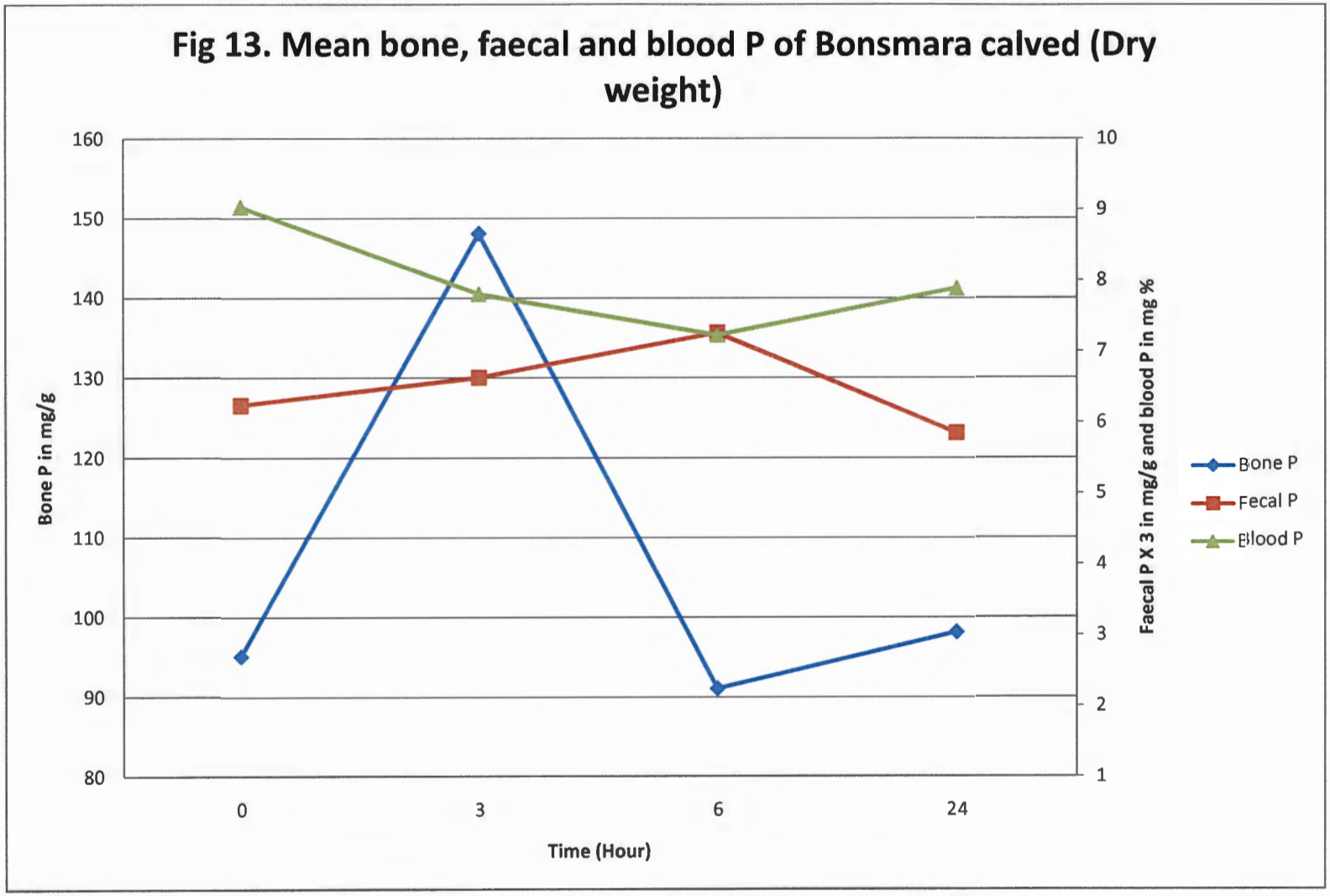


Fig 13. Mean bone, faecal and blood P of Bonsmara calved (Dry weight)
Bone P in mg/g
160
150
140
130
120
110
100
90
80
10
9
8
7
6
5
4
3
2
1
Faecal P X 3 in mg/g and blood P in mg %
0
3
6
24
Time (Hour)
Bone P
Fecal P
Blood P

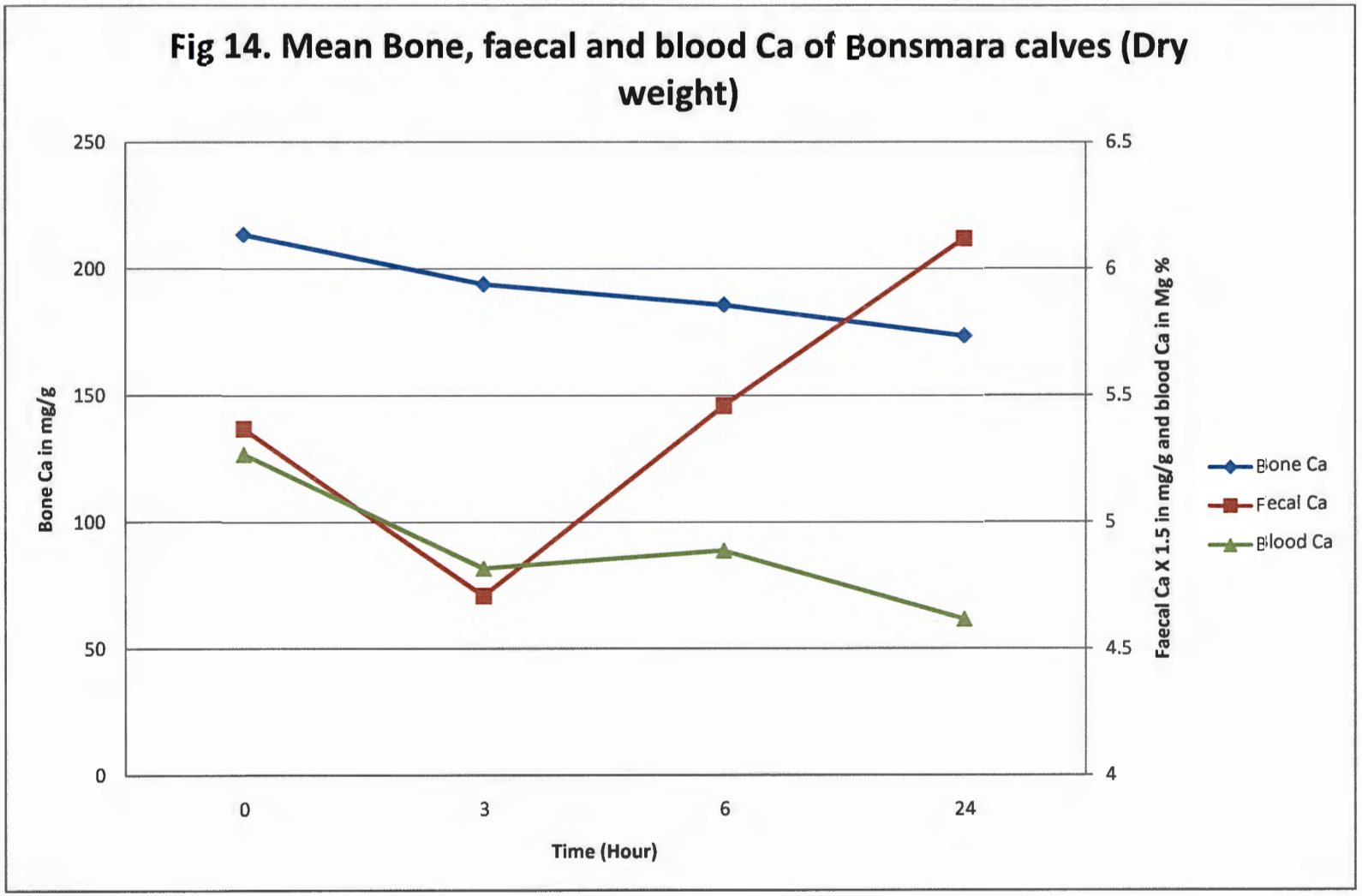


Fig 14. Mean Bone, faecal and blood Ca of Bonsmara calves (Dry weight)
250
200
150
100
50
0
Bone Ca in mg/g
6.5
6
5.5
5
4.5
4
Faecal Ca X 1.5 in mg/g and blood Ca in Mg %
0
3
6
24
Time (Hour)
Bone Ca
Fecal Ca
Blood Ca

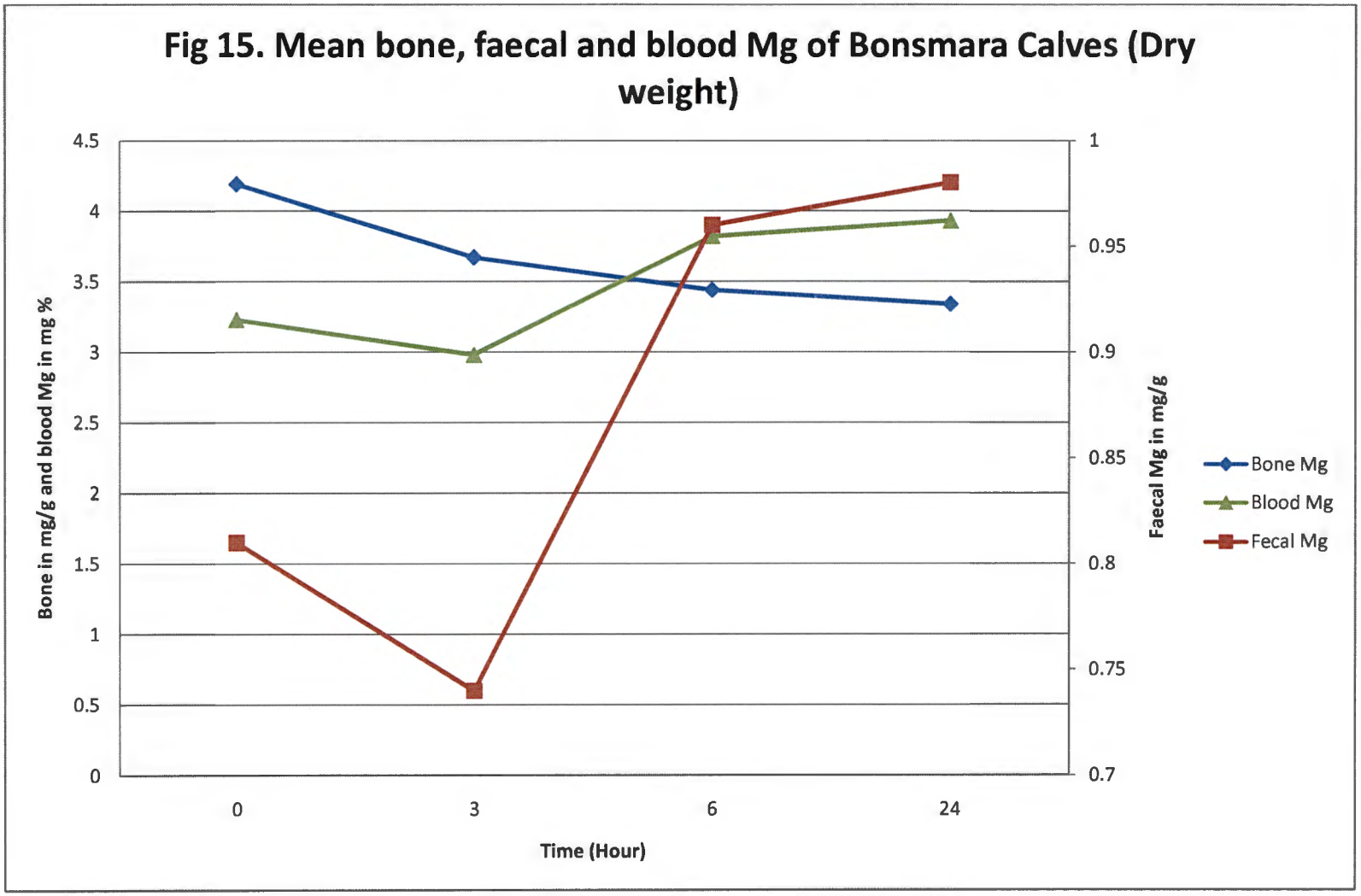


Fig 15. Mean bone, faecal and blood Mg of Bonsmara Calves (Dry weight)
Bone in mg/g and blood Mg in mg %
Faecal Mg in mg/g
Time (Hour)
0
3
6
24
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
1
0.95
0.9
0.85
0.8
0.75
0.7
Bone Mg
Blood Mg
Fecal Mg

