THE USE OF AMMONIUM SULPHATE AS AN ACIDIOGENIC AGENT IN BONE MEAL LICKS TO IMPROVE THE PHOSPHORUS STATUS IN CATTLE.

by

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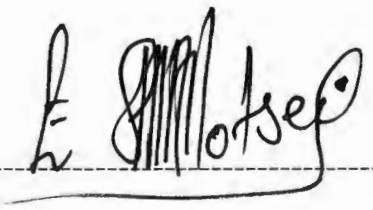
DECLARATION

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.



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Nothing is impossible to a willing heart

ABSTRACT

Sixteen Bonsmara calves (4 males, 12 females) were blocked according to sex and randomly assigned to 2 groups of 8 each. They were offered licks containing bone meal and salt (control) and bone meal and ammonium sulphate at 1.25, 2.5, 5, 10, 15, and 18% (treatment) to evaluate the effect of dietary anions on blood, bone and faecal P, Ca and Mg for a period of 6 weeks. Calves were also fed *ad lib* a roughage containing 50% *Medicago Sativa* and 50% *Cencrus Ciliaris*. Blood, bone and faecal samples were taken on the same day to prevent variation between sampling periods and mineral contents. Bone, blood and faecal P concentrations were significantly ($P<0.05$) higher in the anionic treatment group in most weeks of the experiment compared to the control group. Bone P concentration was significantly ($P<0.05$) increased in the anion treatment group at every sampling period, not only compared with control animals, but also compared to other treatment concentrations of ammonium sulphate, indicating that the ammonium sulphate was able to improve the P content of bone at each of the 6 concentrations used in the lick. A relationship existed between bone and blood Ca where there was resorption from bone with increased blood and faecal Ca and decrease in bone Ca. The response of bone Ca and P was in opposite directions in most weeks of supplementation. When bone Ca increased bone P decreased and when bone Ca decreased bone P increased and this indicate independent absorption and resorption of Ca and P into and out of bone and a wide Ca:P ratio varying from 1.5:1 to 5:1.

Faecal P concentration was significantly ($P<0.05$) increased in the faeces of the treatment animals at all sampling periods except when ammonium sulphate was added at 2.5% and 15%. The mean concentration of P in the blood was significantly ($P<0.01$) higher in the calves on the anionic diet compared with the calves on the control diet and the bone thickness was significantly ($P<0.05$) greater in the calves fed the anionic diet compared with those fed the control diet. It should also be noted that the bone thickness followed bone P and not bone Ca. Bone Mg was

diet. It should also be noted that the bone thickness followed bone P and not bone Ca. Bone Mg was unpredictable when compared to the other two mineral parameters. Bone Mg fluctuated, and it was difficult to build a relationship between bone Mg, Ca and P. When bone Ca increased, bone Mg also increased but only at 1.25% NH_4SO_4 and 5% NH_4SO_4. It was only at 2.5% NH_4SO_4 that bone Ca decreased with a decrease in bone Mg. At the 10%, 15% and 18% NH_4SO_4 level of supplementation these two minerals responded in opposite directions.

Results from this research indicate that the addition of anions, in the form of NH_4SO_4 has a beneficial effect in increasing bone P; and because of simultaneous increases in blood, bone and faecal P when ammonium sulphate was added to the lick at 1.25 and 18%, also has a P sparing effect for animals grazing a P-deficient veld.

Key words: Phosphorus, Calcium, Magnesium, Bone meal, ammonium sulphate lick anions, bone, blood, faeces.

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INTRODUCTION

Our knowledge of many aspects of the biochemistry, physiology and nutrition of phosphorus (P) in domesticated animals has improved significantly in the subsequent 50 years. My research has the express intention of highlighting some of the improvements in our knowledge of the role of anionic diets in bone meal lick to improve the P status in animals, how it was obtained, and some of its limitations. I have concentrated in beef calves.

Research on the effect of dietary cation-anion balance (DCAB) in cattle (calves) for a way to prevent botulism in beef cattle that are free ranging have long been done by many researchers (Beighle *et al*, 1997; Block, 1984; Dishington, 1975; Eckles *et al*, 1932; Du Toit *et al*, 1930). Block (1984) manipulated dietary anions and showed that cows consuming an anionic diet maintained adequate blood Ca during the periparturient period whereas cows consuming a cationic diet were not able to do so. While assigning cows to two different diets, one being cationic and one being anionic for 2 years, he found that cows consuming the anionic diets had no milk fever, but cows consuming the cationic diet had a 47.4 % incidence of the disease (Block, 1984).

A wide dietary Ca:P ratio had no apparent effect on P absorption when P intake was adequate. The availability of P was lowered by a diet deficient in P with a wide Ca:P ratio (Young *et al*, 1966b). Wise *et al* (1962) noted that serum Mg levels were decreased with high levels of dietary P when dietary Ca levels resulted in a "normal" serum Mg levels even at the highest P level.