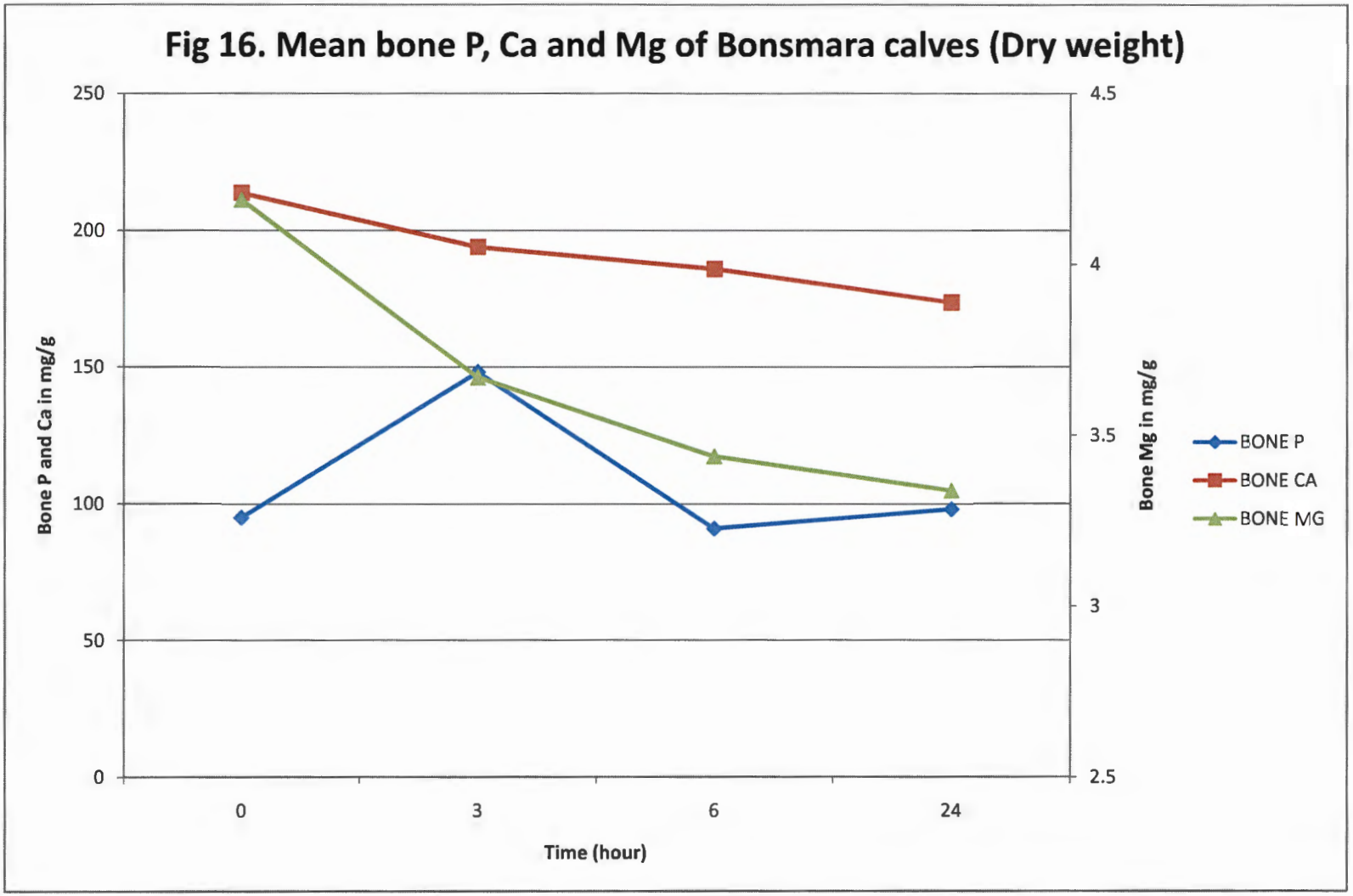


Fig 16. Mean bone P, Ca and Mg of Bonsmara calves (Dry weight)
250
200
150
100
50
0
4.5
4
3.5
3
2.5
Bone P and Ca in mg/g
Bone Mg in mg/g
0
3
6
24
Time (hour)
BONE P
BONE CA
BONE MG

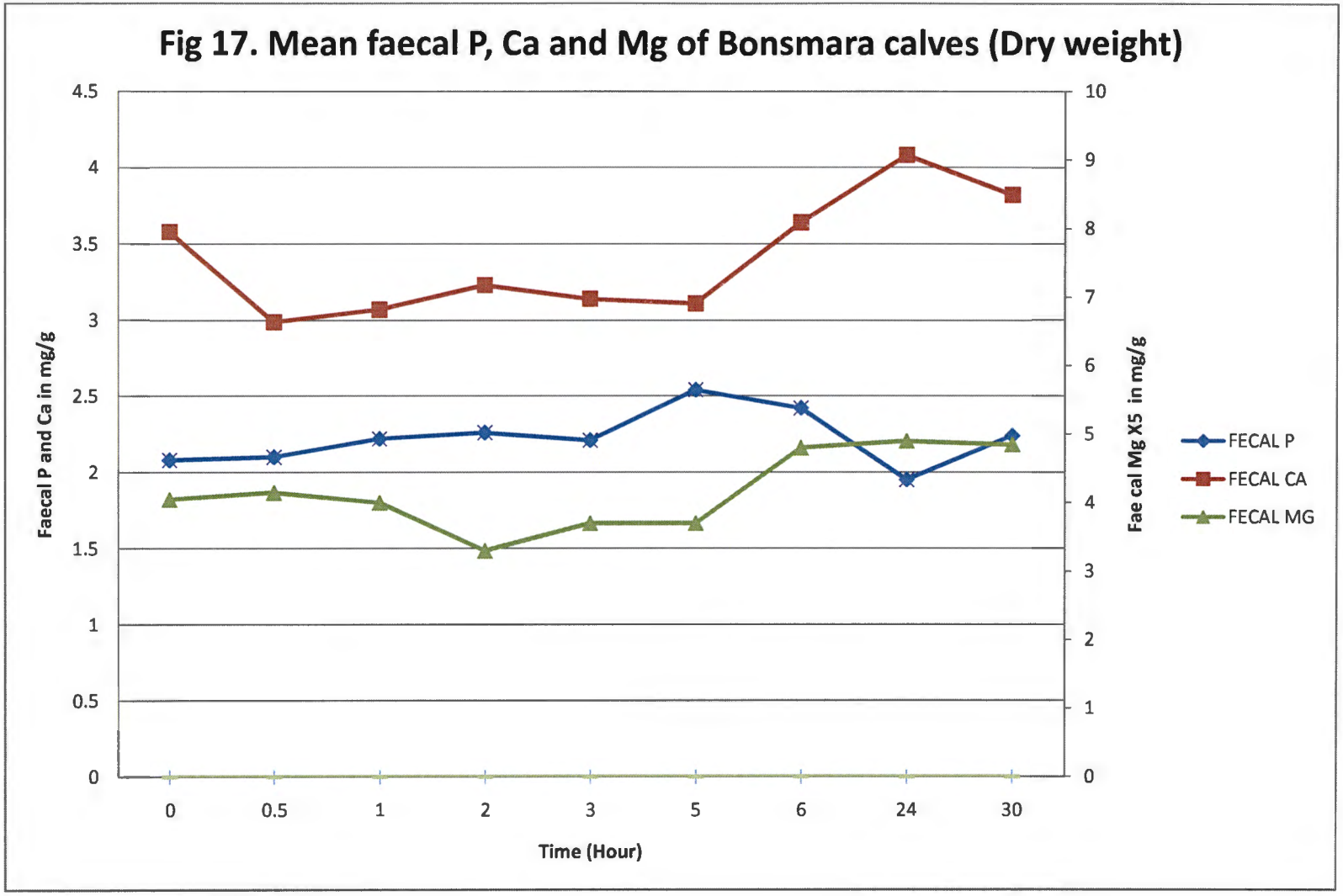


Fig 17. Mean faecal P, Ca and Mg of Bonsmara calves (Dry weight)
Faecal P and Ca in mg/g
Fae cal Mg X5 in mg/g
Time (Hour)
0
0.5
1
2
3
5
6
24
30
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
10
9
8
7
6
5
4
3
2
1
0
FECAL P
FECAL CA
FECAL MG

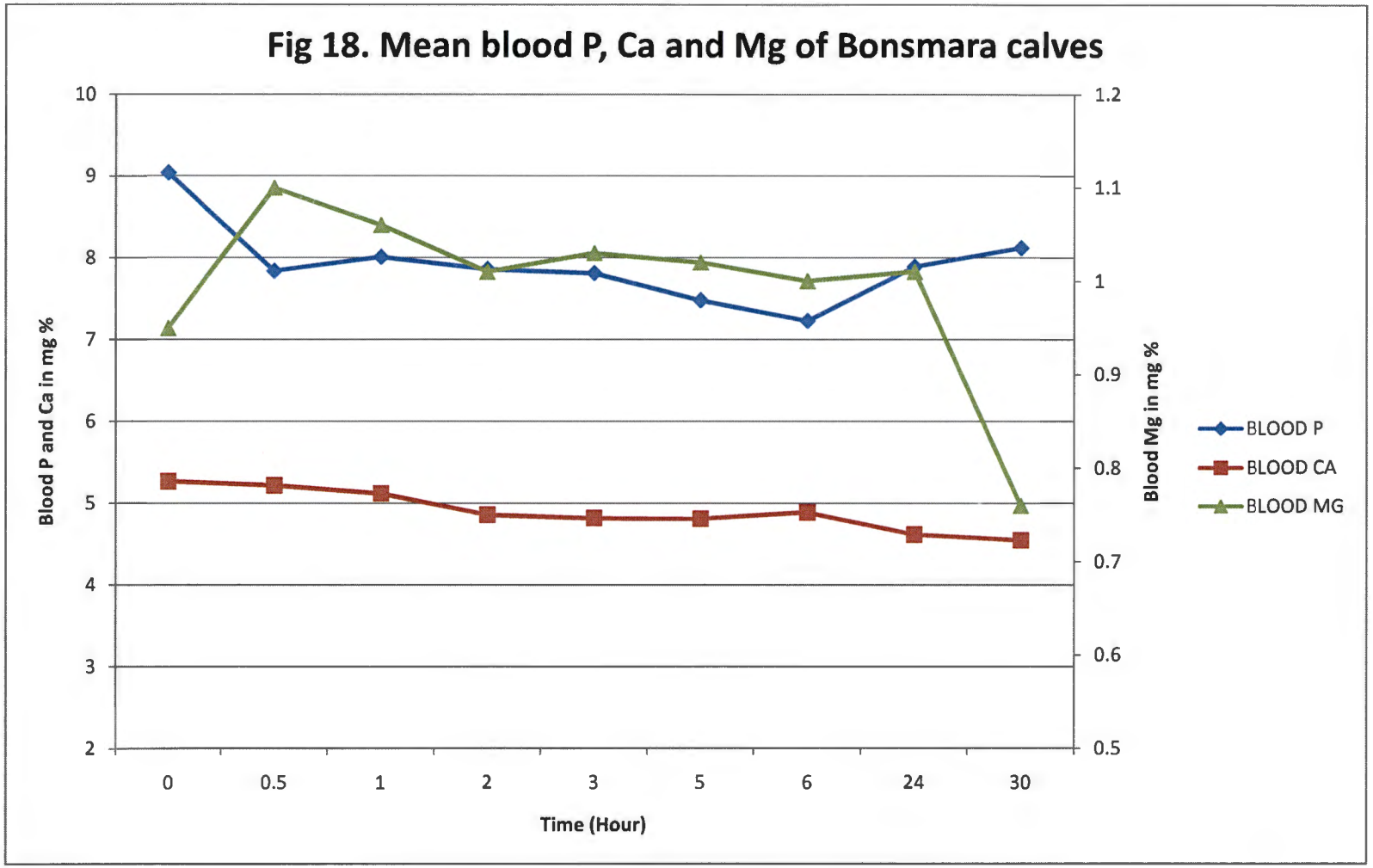


Fig 18. Mean blood P, Ca and Mg of Bonsmara calves
Blood P and Ca in mg %
Blood Mg in mg %
Time (Hour)
0
0.5
1
2
3
5
6
24
30
BLOOD P
BLOOD CA
BLOOD MG