Chapter 1

LITERATURE REVIEW

1. HISTORICAL OVERVIEW

1.1. PICA

1.1.1. STIFF-SICKNESS AND BOTULISM

Theiler *et al.* (1920) described an aetiological chain which linked phosphorus deficiency to both botulism and stiff-sickness. Phosphorus deficiency in the soil leads to phosphorus deficiency in the pasture and hence the phosphorus deficiency in the animal grazing on that pasture. Cattle developed a depraved appetite or "pica" with special favouritism for bone craving or specifically bovine osteophagia as a manifestation for aphosphorosis. Such cattle eat any carcass debris which happens to be lying about in the veld and if such a debris happens to be infected with the toxin from the toxicogenic anaerobe *Clostridium botulinum*, they go down with botulism.

Cattle grazing pastures low in phosphorus (P) show evidence of depraved appetite or pica, retarded growth, low reproductive efficiency, reduced milk yield, frequently walking with stiffened gait and suffer from spontaneous fractures. Collectively this syndrome is known as clinical aphosphorosis (stiff-sickness, styfziekte) and it is actually a form of osteomalacia or rickets in adults and young animals respectively (Theiler and Green, 1932), because symptoms can be cured by provision of high phosphorus content supplements. Pathologically, both conditions are characterised by increased osteoclastic cell activity and osteoid (protein) formation. The osteoclastic cells remove the mineral from the bone while the new osteoid material is laid down, presumably as a response to the normal mechanical stresses applied to the bone (Ternouth, 1990). Thus there is enlargement and

softening of the bone occurring at the same time. If this condition is not treated by provision of supplements, the animal will become progressively worse as the joint becomes more and more painful and eventually may die due to starvation as a sequel to bone fracture disablement (Theiler and Green, 1932).

The deficiency of phosphorus in ruminants is primarily reflected in retarded growth, poor reproductive performance, reduced milk yield and wool growth and impaired skeletal and dental health (McDowell, 1985) and affected cattle manifest depraved appetites or pica and frequently chew on bones which may result in a higher incidence of botulism (McDowell, 1985).

1.1.2. PASTURE

Because analysis to determine the variable forms of soil minerals is more often unreliable and difficult to interpret, (Lamand, 1979, cited by McDowell, 1987) it is postulated that forage analysis was more reliable to estimate nutrient availability for grazing ruminants. However, carefully collected forage samples represent the diet of grazing livestock and are extremely variable in predicting livestock mineral status.

This, however, has several disadvantages such as, uncertainty of sample representing what livestock consumed, difficulty of estimating forage intake, variation in the availability of forage elements and the possibility of soil contaminated forage samples (McDowell, 1987). The work carried out earlier by Du Toit *et al.* (1940) indicated that an approximation of the daily mineral intake of the grazing animals may be arrived at by analysing the representative samples of those species of grasses which the animal ate.

They further showed that grasses were particularly low in phosphorus ranging between 0.12-0.17 % in summer and 0.05-0.07 % in winter.

Barnes *et al,* (1955) collected pasture samples over a traverse of about 60 miles and found the P content to be 0.07 %. Although the collection was limited, it did however, indicate the P levels that can be expected, as was also reflected in the low P levels in the soil (0.05%).

Rogers *et al,* (1986) suggested that pasture samples may indicate the P status of grazing ruminants by analysing pasture samples grazed by those animals. More than 80% of the samples had more than 0.3% P and 3% of the samples had less than 0.3% P which were higher than the minimum levels recommended (0.16%) for mature, non-lactating sheep and cattle at maintenance (NRC, 1963, 1964, cited by Playne, 1969) and Blood and Radostits (1989) have reported that the deficiency of P occurs when pasture P is less than 0.3%.

Recent studies carried out by Mazengera, (1992) when analysing the mineral content of veld grass of several districts of the former Republic of Bophuthatswana, indicated a mean content of less than 0.1% in both summer and winter which according to Groenewald and Boyazoglu (1980) was deficient and therefore such districts posed a severe and extensive P deficiency throughout the year.

1.1.3. FAECAL

Faecal levels for some minerals provide a means of status assessment as high excretion rates reflect dietary adequacy (McDowell, 1987). Faecal P concentration is associated with dietary P since nearly all P is excreted through the faeces (Bromfield and Jones, 1970). Cohen (1974) reported that

faecal P was linearly related to dietary P levels and thus could be used to predict dietary P intake as earlier reported by Moir (1960). Later, Holochek *et al,* (1985), using six forages with wide ranges of P content and fed to six steers concluded that dietary P concentration of grazing cattle could accurately be predicted from faecal P concentration using generalised simple linear regression equations. The P intake of cattle was associated (r^2=0.94) with total daily faecal P output as had earlier been noted by Cohen (1974).

Jones *et al,* (1984) showed that faecal P levels were lower in winter (0.3%) than in summer, and therefore it follows that P levels in the diet were also lower in winter when there is less green material in the pasture. Even so, the average level of 0.3% P in faeces during winter was still indicative of diets containing in excess of 0.12% P suggested as a minimum requirement for young growing cattle (Little, 1980). This faecal measurement suggests that there was adequate P for animal growth, although not necessarily for lactating cows.