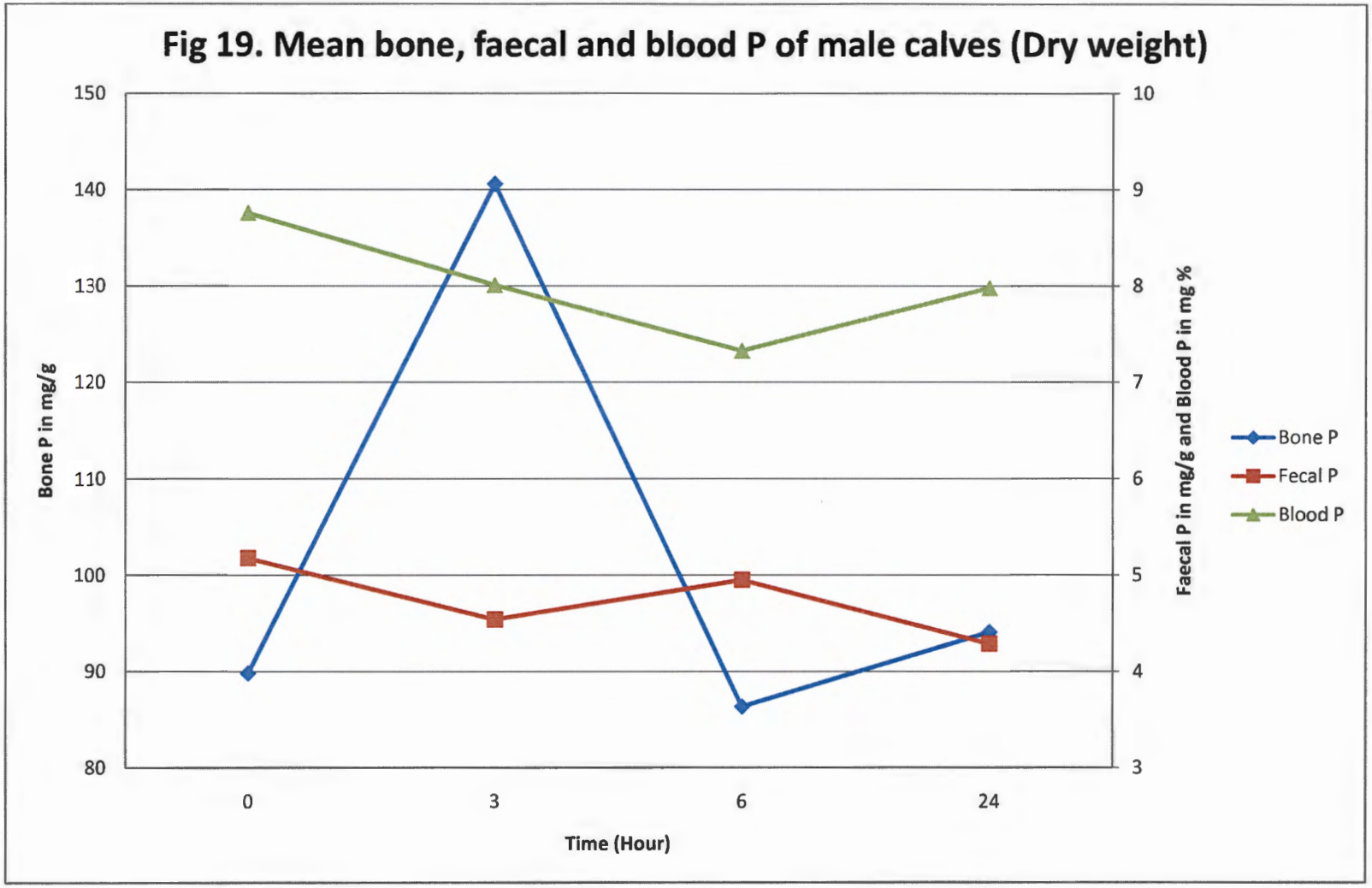


Fig 19. Mean bone, faecal and blood P of male calves (Dry weight)
150
140
130
120
110
100
90
80
10
9
8
7
6
5
4
3
0
3
6
24
Time (Hour)
Bone P in mg/g
Faecal P in mg/g and Blood P in mg %
Bone P
Fecal P
Blood P

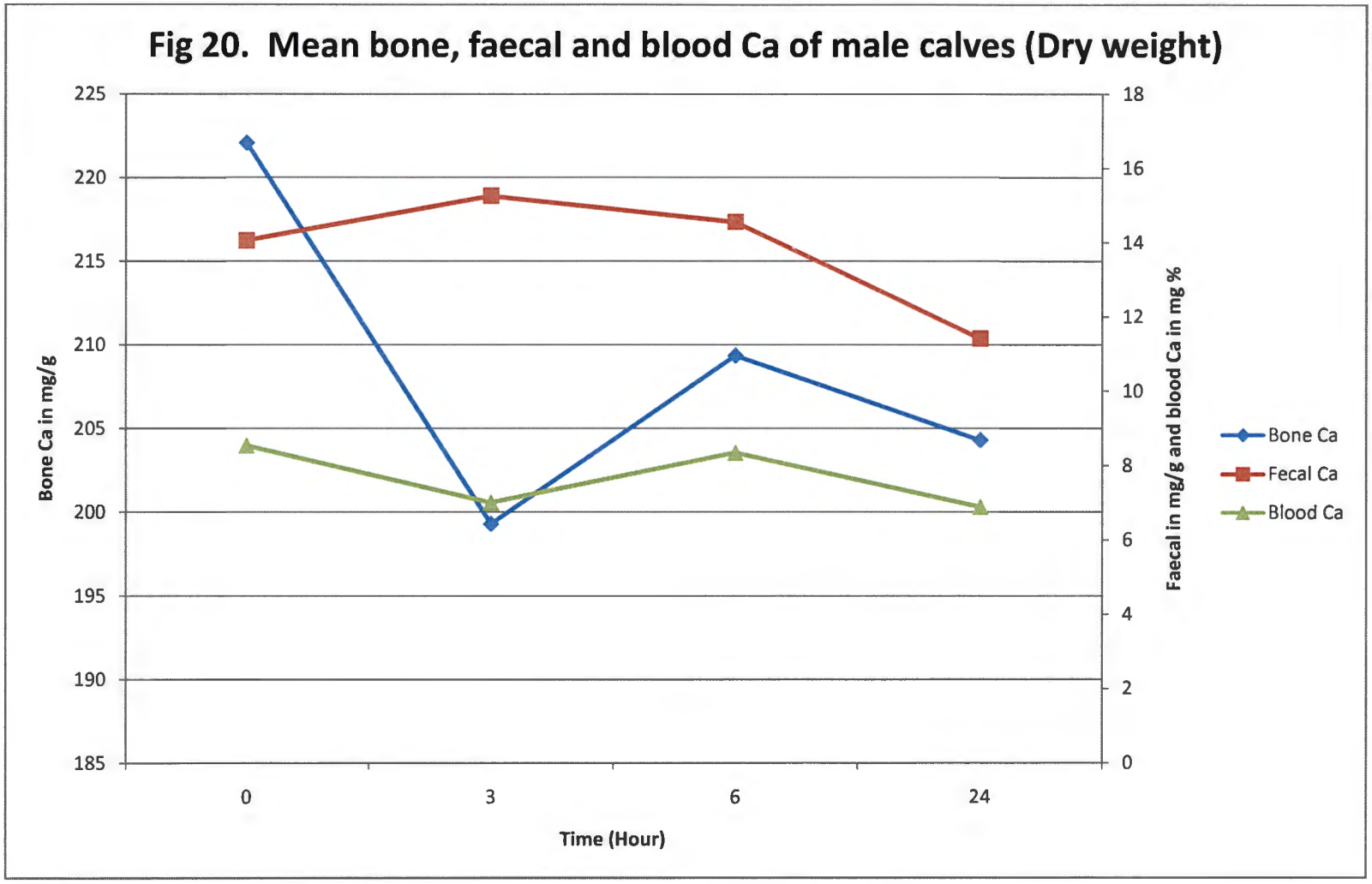


Fig 20. Mean bone, faecal and blood Ca of male calves (Dry weight)
225
220
215
210
205
200
195
190
185
Bone Ca in mg/g
18
16
14
12
10
8
6
4
2
0
Faecal in mg/g and blood Ca in mg %
0
3
6
24
Time (Hour)
Bone Ca
Fecal Ca
Blood Ca

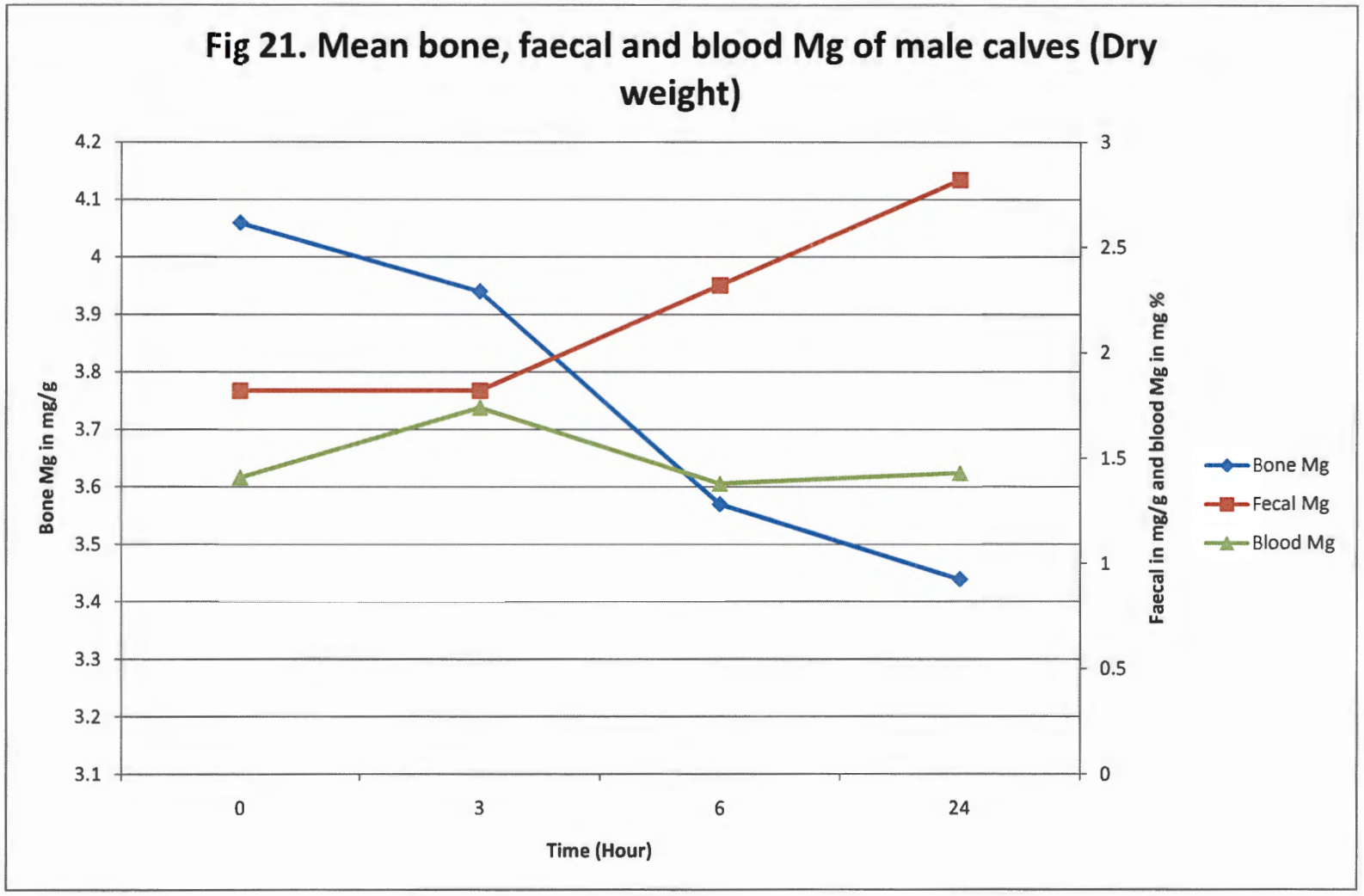


Fig 21. Mean bone, faecal and blood Mg of male calves (Dry weight)
Bone Mg in mg/g
4.2
4.1
4
3.9
3.8
3.7
3.6
3.5
3.4
3.3
3.2
3.1
Faecal in mg/g and blood Mg in mg %
3
2.5
2
1.5
1
0.5
0
0
3
6
24
Time (Hour)
Bone Mg
Fecal Mg
Blood Mg

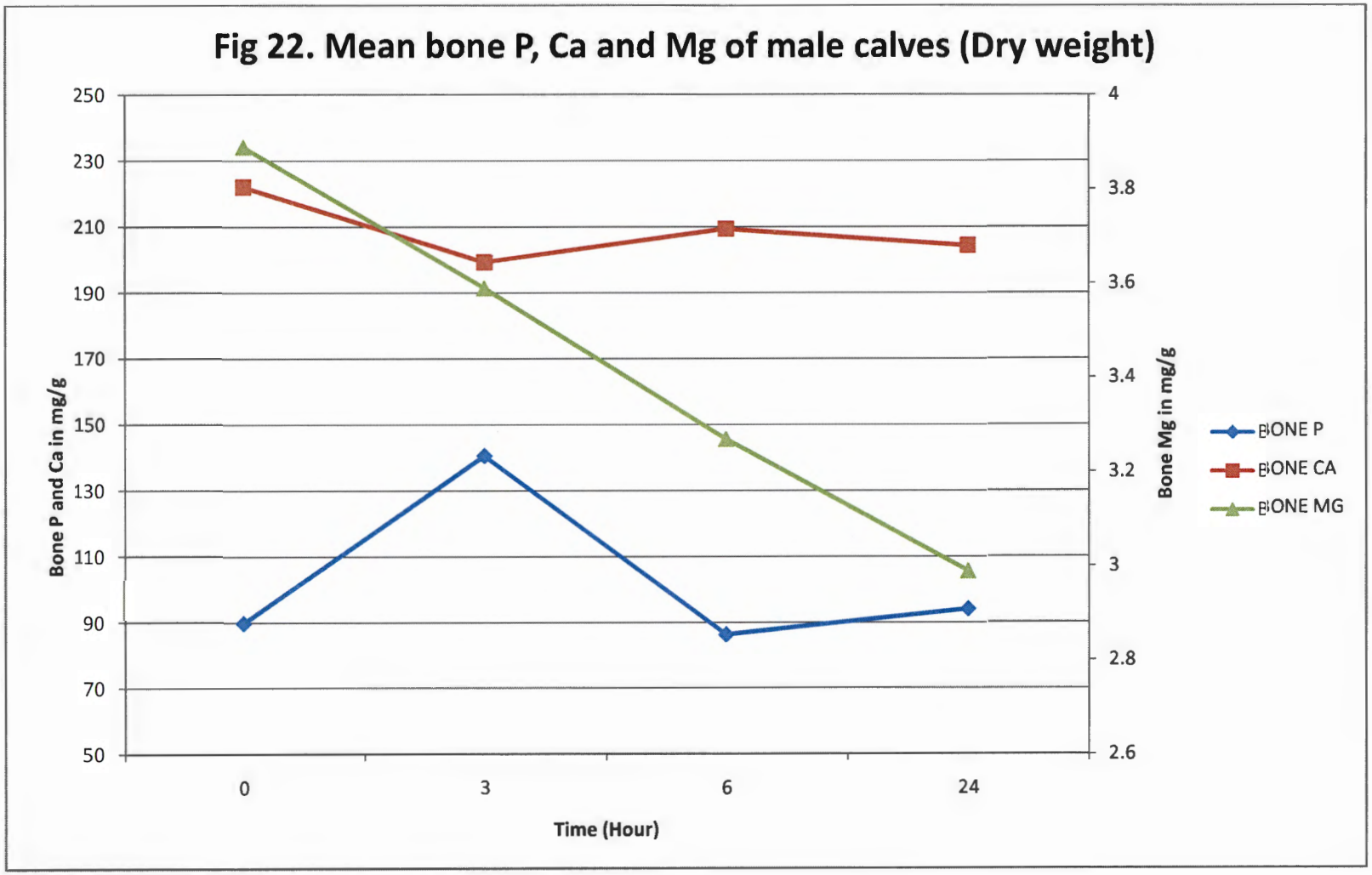


Fig 22. Mean bone P, Ca and Mg of male calves (Dry weight)
250
230
210
190
170
150
130
110
90
70
50
4
3.8
3.6
3.4
3.2
3
2.8
2.6
Bone P and Ca in mg/g
Bone Mg in mg/g
0
3
6
24
Time (Hour)
BONE P
BONE CA
BONE MG

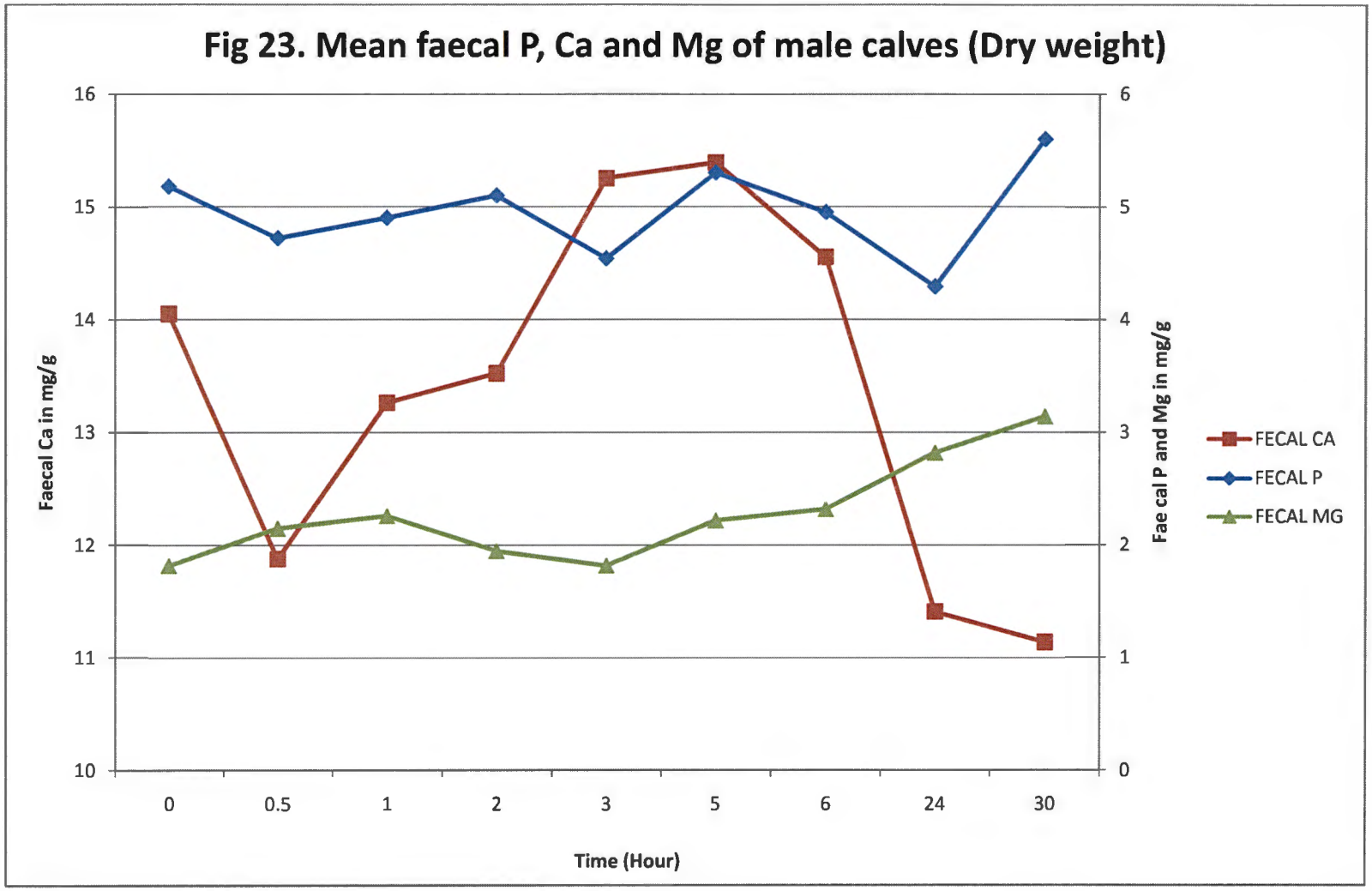


Fig 23. Mean faecal P, Ca and Mg of male calves (Dry weight)
Faecal Ca in mg/g
Fae cal P and Mg in mg/g
Time (Hour)
16
15
14
13
12
11
10
6
5
4
3
2
1
0
0
0.5
1
2
3
5
6
24
30
FECAL CA
FECAL P
FECAL MG