Faeces as an indication of mineral status of grazing ruminants may have some disadvantages (limitations) rendering them unreliable. For instance, when considering faecal P in mature tropical and subtropical forages, a large portion of minerals in the faeces represents undigested minerals and excretions and are therefore not reflected by metabolic need especially for P (McDowell, 1987). The faecal P concentration may also be of limited value as an indicator of animal mineral status due to site and animal class variability. For instance, Winter (1988) showed that at one location, faecal P concentrations were increased to about 0.3% with P supplementation, while elsewhere responses were obtained when faecal P levels were as high as 0.4%.

Read *et al,* (1986) showed that in early lactation, range cattle at Glen receiving no P had higher faecal concentrations than the supplemented group (0.286 v/s 0.272%). Also in the lactating or weaning animals there was no statistical significance between the P supplemented and unsupplemented group (0.266 v/s 0.239%). So these discrepancies cast a doubt on the reliability of this indicator. Their results seemed to agree with the conclusion of Winks *et al,* (1977) that faecal P alone was a poor indicator of dietary P when faecal P was relatively high and ceased when levels were considerably lower.

1.1.4. BLOOD PHOSPHORUS

The plasma content of minerals such as inorganic P and Ca can be regarded as the result of absorption from the gastro-interstinal tract exchange with bone and excretion in faeces and urine (Breves, 1982).

In their study carried out earlier Wise *et al,* (1962) found a marked decline in serum inorganic P during the initial two week period among calves fed the basal diet and those receiving the soft phosphate with colloidal clay.

Throughout the experiment of Benzie *et al,* (1957) blood inorganic P values in ewes fed a low P ration were significantly lower than the values in ewes fed on the moderate P ration. Plasma inorganic P concentrations were higher for calves fed the high P diet than for those fed the Low P diet (Teh *et al,* 1982). Braithwaite (1985) found that serum P concentration progressively increased with increased P intake in lambs fed diets which were grossly deficient, moderately deficient and adequately supplied with P to meet recommendations for lambs growing at 0.2 kg/day.

In an experiment conducted by Wise et al, (1962) using fifty Holstein calves in two experiments throughout a test period of 56 days, in experiment I, they gave 0.01 % P in the basal diet and found a terminal serum inorganic P to be approximately 4.7 mg %. A terminal level of 5.9 mg % was observed on the soft phosphate diet while serum inorganic P on the other three diets containing 0.19 % P (Basal + Defluorinated Phosphate, rock; Basal + Curacao Island Phosphate and Basal + colloidal clay) ranged from 6.2 to 6.9 mg % (Wise. 1962).

In an experiment carried out by ARC (1965) to monitor the response of the bovine kidney to increasing plasma inorganic P concentrations, urinary excretion of P began to increase when the plasma inorganic P concentration exceed 7 mg/100 ml.

1.1.5. PREGNANCY AND LACTATION

While studying the changes in cortical bone mineral in the dairy cow in response to demands of lactation and pregnancy using rib bone biopsies in serial sampling, Beighle (1999) found cortical bone P concentration in rib bone during the lactation period to be not significantly different but Ca concentrations being significantly higher at parturition and during the first 30 days of lactation compared to the next 30 days and between 90 and 120 days. He reported that cows resorb bone Ca during the middle of lactation and not during the periparturient period as previously thought.

1.1.6. BONE MEAL

Bone meal as one of the cheapest P supplements has been reviewed by many researchers (Theiler *et al,* .1924,1928; Wyoming Agric Experimental

Station, 1945). It also has properties of good palatability and utilisation and is also a good source of trace elements (McDowell and Conrad, 1977).

1.1.6.1. EFFECT ON PREVENTION OF DISEASE AND REDUCTION OF MORTALITY RATES

Investigations have proven the beneficial effects of bone meal in prevention of diseases (stiff-sickness and botulism) pica and osteophagia (Theiler *et al,* 1924, 1928; Barnes and Japhcott, 1955; Bisschop, 1964; Blood and Henderson, 1968; Blood *et al,* 1983).

Carson *et al,* (1978) reported a reduction in the incidence of retained placenta post-parturient metritis, and dystocia, in a dairy herd fed steamed bone meal. At the end of 3 months, the incidence of dystocia had been reduced from 75% to 10%; retained placenta from 35% to 8% and post parturient metritis from 70% to 10%.

In research carried out by Du Toit and Bisschop (1929) over a period of 3 years among cattle fed bone meal, a mortality rate of 66% was reduced to 9% from various causes such as botulism, cachexia, fractures and poisoning.

1.1.6.2. IMPROVEMENT OF GROWTH AND WEANING WEIGHT OF CALVES

Bone meal has been shown to have a very beneficial effect on production. In experiments carried out by Theiler *et al,* (1924) it was shown that grazing cattle supplemented with bone meal gained more weight throughout the 15 months of the study than the controls not given the supplement. However, during the winter season, both groups lost weight but the bone meal group maintained the advantage gained in summer and spring and were 45.5 kg heavier than the controls, indicating the significance of winter supplementation.

Theiler *et al,* (1924) demonstrated that weaned calves fed P, could gain 61 kg more weight over a 12-month period than unsupplemented ones. Young oxen gained 68 kg per year more than their controls, while supplemented cows showed a weight advantage of 48 kg over an 11-month feeding period.

During the first year, feeding bone meal proved of no advantage from the stand point of weaning of calves. On the other hand, the second crop of calves from cows and heifers fed bone meal averaged 45.1 and 55.8 pounds heavier, respectively, than the calves from cows and heifers fed no mineral supplement (Wyoming Agric Experiment Station, 1945).

DuToit and Bisschop (1929) showed that by continuous bone meal supplementation, 2.5 year old oxen had a 30% weight advantage over their controls while bone meal supplemented cows weighed 20% more and produced more calves.

In an experiment carried out by the Wyoming Agric Experimental Station (1945), there was an advantage of slightly less than six pounds in favour of

one group for the first year, but by the end of the second year the cows and heifers which were fed bone meal had gained 26.4 pounds, or almost 17 per cent, more than the group which received no mineral supplement, however the means were not statistically significant.

1.1.6.3. EFFECT ON REPRODUCTIVE PERFORMANCE

The use of bone meal in improving reproduction in farm animals was noticed by many researchers. Theiler *et al,* (1928) used bone meal in cattle and showed a marked increase in conception rates of 80% compared to 51% in the controls. Other researchers have also reported an improvement in age at sexual maturity, pregnancy rates, calving percentage and regular occurrence of estrum (DuToit and Bisschop, 1929; cited by McDowell, 1985; Betteridge, 1989).

From the standpoint of reproduction, the cows and heifers which were fed bone meal were superior in practically every respect. Each year they weaned a larger percentage of calves, fewer cows failed to settle to service, fewer cows lost their calves, and there were fewer cases of calving difficulty. Twenty per cent more of the yearling heifers fed bone meal raised calves to weaning age than the heifers of the same age fed no mineral supplements (Wyoming Agric Experimental Station, 1945).

1.1.7.4. EFFECT ON CONCENTRATION OF PHOSPHORUS AND CALCIUM IN THE BLOOD

In research carried out in the United States, blood analyses at different intervals during the course of the experiment showed that the cows fed bone meal had appreciably higher levels of blood P than those fed no mineral supplement. While this was true, the cows and heifers which were fed no

bone meal showed no deficiency of P in the blood until the end of the first year. At this time the mean concentration of inorganic P was 3.8 mg%, compared with 4.3 mg% for cows and heifers which were fed bone meal (Wyoming Exper. Station,1945). They further found that the cows fed bone meal showed a somewhat higher concentrations of calcium in blood, but, as a whole the difference was non-significant. Therefore feeding bone meal had no significant effect upon the level of calcium in the blood.

1.2. DIETARY IONS

Samples of blood plasma showed that cows consuming an anionic diet maintained adequate blood Ca and P throughout parturition whereas the blood of cows consuming a cationic diet decreased in these minerals around calving (Block, 1984). While assigning cows to two different diets, one being cationic and one being anionic for 2 years, Block (1984) found that cows consuming the anionic diets had no milk fever, but cows consuming the cationic diet had a 47.4 % incidence of milk fever.

1.2.1. Calcium and Phosphorus as ions

In a study carried out by Braithwaite (1975), apparent P absorption was directly related to P intake and maximum P absorption was also higher for the young animals. Calcium retention was directly related to calcium absorption. Calcium and Phosphorus were retained in a constant ratio of 1.25:1 and the results suggested that P retention was controlled by the rate of calcium retention.

A wide dietary Ca:P ratio had no apparent effect on P absorption when P intake was adequate. The availability of P was lowered by a diet deficient in P with a wide Ca:P ratio (Young *et al.* 1966). Wise *et al.* (1962) noted that

serum Mg levels were decreased with high levels of dietary P but when dietary Ca levèls resulted in a "normal" serum Mg level even at the highest P level.

1.2.2. SULPHATE ION

The role of the sulphate radical as an anion was recognised by Du Toit *et al,* (1930) when they reported that magnesium hydroxide bound calcium in the tissues while magnesium sulphate favoured its elimination. A high level of magnesium sulphate in the diet was responsible for a delay in the development of the typical signs of P deficiency (Eckles *et al,* 1932). Eckles *et al,* (1932) further demonstrated that when the magnesium sulphate was withdrawn from a diet low in P there was a rapid deterioration in the condition of the animals and a dramatic exhibition of pica.

1.2.3. ACIDIC DIETS

The important role of the acidiogenic agents in improving P uptake have been reviewed by many researchers (Eckles *et al,*1932; Beighle *et al,*1997). In evaluating the acute effect of the acidogenic diet of -11.1meq/100g of diet dry matter, compared with a basiogenic diet of +25.6 meq/100g or a control diet of +16.5 meq/100g of diet dry matter on blood, bone and faecal P, Ca and Mg for a period of 9 weeks. Beighle *et al,* (1997) found a relationship between blood and bone Ca existing, in which there was resorption from bone with increased blood Ca in responses to the anionic diet. They found that the anionic treatment group demonstrated simultaneous increases in bone, blood and faecal P concentrations at various stages of the experiment compared to the cationic and control treatment groups (Beighle *et al,* 1997).