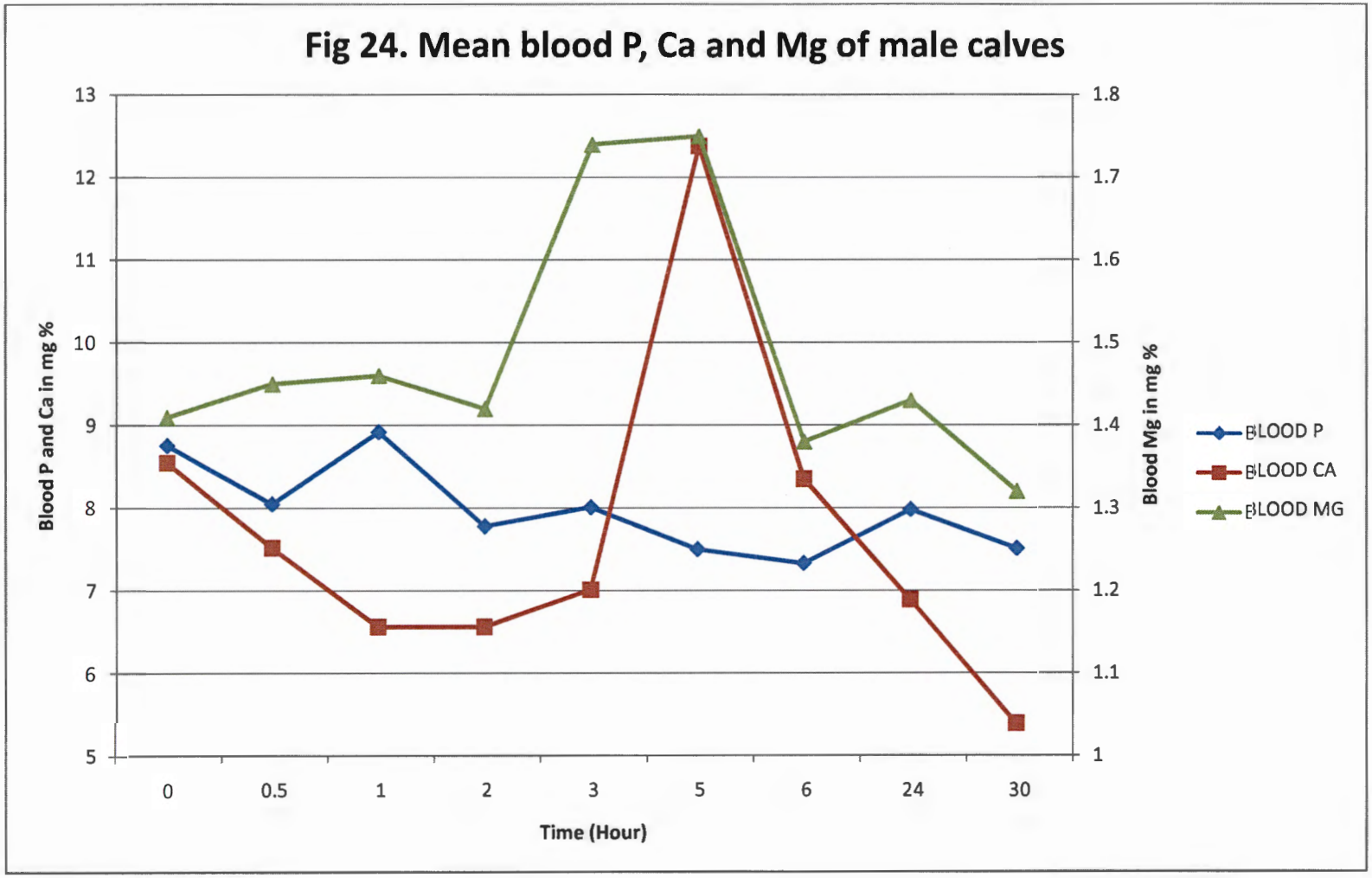


Fig 24. Mean blood P, Ca and Mg of male calves
Blood P and Ca in mg %
Blood Mg in mg %
Time (Hour)
0
0.5
1
2
3
5
6
24
30
BLOOD P
BLOOD CA
BLOOD MG

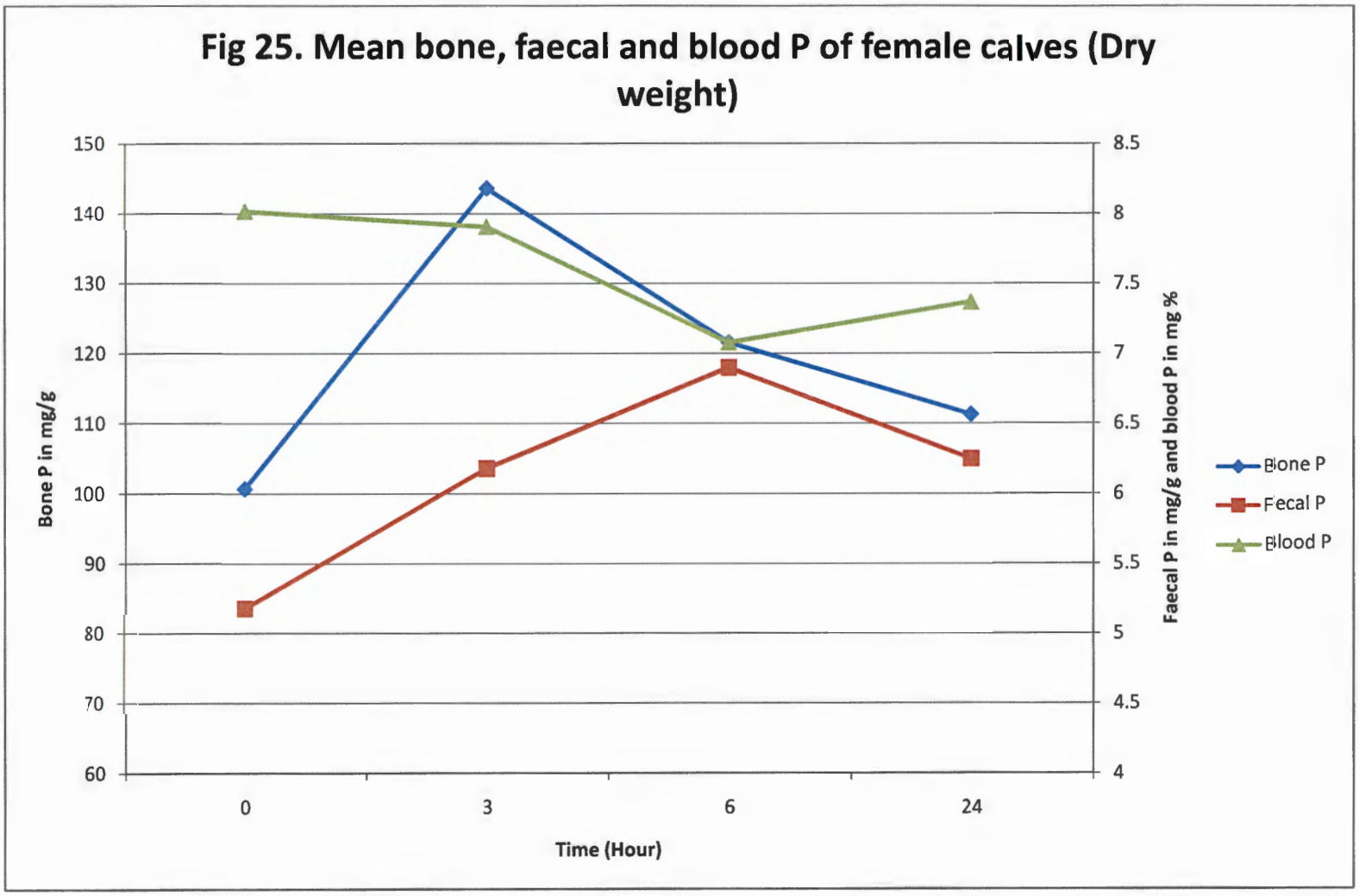
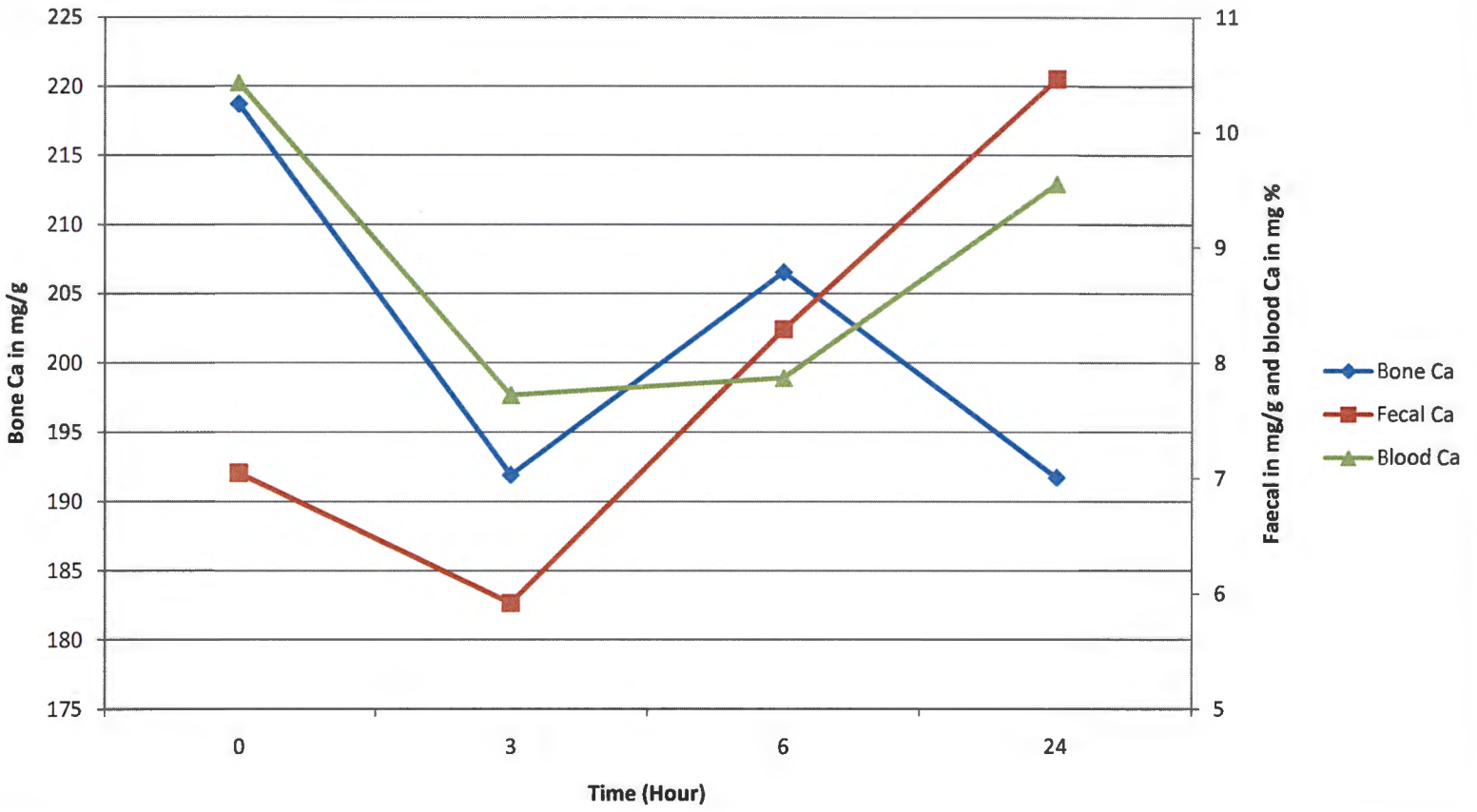


Fig 25. Mean bone, faecal and blood P of female calves (Dry weight)
Bone P in mg/g
Faecal P in mg/g and blood P in mg %
Time (Hour)
150
140
130
120
110
100
90
80
70
60
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
0
3
6
24
Bone P
Fecal P
Blood P

Fig 26. Mean bone, faecal and blood Ca of female calves (Dry weight)