CHAPTER 2

2. IMPORTANCE OF PHOSPHORUS

2.1. GRAZING LIVESTOCK

Importance of P in improving the animals mineral status

Phosphorus, like calcium is a major component of bones. In addition, it is involved in numerous metabolic pathways. Because it has such a wide range of functions, deficiencies in phosphorus tends to cause general disabilities and failure to perform. Much of the phosphorus in plants is bound up in phytases which are only digestible by ruminants. In contrast to sodium and calcium, phosphorus is very expensive to include in rations (Chamberlain and Wilkinson, 1996).

As NRC (1996) pointed out, approximately 80% of phosphorus is found in bones and teeth and the remainder is distributed in soft tissues. Phosphorus functions also in cell differentiation and growth as a component of DNA and RNA, energy utilisation and transfer as a component of ATP, ADP and AMP, phospholipid formation and maintenance of acid-base and osmotic balance. Again phosphorus is required by ruminant micro-organisms for their growth and cellular metabolism in the rumen.

About 21 years ago, Underwood (1981) found that P deficiency resulted in reduced growth and feed efficiency, decreased appetite, impaired reproduction, reduced milk production and weak fragile bones. These are the same problems encountered in the North West Province. The skeleton provides a large reserve of phosphorus that can be drawn on during periods of inadequate phosphorus intake in mature animals. Skeletal reserves can

subsequently be replaced during periods when phosphorus intake is high. The P content of dry fat free rib-bone was found to be greater during the season of active pasture growth (summer) than at other times (Cohen, 1972).

2.2. INCREASED DEMANDS FOR PHOSPHORUS

Although lack of sufficient energy and protein is often responsible for sub-optimum livestock production, it has been shown that animals deteriorate in spite of an abundant feed supply when deficient in minerals (Davis, 1951). Cattle grazing on forages in severe phosphorus, cobalt or copper deficient areas are even more limited by lack of these elements than by lack of either energy or protein (McDowell *et al,* 1984). The mineral requirements in livestock are affected by many factors, including level of production, age, chemical form of the element, interrelation with other nutrients, breed and adaptation. However, mineral requirements are highly dependent on the level of productivity (ARC, 1980).

Both low and excessive amounts of minerals disturb the body's physiological processes and may lead to illness or death. The animal will show toxic symptoms after ingesting excess minerals over a long time (McDonald *et al,* 1988). The presence of low mineral levels in blood will also show deficiency symptoms specific to those mineral elements.

McDowell, (1992) indicated that parasitism by *Ostertagia* species affected absorption and utilisation of P in young calves and generally, diseases or parasites affecting the gastrointestinal tract reduces intestinal absorption of minerals especially if such disease causes diarrhoea or vomiting (McDowell, 1992).

In 1992, McDowell reported phosphorus deficiency as being the most prevalent minèral deficiency throughout the world. Studies in South Africa and Texas on cattle that grazed forages low in phosphorus, showed large improvements in fertility and calf weaning weights with phosphorus supplementation (Dunn and Moss, 1992). Du Toit *et al,* (1940) while analysing the grasses and bushes of South Africa for various minerals showed that the herbage declined in nutritive value during the dry season especially in energy, protein, carotene, P and other elements. The dry season caused less forage and thus less minerals to be consumed by the grazing ruminant. It was the extreme deficiency of P in South African soils and grasses and the wide spread incidence of botulism caused by cattle eating remains of dead animals which first stimulated interest in P supplementation in South Africa.

2.3. IMPROVING SUPPLEMENTATION

In experiments by Theiler *et al,* (1924) it was indicated that grazing cattle supplemented with bone meal gained more weight throughout the 15 months of the study than the controls not given the supplement. However, during the winter season, both groups lost weight but the animals receiving bone meal maintained the advantage gained in summer and spring and were 45.5 kg heavier than the control, indicating the significance of winter supplementation.

Van Schalkwyk and Lombard (1969) noted that winter supplementation of steers was advantageous because they outgrew the controls, indicating that P supplementation promoted the growth of young steers in both winter and summer seasons. They also found that during the subsequent 3 months, when the winter group received no supplementation, it was 35% heavier

than the controls, showing the need of animals to build P reserves in winter which may be necessary during the period of growth.

McDowell, (1992) suggested the contrary in that during the winter or dry season when forages stop growing and become high in fibre and lignin, that mineral intake and supplementation were necessary to counteract low mineral availability from forage sources.

CHAPTER 3

3. SOURCES OF PHOSPHORUS TO THE ANIMAL

3.1. INDIRECT SOURCE

3.1.1. SOIL

Sources of mineral elements found in plants and most naturally occurring mineral deficiencies in livestock are associated with the specific regions directly related to both soil mineral concentration and soil characteristics (McDowell, 1985). Plants take in only a small fraction of the total mineral concentration in soils and the "availability" of these minerals in soils depends upon their effective concentration in the soil solution. Most of the South African grasses are phosphorus deficient due to poor P in the soil (0.0005-0.002% Phosphoric oxide) (Theiler *et al,* 1927).

High Ca concentration in the grasses and concurrent low P levels lead to a high Ca:P ratio which may cause a reduction in the P absorption (ARC, 1980). McDowell et al, (1987) postulated that greater responses to P supplements can be brought about by provision of a more favourable Ca:P ratio. In the presence of excessively wide ratio, calcium binds with the low level P to form an insoluble form to be voided unutilised. For this reason regulations usually call for a Ca:P ratio not wider than 2:1 (Groenewald and Boyazoglu, 1980).

Alkalinic geological formations are more abundant in most trace elements than the older, more acidic, coarse, sandy formations. The soil drainage and pH also influence the concentrations of minerals in the soils. Thus the effect

of P fertilisation on plant mineral uptake is dependent to a considerable degree on the properties of soils (McDowell, 1985).

3.2. DIRECT SOURCE

3.2.1 PLANT PHOSPHORUS

It is accepted that legumes and herbs are richer in a number of mineral elements than are grasses (McDowell, 1985). Various herbs may contain higher concentrations of certain trace elements than do legumes, due to their deep root system.

Dutoit *et al,* (1930) reported on differences in mineral levels between grass and bush pastures. They indicated that grasses showed fair figures for P and protein during the period of active growth associated with the rainy season. These values quickly dropped to extremely low levels after the cessation of the rains into the cold winter months.

Hemingway (1967) reported the presence of more P in legumes than grasses grown on soils with adequate levels of available P. Legumes contained between 0.35-0.4 % P as compared to grasses, 0.2-0.25 % P. It has also been shown that cereals contain less Ca than P and roughages on the other hand have more Ca than P content (Groenwald and Boyazoglu, 1980).

Theiler *et al,* (1927) showed that very young green grass generally contained sufficient P (0.5%) to prevent osteophagia but for a short time because this was soon followed by summer rains which brought relatively abundant, rapidly growing grass of falling P content due to lignification. By the time plants lost their seeds, the P content had fallen to 0.08 % and more

than 80% of the cattle manifested osteophagia throughout the long rainless autumn and winter months.

3.2.1.1 EFFECT OF CLIMATE AND SEASONAL VARIATION ON PLANT PHOSPHORUS

The growth of plants is to some extent influenced by the climate and seasonal variation. When working on phosphorus deficient herbage of the veld, Theiler *et al,* (1924) found that the P decreased steadily from November onwards. In November (Spring) when the grass is young and green the percentage was 0.6 % as compared to 0.09 % in June (Winter) of the next year. As the grass matures carbohydrate formation in the grass proceeds so much faster than P absorption from the soil that the percentage of phosphoric acid rapidly falls. The low P in the soil being complicated by drought and the osteophagia or craving for bone shown by many cattle was attributable to this low P content in herbage.

3.2.2. WATER PHOSPHORUS

Normally, water is not a major source of minerals. Nevertheless, although highly variable, all mineral elements essential as dietary nutrients occur to some extent in drinking water (McDowell et al, 1985). However, their concentration is quite inadequate for meeting daily dietary requirements. Ions mostly present in highly saline waters are Na, Cl, Ca, So_4, HCO_3 (NRC, 1974; cited by McDowell, 1985). It has been reported that naturally high salt concentration in drinking water for cattle decreases mineral supplement intakes (McDowell, 1992). Livestock have a natural craving for salt but if this desire is fulfilled from drinking water high in salt, grazing livestock will consume less or none at all of a free choice mineral lick mixture based on salt (McDowell, 1992).

3.3. PHOSPHORUS SUPPLEMENTS AND SUPPLEMENTATION

Van Schalkwyk and Lombard (1969) noted that the winter supplementation of steers was advantageous because they outgrew the controls, indicating that P supplementation promoted the growth of young steers in both winter and summer seasons. They also found that during the subsequent 3 months, when the winter group received no supplementation it was 35% heavier than the controls, showing the need of animals to build P reserves in winter which may be necessary during the period of growth.

Cohen (1972), When experimenting with beef cattle found that P supplements did not increase weight gains neither in summer nor in winter months when the steers were kept on free range or penned. He attributed the lack of response to be due to the inability of the P supplement to stimulate increased feed intake, dry matter digestibility or metabolic efficiency as previously noted by Playne (1969).

Cohen (1972) came to the conclusion that neither summer nor winter P supplementation may improve liveweight performance when beef cattle graze pasture low in protein. However, Cohen (1973a) observed that during the winter months when grazing cattle lose weight, supplemented steers maintained their blood inorganic P within the normal accepted range of adequacy (4-8 mg/100 ml). Cohen (1973b) therefore, postulated that P supplementation in winter months is essential to maintain adequate blood inorganic P that would otherwise be drawn from muscles as a result of catabolism and also from bone.

Finally Cohen (1973b) showed that it was during the season of active pasture growth (summer) that the P content of dry-fat-free bone was greater

and there was need therefore to maintain adequate skeletal reserves in winter by supplementation. Thus, although P supplementation in winter may have no effect on live weight of grazing range cattle, because of other complicating factors like low diet protein (Cohen, 1972), its effect on mineralization of bone tissue may justify its use, particularly in areas where poor bone development and lameness occur, to maintain normal bone homeostasis.