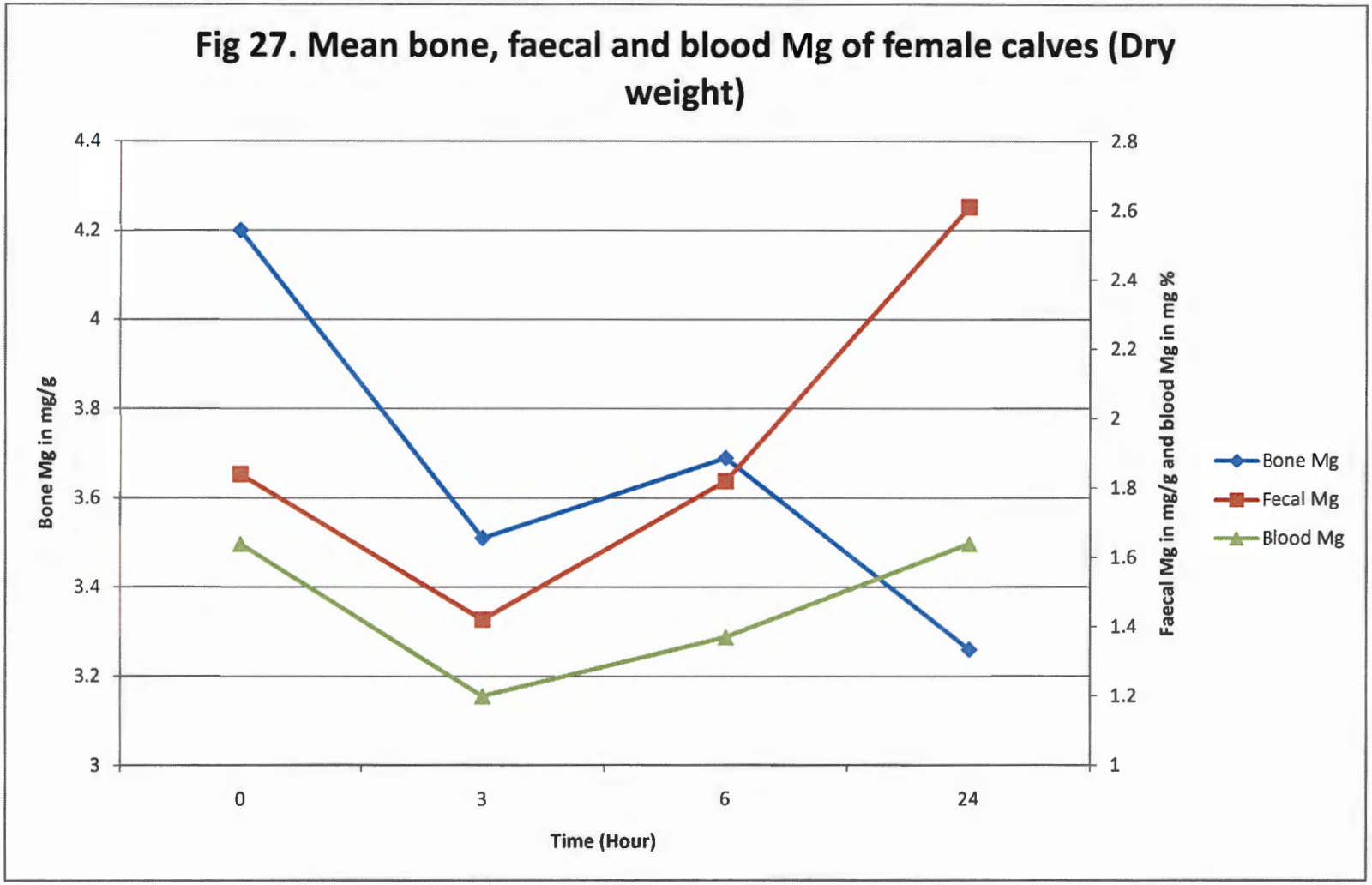


Fig 27. Mean bone, faecal and blood Mg of female calves (Dry weight)
Bone Mg in mg/g
Faecal Mg in mg/g and blood Mg in mg %
Time (Hour)
4.4
4.2
4
3.8
3.6
3.4
3.2
3
2.8
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
0
3
6
24
Bone Mg
Fecal Mg
Blood Mg

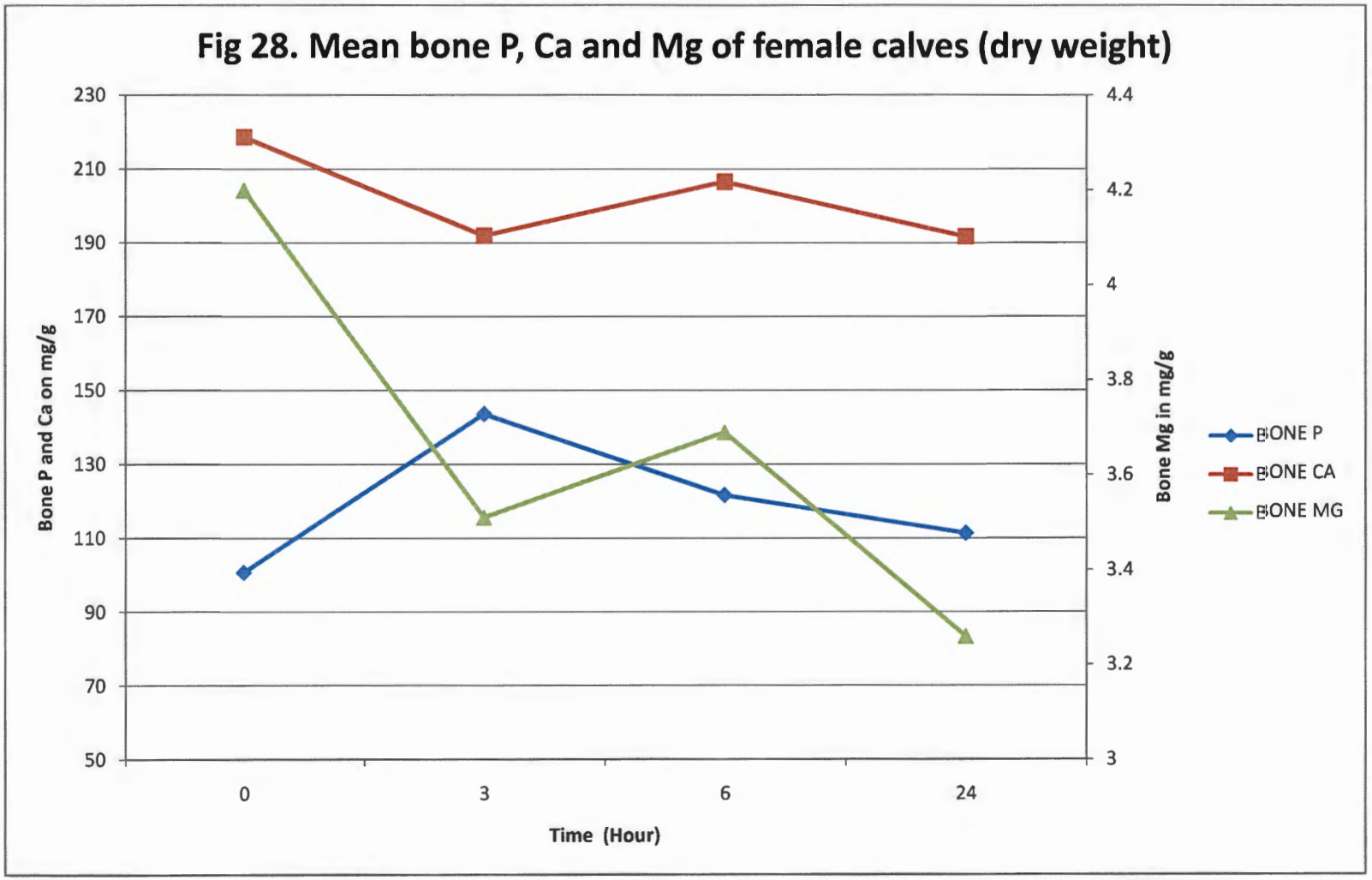


Fig 28. Mean bone P, Ca and Mg of female calves (dry weight)
230
210
190
170
150
130
110
90
70
50
4.4
4.2
4
3.8
3.6
3.4
3.2
3
Bone P and Ca on mg/g
Bone Mg in mg/g
0
3
6
24
Time (Hour)
BONE P
BONE CA
BONE MG

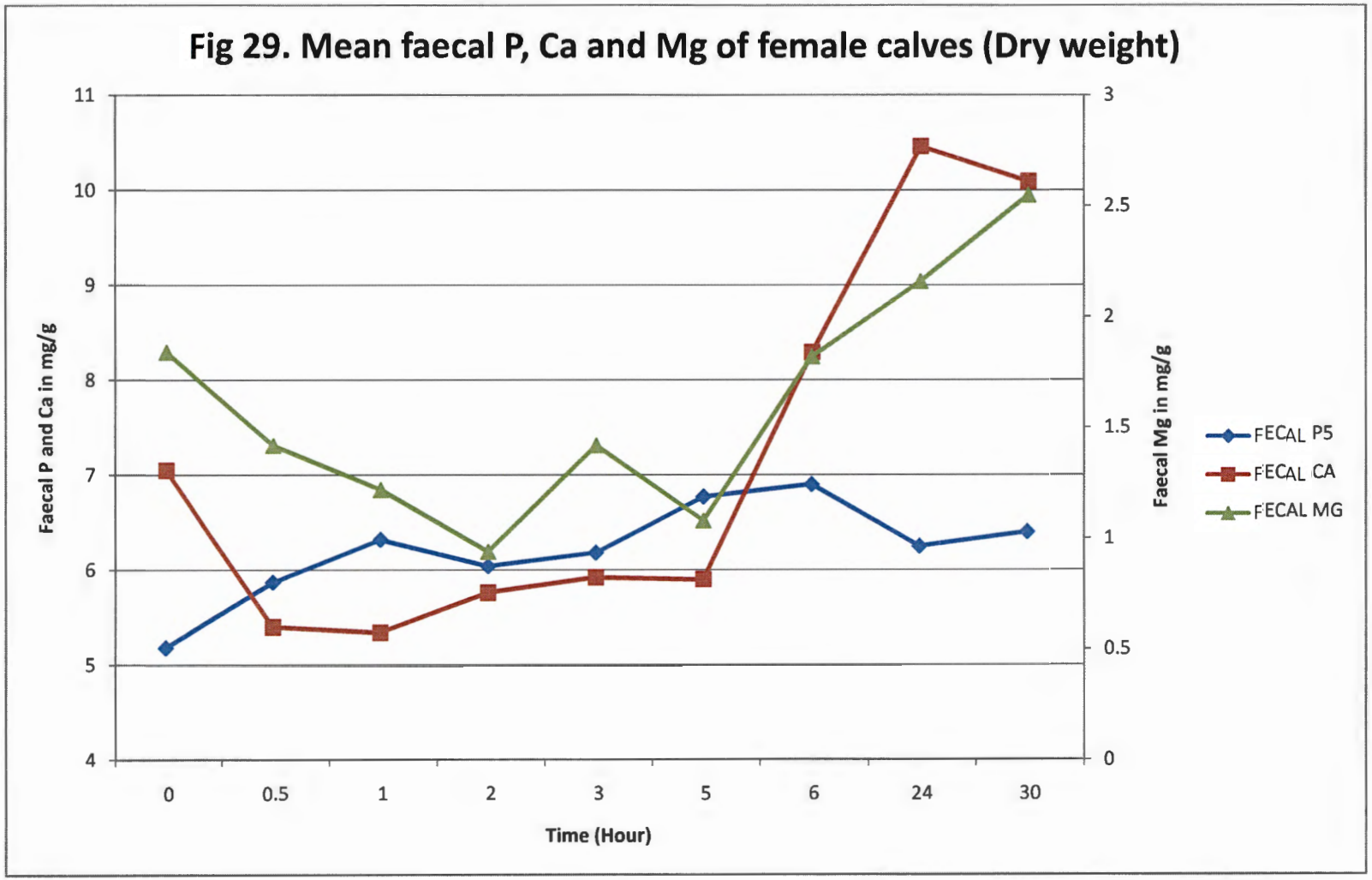


Fig 29. Mean faecal P, Ca and Mg of female calves (Dry weight)
Faecal P and Ca in mg/g
Faecal Mg in mg/g
Time (Hour)
11
10
9
8
7
6
5
4
3
2.5
2
1.5
1
0.5
0
0
0.5
1
2
3
5
6
24
30
FECAL P5
FECAL CA
FECAL MG