CHAPTER 4

4. PHOSPHORUS DEFICIENCIES

Both low and excessive amounts of minerals disturb the body's physiological processes and may lead to illness or death and the animal will show toxic symptoms after being exposed to excess minerals over a long time (McDonald *et al,* 1988). Subminimum or low mineral levels in blood will also show deficiency symptoms specific to those mineral elements.

4.1. FEED CONSUMPTION

In a research conducted by Benzie *et al,* (1957) using sixty cheviot ewes of three and a half years, the basal ration was designed to provide between 1.0 and 1.5g of P daily and was converted to a ration of moderate P content by adding 3.0g of P (as disodium hydrogen phosphate) daily. The mean daily P intake from early gestation to mid-lactation was 1.3-1.4 g on the low-P ration and 4.2-4.5 g on the moderate P ration, and from mid-lactation to mid-dry period 1.5g on the low P ration and 4.4 - 4.5g on the moderate P ration (Benzie *et al,* 1957).

The liveweights of all animals decreased considerably during late pregnancy and early lactation, the decrease in ewes on the low phosphorus ration being significantly greater than the decrease in ewes on the moderate P ration, but between mid-lactation and the end of the experiment, ewes fed on the low P ration to mid-lactation and moderate-P thereafter gained significantly more weight than those fed the low P ration throughout (Benzie *et al,* 1957).

Wise *et al,* (1958) demonstrated that in young calves with an initial weight of 96 kg, 0.222% P was adequate for maximum weight gains, but increasing

P to 0.30% increased bone ash. Call *et al,* (1978) fed Hereford heifers (168 kg initial weight), beginning at approximately 7 months of age, diets contain 0.14 or 0.36% P for two years. No differences between the two groups were detected in growth, rib bone morphology and P content, age at puberty, conception rate, or calving interval.

The second study used Hereford heifers fed low P and received 6 - 12.1g/P/day, while the controls received 20.6 to 38.1g/P/day with phosphorus intake increased as the animals grew larger. Females with low phosphorus intake remained healthy and growth and reproduction were similar to those observed in P supplemented animals. When phosphorus intake of 6-12.1g/P/day was reduced to 5.1 to 6.6g/p/day, clinical signs of deficiency occurred within 6 months (Call *et al*, 1986). Reproduction was not impaired until cows were fed the very low phosphorus for more than one year (Call *et al,* 1986).

4.2. REPRODUCTION

Phosphorus deficiency has been claimed to have a special role in reducing fertility in livestock (Theiler *et al,* 1928). However there is no information to support this claim in that there is no evidence of impaired reproduction due to phosphorus deficiency in forage diet that is not accompanied by a general state of undernutrition or by a deficiency of energy, protein, or some other nutrient (Underwood, 1981).

4.3. LACTATION

It is accepted that cattle with a high milk yield are potentially more liable to develop signs of aphosphorosis than dry cows or those producing less milk. Theiler *et al,* (1924) demonstrated a 40% increase in milk production in five cows receiving bone meal as compared to a similar control group on veld grazing only, while McDowell (1985) reported an increase of up to 24% milk production in P deficient farms where bone meal was supplemented.

Cows fed adequate P (98% of requirements) yielded 1.8 kg/day more milk than those on high P diet (Carstairs et al, 1979). Beighle (1999) reported that cortical bone Ca and Mg values decrease as milk production increases up to 20 kg/day.

Braithwaite (1985) found bone mineral stores that were mobilised in late pregnancy and early lactation were replaced in mid to late lactation in ewes given the plentiful Ca and P intake but not in the ewes given a restricted intake. Bone mineral stores of Ca and P being lost in the normal way during late pregnancy and early lactation but were not replaced, as normal in mid to late lactation. He found the ewes still in deficit of 125g P and 100g Ca at the end of the lactation.

CHAPTER 5

5. HOMEOSTASIS

5.1. SULPHUR ANION

The role of the sulphate radical as an anion was recognised by Du Toit *et al,* (1930) when they reported that magnesium hydroxide bound Ca in the tissues while magnesium sulphate favoured its elimination. A high level of magnesium sulphate in the diet was responsible for a delay in the development of the typical signs of P deficiency (Eckles *et al,* 1932). Eckles *et al,* (1932) further demonstrated that when the magnesium sulphate was withdrawn from a diet low in P there was a rapid deterioration in the condition of the animals and a dramatic exhibition of pica.

5.2. ACIDIC DIETS

Block (1984) suggested that in addition to intestinal effects of a diet balanced for excess anions, there must be a systemic response by 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 investigation.

In evaluating the acute effect of the acidiogenic diet of -11.1meq/100g of diet dry matter, compared with a basiogenic diet of +25.6 meq/100g or a control diet of +16.5 meq/100g of diet dry matter on blood, bone and faecal

P, Ca and Mg for a period of 9 weeks, Beighle *et al,* (1997) found a relationship between blood and bone Ca existing, in which there was resorption from bone with increased blood Ca in responses to the anionic diet. They also found that the anionic treatment group demonstrated simultaneous increases in bone, blood and faecal P concentrations of various stages of the experiment compared to the cationic and control treatment groups (Beighle *et al,* 1997).