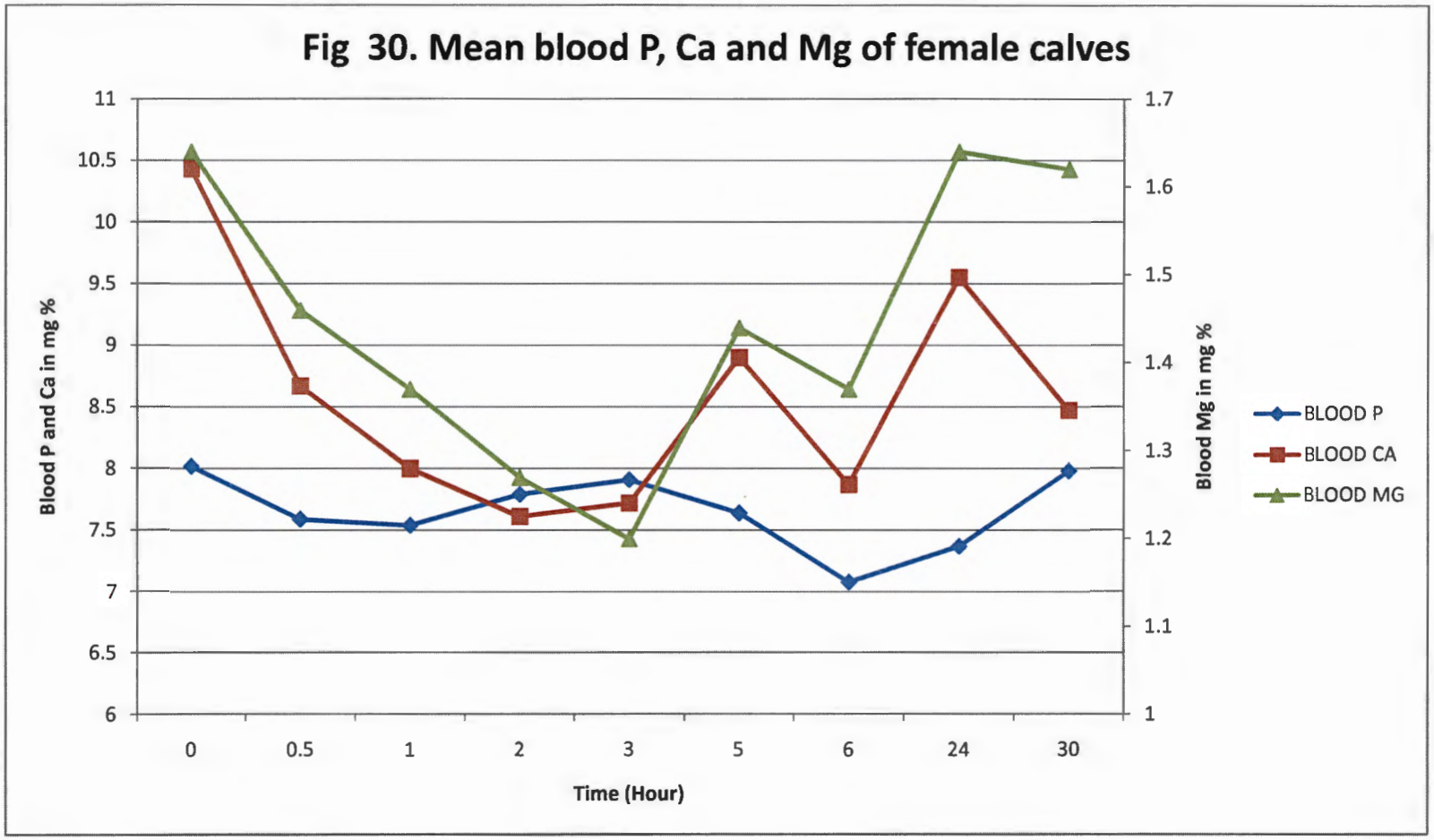


Fig 30. Mean blood P, Ca and Mg of female calves
Blood P and Ca in mg %
Blood Mg in mg %
Time (Hour)
11
10.5
10
9.5
9
8.5
8
7.5
7
6.5
6
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1
0
0.5
1
2
3
5
6
24
30
BLOOD P
BLOOD CA
BLOOD MG

CHAPTER 6

6. BIBLIOGRAPHY

Agnes, F., Sartorelli, P., Bisso, M.C. and Dominoni, S. 1993. Ionised calcium in calf serum: relation to total serum calcium, albumin, total protein and plasma. J. Vet. Med. Series A, 80: 605-608.

Aiello, S. E. and Moses M. A. 2003. Merck Vet Manual Online. Special clinical concerns.. Merck & Co., Inc and Merial LTD

Allen, W. M., and Sansom, B. F. 1985. Milk fever and calcium metabolism. J. Vet. Pharmacol. Therap. 8: 19-29.

Association of Official Analytical Chemists (AOAC). 1980. Official Methods of Analysis. "Minerals in Feeds by Atomic Absorption Spectrophotometry-Official Final Action, Washington D.C.

Barragry, T. B. 1994. Veterinary Drug Therapy. Philadelphia: Lea & Febiger. 530-545.

Beighle, D. E., Tucker, W. B., and Hemken, R. W. 1989. Interactions of dietary cation-anion balance and phosphorus: effects on growth and serum inorganic phosphorus in dairy calves. J. Dairy Sci. 71(12): 3362-3368.

Beighle, D. E., Boyazoglu, P. A., and Hemken R. W. 1993. Use of bone in serial sampling for mineral analysis. J. Dairy Science. 76: 1087-1052.

Beighle, D. E., Boyazoglu, P. A., and Hemken R. W., Serumaga-Zake P. A. 1994. Determination of calcium, phosphorus, and magnesium values in rib bones from clinically normal cattle. Am. J. Vet. Res. 55 (1): 85-89.

Beighle, D. E., Boyazoglu, P. A., and Hemken R. W. 1995. Short-term effects of dietary cation:anion balance on bone mineral homeostasis in the bovine. J. S. Afr. Vet. Ass. 66(2): 56-60.

Beighle, D. E., Boyazoglu, P. A., and Hemken R. W. 1997. Acute effects of anionic diets on bone mineral homeostasis in the bovine. J. S. Afr. Vet. Ass. 68: 73-77.

Beighle, D. E. 1999. The effect of gestation and lactation on bone calcium, phosphorus and magnesium in dairy cows. J. S. Afr. Vet. Ass. 70(4):142-146.

Beighle, D. E. 2000. Bone, blood and faecal response to an acidogenic lick for range cattle using different concentrations of ammonium chloride. J. S. Afr. Vet. Ass. 71(4): 215-218

Beitz, D. C., and Allen, R. S. 1984. Digestion and absorption. In Swenson M J Dike's Physiology of Domestic Animals. 10th ED. Comstock Publishing Association, London 373.

Berne, R. M. and Levy, M. N. 1998. Physiology 4th Ed. Mosby Company, St. Louis, 60-64, 69-71, 282-285, 307-314, 661-664, 789-791, 848-870.

Block, E. 1984. Manipulating dietary anions and cations for prepartum dietary cows to reduce incidence of milk fever. J. Dairy Sci. 67:2939-2948.

Bondi, A. A. 1987. Animal Nutrition. John Wiley and Sons, New York. 172-173, 178-185, 190-193.

Bortolussi, G., Ternouth , J. H., McMeniman, N. P. 1996. Dietary nitrogen and phosphorus depletion in cattle and their effects on liveweight gain, blood metabolite concentrations and phosphorus kinetics. J. Agric. Sci. (Camb) 126: 493-501

Borucki Castro, S. I., Phillip, L. E., Girard, V., and Tremblay, A. 2004. Altering dietary cation-anion difference in lactating dairy cows to reduce phosphorus excretion to the environment. J. Dairy Sci. 87: 1751-1757

Braithwaite, D. G. 1980. The effect of dose rate of 1-alpha-hydroxycholecalciferol on calcium and phosphorus metabolism in sheep. Br. J. Nutr. 44(2):183-191.

Burrows, G. E., Barto, P. B., and Martin, B. 1987. Comparative pharmacokinetics of gentamicin, neomycin, and oxytetracycline in newborn calves. J. Vet. Pharmacol. Ther. 10: 54-63.

Bushinsky, D. A., Nalbantian-Brandt, C., and Favus, M. J. 1989. Elevated Ca^{2+} does not inhibit the $1.25(OH)_2D_3$ response to phosphorus restriction. Am. J. Physiol. Renal Physiol. 256: F285-F289.

Capen, C. C. and Rosol, T. J. 1989. Calcium regulating hormones and diseases of abnormal mineral metabolism. In: Clinical biochemistry of domestic animals. Kaneko J. J. (ed.). 8th ed. Academic Press, Inc. San Diego, California, USA, 678-752.

Capen, C. C. 2004. Patho physiology of calcium-phosphorus metabolism in bone. In Dunlop, R. H. Veterinary Pathophysiology. Blackwell Publishing. Iowa. 402-421.

Carstairs, J. A., Neitzel, R. R., and Emergy, R. S. 1980. Energy and phosphorus status as factors affecting postpartum performance and health of dairy cows. J. Dairy Sci. (In Press).

Chapus-Lardy, L., Fiorini, J., Toth, J. and Dou, Z. 2004. Phosphorus concentration and solubility in dairy feces, variability and affecting factors. J. Dairy Sci. 87(12):4334-4341.

Chan, P. S., West, J. W., and Bernard, J. K. 2006. Effect of prepartum dietary calcium on intake and serum and urinary mineral concentrations of cows. J. Dairy Sci. 89:704-713.

Cheecke, P. R. 1991. Applied Animal Nutrition: Feeds and Feeding, Macmillan Publishing Co. New York, 201-204, 208-209.

Chester-Jones, H., Fontenot, J. P., Veit, H. P. and Webb, K. E. 1990. Physiological and pathological effects of feeding high levels of magnesium to steers. J. Anim. Sci. 68: 8800-8813.

Cohen, R. D. H. 1973a. Phosphorus nutrition of beef cattle. II. Regulation of blood, hair and bone of grazing steers. Aust. J. Exp Agric. Anim. Husb. 13:5-8

Davey, L. A., Ferber, M. T., and Kaye, B. 1985. Comparisn of the serum pharmacokinetics of a long acting and a conventional oxytetracycline injection. Vet. Rec. 117: 426-429.

Desmecht, D. J., Linden, A. S., Godeau, J. M., and Lekeux, P. M. 1995. Experimental production of hypocalcaemia by EDTA infusion in calves: a critical appraisal assessed from the profile of blood chemicals and enzymes. Comp. Biochem. Physiol. 110A, 115-130.

Dias, R. S., Kebreab, E., Vitti, D. M. S. S., Roque, A. P., Bueno, I. C. S., and France, J. 2006. A revised model for studying phosphorus and calcium kinetics in growing sheep. Am. J. Anim. Sci. 84: 2787-2794.

Dou, Z., Knowlton, F. K., Kohn, R. A., Wu, Z., Satters, L. D., Zhang, G., Toth, J. D., and Ferguson, J. D. 2002. Phosphorus characteristics of dairy faeces affected by diets. J. Env. Quality. 31:2058-2065.

Erickson, G. E., Klopfenstein, T. J., Milton, C. T., Hanson, D., and Calkins, C. 1999. Effect of dietary phosphorus on finishing steer performance, bone status, and carcass maturity. J. Anim. Sci. 77: 2832-2836.

Fernandez, F. J., and Kahn, H. L. 1971. Clinical Methods for Atomic Absorption Spectroscopy. Clin. Chem. Newsl. 3, 24.

Georgeovskii, V. I., Annenkov, B. N. and Samokhin, V. I. 1982. Mineral nutrition of animals. Studies in the agricultural and food sciences. Butterworths, Moscow, 475.

Goff, J. P. and Horst, R. L. 1993. Oral administration of calcium salts for treatment of hypocalcernia in cattle. J. Dairy Sci. 76: 101-108.

Goff, J. P. 2000. Phathophysiology of calcium and phosphorus disorders. Veterinary clinics of North America: Food Animal Practice. 16: 319-337, vii.

Goff, J. P., Rulz, R., and Horst, R. L. 2004. Relative acidifying activity of anionic salts commonly used to prevent milk fever. Am. J. Dairy Sci. 87: 1245-1255.

Goff, J. P. 2008. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. The Vet. J. 176:50-57.

Gonul, R., Kayar, A., Bilal, T., Erman, O. R. M., Cagla Parkan, D. V. M., Dodurka, T. H. 2009. Comparison of mineral levels in bone and blood serum of cattle in Northwestern Turkey. J. Anim. And Vet Adv. 8 (7): 1263-1267.

Grunberg, W., Morin, D. E., Drackley, J. K., Barger, A. M., and Constable, P. D. 2006. Effect of continuous intravenous administration of a 50% dextrose solution on phosphorus homeostasis in dairy cows. J. Am. Vet. Med. Assoc. 229 (3): 4123-420.

Hall, W. F., Kniffen, T. S., and Bane, D. P. 1989. Plasma concentrations of oxytetracycline in swine after administration of the drug intramuscularly and orally in feed. J. Am. Vet. Med. Assoc. 194(9): 1265-1268.

Horst, R. L., and Littledike, E. T. 1982. Comparison of plasma concentrations of vitamin D and its metabolites in young and aged domestic animals. Comp. Biochem. Physiol B. England. 73:485-489.

Horst, R. L. 1986. Regulation of calcium and phosphorus homeostasis in the dairy cow. J. Dairy Sci. 69:608-616.

Horst, R. L., Goff, J. P., and Reinhardt, T. A. 1994. Calcium and vitamin D metabolism in the dairy cow. J. Dairy Sci. 77:1936-1951.

Howard and Smith, 1999. Current Veterinary.Therapy, food animal practice. 4th ed. Saunders

.

Jackson, J. A., Hopkins, D. M., Xin, Z., and Hemken, R. W. 1992. Influence of cation-anion balance on feed intake, body weight gain, and humoral response of dairy calves. J. Dairy Sci. 75:1281-1286.

Jorgensen, R. J., Nyengaard, N. R., Daniel, R. C. W., Mellau, L. S. B., and Enemark, J. M. D. 1999. Induced hypocalcaemia by Na_2EDTA infusion. A Review. J. Vet. Med. A 46. 389-407.

Kahn C. M. and Line S. 2005. Merck Vet Manual of Diagnosis and Therapy.. Merck Research Laboratories. Merck & Co., Inc. Whitehouse Station, N. J.

Karn, J. K. 2001. Phosphorus nutrition of grazing cattle: a review. Animal feed Science and Technology. 89:133-153.

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

Knowlton, K. F., and Herbein, J. H. 2002. Phosphorus partitioning during early lactation in dairy cows fed diets varying in phosphorus content. J. Dairy Sci. 85(5):1227-1236.

Lambs, L., Reverend, B. D., Kozlowski, H., and Berthon, G. 1988. Metal ion-tetracycline interactions in biological Fluid. Part 9. Circular dichroism spectra of calcium and magnesium complexes with tetracycline, oxytetracycline, doxycycline and chlortetracycline and a discussion of their binding modes. Inorgan chem. 27:3001-3012.

Legradi, G., Emerson, C. H., Ahima, R. S., Flier, J. S., and Lechan, R. M. 1997. Leptin prevents fasting-induced suppression of prothyrotropin-releasing hormone messenger ribonucleic acid in neurons of the hypothalamic paraventricular nucleus. Endocrinology. 138:2569-2576.

Leonhard-Marek, S. and Martens, H. 1996. Effects of potassium on magnesium transport across rumen epithelium. Am. J. Physiol. 271: G1038-G1038.

Leonhard, S., Martens, H. and Gäbel, G. 1989. New aspects of magnesium transport in ruminants. Acta Vet. Scand. 86:186-151 (Suppl.).

Little, D. A. 1984. Definition of an objective criterion of body phosphorus reserves in cattle and its evaluation in vivo. Can. J. Anim. Sci. 64:229-231

Lloyd, L. E., McDonald, B. E., and Crampton, E. W. 1978. Fundamentals of Nutrition 2nd ED. W. H. Freeman & Co. San Francisco. 224-229, 250-253, 259-262

Martens, H. 1993. Minerals and vitamins in animal nutrition. Internordic postgraduate course in animal nutrition. 2.8 - 13.8, Helsinki.

Martin, R. B. 1985. Tetracyclines and daunorubicin in metal ions in biological systems. In: Antibiotics and their complexes. Sigel H, editor. Vol. 19. New York: Marcel Dekker, Inc., 19-40.

Martins, H., and Schweigel, M. 2000. Pathophysiology of grass titany and other hypomagnesemias. Implications for clinical management. Vet. Clin. North Am. Pract. 16(2):339-368.

Masuyama, R., Nakaya, Y., Tanaka, S., Tsurukami., Nakamura, T., Watanabe., Yoshizawa, T., Kato, S., and Suzuki, K. 2001. Dietary Phosphorus restriction reserves the impaired bone mineralization in vitamin D receptor knockout mice. J. Nutr Sci. 142(1):494-497.

McDonald, P., Edwards, R. A, Greenhalgh, J. F. D., and Morgan, C. A. 1995. Animal Nutrition. Longman Scientific & Technical, New York. 72-75.

Mellau, L. S. B. Jorgeson, R. J. and Enemark, J. M. D. 2001. Plasma calcium, inorganic phosphate and magnesium during hypocalcemia induced by a standardized EDTA infusion in cows. Acta Vet. Scand. 42: 251-260.

Mevius, D. J., Nouws, J. F. M., and Breukink, J. F. 1986. Comparative Pharmacokinetics, bioavailability and renal clearance of five parenteral oxytetracycline-20% formulations in dairy cows. Vet Q. 8(4): 285-294.

Milinkovic-Tur, S., D., Stojevic, Z., Gradinski,-vrbanac, B., and Simpraga, M. 2000. Magnesium absorption from different compartments of the swine large intestine. Acta Vet. Brno. 69, 3-9.

Minson, D. J. 1990. Forage in ruminant nutrition. Academic press. San Diego, CA.

Mosel, M.V., A. T. H. Klooster, F. V. Mosel and J. V. Kuilen. 1993. Effect of reducing dietary ((Na+K)-(Cl+SO4)) on rate of Calcium mobilization by dairy cows at parturition. Res. Vet Sci., 54: 1-9. PMID: 8434135.

Mosel, M.V., Wouterse, H. S.,and Vant Klooster A. T. H. 1994. Effect of reducing dietary ((Na+K)-(Cl+SO4)) on bone 'n da'ry cows at parturition. Res. Vet Sci., 56: 270-276. PMID: 8073176.

Motsei, L. E. and Beighle, D. E. 2006. Bone mineral response to ammonium sulphate offered as a lick supplement in beef calves. J. of S. A. Vet. Ass. 77(1): 19-23.

National Research Council. 1987. Vitamin tolerance of Animals. National Academy Press, Washington, DC.

National Research Council. 1988. Nutrient requirements of Bovine. 9th edn. National Academy Press, Washington, DC.

Newman, E.C., and Frank, C. W. 1976. Circular dichroism spectra of tetracycline complexes with Mg^{2+} and Ca^{2+}. J. Pharm. Sci. 65: 1728-1732.

Nouws, J. F. M., Van Ginneken, C. A. M., and Zig, G. 1983. Age-dependent pharmacokinetics of oxytetracycline in ruminants. J. Vet. Pharm. Ther. 6: 59-66.

Overton, T. R., and Waldron, M. R. 2004. Nutritional Management of Transition Dairy Cows: Strategies to Optimize Metabolic Health. J. Dairy Sci. 87:E105-E119.

PerkinElmer, INC. 2000. Analytical Methods for Atomic Absorption Spectrometry. Release E, PerkinElmer Instruments LLC, Part number 0303-0152, Singapore. 154-155.

Phillis, J. W. 1976. Veterinary Physiology. Wright and Scientechnica, Bristol. 646-647, 649-652.

Pond, W. G., Church, D. C., and Pond K. R. 1995. Basic Animal Nutrition and Feeding, 4th ED. John Wiley & Sons, New York. 169-175, 190-194, 231-236.

Pugh, D. G., Rankins, D. L. Jr, Eason, J. T., Wenzel J. G. W., and Spano, J. S. 1994. The effect of feeding broiler litter on the serum calcium, phosphorus, and magnesium concentrations of beef brood cows. Vet. Clin. Nutr. 1:18.

Puls, R. 1994. Vitamin Levels in Animal Health. 1st ED. Sherpa International, Clear Book. 82-86.

Radostits, O., Blood, D., and Gay, C. 1994. Veterinary Medicine. 8th ED. London: Bailliere Tindall.

Rahnema, S., Wu, Z., Ohajuruka, O. A., Weiss, W. P., and Palmquist, D. L. 1994. Site of mineral absorption in lactating cows fed high-fat diets. J. Anim. Sci. 72:229-235.

Ramberg, C. F., Johnson, E. K., Fargo, R. D., and Kronfeld, D. S. 1984. Calcium homeostasis in cows, with special reference to parturient hypocalcemia. Am. J. Physiol. Regul. Integr Comp. Physiol. 246: R698-R704.

Reichel, H., Koeffler, H. P., and Norman, A. W. 1989. The role of the vitamin D endocrine system in health and disease. J. Med. 320: 980-991.

Reinhardt, T. A., Horst, R. L., and Goff, P. 1988. Calcium, phosphorus, and magnesium homeostasis in ruminants. Vet. Clin. North Am. Food Anim. Prac. 8: 2. 331-350.

Remond, D., Meschy F. and Bovin R.. 1996. Metabolites, Water and Mineral Exchanges Across the Rumen Wall: Mechanisms and Regulation. Ann. Zootech. 45:97

Riond, J. L., Kocabagli, N., Spichiger, U. E. and Wianner, M. 1995. The concentration of ionized magnesium in serum during the periparturient period of non-paretic dairy cows. Vet. Res. Com. 19: 195-203.

Riviere, J., Craigmill, A. L., and Sundlof, S. F. 1999. Handbook of comparative pharmacokinetics and residues of veterinary antimicrobials. Boca Raton, F. L: CRC Press, Inc. 175-226.

Schifferli, K., Galeazzi, R. L., and Nicolet, J. 1982. Pharmacokinetics of oxytetracycline and therapeutic implications in veal calves. J. Vet. Pharmacol. Ther. 5:247-257.

Sjaastad, O. V., Hove K., and Sand, O. 2003. Physiology of Domestic Animals. Scandinavian Veterinary Press, Oslo. 102-104, 237-238, 245-252, 274-279, 286-292.

Speer, H. L., Rosenthal, J, Silva G. H., and Pennsy, M. L. 1984. Fluorescence analysis of the tetracyclines in foodstuffs enhanced by metal chelation. Anal. Chem. 44:431-441.

Taylor, M. S., Knowlton, K. F., McGilliard M., Seymour, W. M., and Herbein, J. H. 2008. Blood mineral hormone and osteocalcin responses of multiparous Jersey cows to an oral dose of 25-Hydroxyvitamin D_3 or vitamin D_3 before parturition. Amer. J. Dairy Sci. 91:2408-2416.

Terhune, T. N., and Upson, D. W. 1989. Oxytetracycline pharmacokinetics, tissue depletion, and toxicity after administration of a long acting preparation at double the label dosage. J. Am. Vet. Med Assoc. 194(7): 911-917.

Thiede, M. A. 1994. Parathyroid hormone-related Protein: a regulated calcium-mobilizing product of the mammary gland. J. Dairy Sci. 77:1952-1963.

Toutain, P. L., and Raynaud, J. P. 1983. Pharmacokinetics of oxytetracycline in young cattle: comparison of conventional versus long-acting formulation. Am. J. Vet. Res. 44(7):1203-1209.

Underwood, E. J., and Suttle, N. F. 1999. The Mineral Nutrition of Livestock. CABI Publishers, Wallingford UK. 3rd ED, 67-98 and 375-391.

USP Dictionary of USAN and international drug names. 2002 ED. Rockville, MD: The United States Pharmacopeial Convention, Inc., 2002.

Uysal, A., 1998. Metabolic Diseases In; Veterinaly internal Medicine Student Lesson Notes, Chapter II, Instanbul University Veterinary Faculty Press, lesson Notes Number: 79, Instanbul, pp:1-73

Vernillo, A.T, Rifkin, B. R. 1998. Effect of tetracyclines on bone metabolism. Adv. Dent Res. 12(2):56-62

Vitti, D. M., Kebreab, E., Lopes, J. B. , Abdalla, A. L., De Carvalho, F. F., De Resende, K. T., Crompton, L. A., and France, J 2000. Akinetic model of phosphorus metabolism in growing goats. J. Anim. Sci. 78: 2706-2712.

Wang, C., and Beede D. K. 1990. Effect of supplemental protein on acid base status and calcium metabolism of non-lactating Jersey cows. J. Dairy Sci. 73:3178-3186.

Wang, C., and Beede D. K. 1992. Effects of ammonium chloride and sulphate on acid base status and calcium metabolism of dry Jersey cows. J. Dairy Sci. 75:820-828.

Wasserman, R. H. 2004. Vitamin D and the dual process of intestinal calcium absorption. J. Nutr. 134: 3137 – 3139

White, J. R., and Pearce, F. L. 1982. Characteristics of histamine secretion from rat peritoneal mast cells sensitized to the nematode Nippostrongylus brasiliensis. J. Immunol. Blackwell Scientific Publications.46:353-359.

World Health Organization. 2005. Chlortetracycline and tetracycline. Food Additives Series 36. Inchem IPCS.