5.3. PHOSPHORUS ABSORPTION

5.3.1. INCREASED ABSORPTION

In their estimate of requirements, AFRC (TCORN, 1991) assumed an absorption co-efficience of 64% for phosphorus in forages and 70% for phosphorus in concentrates. The efficiency of absorption of dietary P remained high and fairly constant throughout the whole experimental period. The rate of absorption varied in direct relation to the P intake. Thus, absorption tended to be high at peak lactation but low in early pregnancy and the dry period (Braithwaite, 1985).

In a feeding trial done by Breves (1982) that was performed using 4 sheep on a diet that was low in P to examine the effect of an insufficient P supply on the activity of rumen metabolism, for P repletion, the animal received abomasal P infusions for control of the P status when blood samples were taken. The observed elevation of plasma calcium in P deficiency should therefore have been the effect of increased absorption from the intestines or increased mobilisation from bone.

Braithwaite (1985) found apparent P absorption being directly related to P intake and maximum P absorption also being higher for the young animals.

5.3.2. SITE OF ABSORPTION

Excess P may interfere with feed digestibility, metabolism at the tissue level, or unknown factors (Carstairs, 1979). The concentration of soluble P in digesta decreased until the 7 meter site in the small interstine and then remained stable. A very low concentration of soluble P was found in the faeces. The upper small intestine (1-3 m from the pylorus) appeared to be the major site of calcium and phosphorus absorption. A large net secretion of P occurred between the mouth and the duodenum. Net absorption of P occurred 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 (Ben-Ghedalia *et al,* 1975).

5.3.3. PHOSPHORUS SECRETION AND EXCHANGE INSIDE THE BODY

Calcium and phosphorus are lost from the body by excretion into the intestine and in the urine. Losses into the intestine occur largely in digestive juices and although the excreted Ca and P might be expected to mix with dietary Ca and P and be reabsorbed at the same efficiency, a lack of correlation between endogenous Ca in the faeces and the Ca intake or total faecal Ca suggest that some discrimination occurs (Braithwaite, 1976).

The rate of excretion of endogenous faecal P is directly related to P intake and rate of absorption (Young *et al,* 1966) 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 concentration (Preston, 1964) and that it plays an important role in P homeostasis.

Maternal demands for Ca and P are increased during pregnancy and lactation as a result of the additional requirements for the foetus and for milk. Neither the Ca nor the P of the foetus is exchangeable with that of the mother and transfer across the placenta is virtually a one-way process (Braithwaite *et al,* 1975)

Faecal excretion of P per unit of feed eaten was non significantly affected by the phosphorus content of the feed eaten, nor by the level of feed intake. Average value was 0.059g of organic phosphorus per 100g of dry matter eaten. Most of the remainder of the P being excreted is in the inorganic form in the faeces (Barrow and Lambourne, 1962). They further stated that the proportion of the faecal phosphorus in the inorganic form depends on the P content of the feed but these proportions also depend on the levels of feed intake, especially if this was so low that the animal was in negative balance.

5.4. BONE HOMEOSTASIS

Hormones play a major role in regulation of phosphorus and calcium homeostasis. Breves (1982) in analysing the plasma inorganic P and Ca concentrations in sheep with experimental P deficiency found Parathyroid hormone (PTH) and dihydroxycholecalciferol [1,25-$(OH)_2$-D_3] playing a major role in the regulation of P and Ca homeostasis.

A combination of a mineral balance and a radioisotope technique has been used to study the relationship between dose rate of 1-∝-hydoxycholecalciferol (1-∝-OH-D3) and the magnitude and the duration of its effect on the various processes of Ca and P metabolism in adult weather sheep. The rates of absorption and retention of P were increased by treatment and maximum responses occurred at the lowest dose rate (Braithwaite, 1975). He further found 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 20kg/day. However, the cortical bone mineral values in animals producing over 20kg/day were greater and cortical bone thickness was lower compared to those animals producing less than 20 kg.

Histological analysis of bones of lambs fed on diets low in P and adequate in Ca or adequate in both showed the presence of lesions characteristic of late rickets in some sheep and of severe osteoporosis in others (Young *et al,* 1966).

5.4.1. PHOSPHORUS MOBILISATION FROM BONE

In comparing P metabolism throughout pregnancy and lactation in ewes fed according to the ARC (1980) recommendations and in ewes fed a plentiful supply of dietary Ca and P, Braithwaite (1983) found that bone mineral stores were mobilised in late pregnancy and early lactation irrespective of the rate of P absorption. He further postulated that these changes in bone stores occurred as a result of changes in Ca requirements rather than in P requirements. On the other hand Benzie *et al,* (1957) found a greater amount of bone repair taking place with a low-P than with a low-Ca ration. The values obtained to prove this were the increase in ash weight being 259g on low-P diet compared to 71 on low-Ca diet.

Phosphorus was mobilised from the bone and soft tissues in late pregnancy and early lactation, when the rate of resorption of P was increased to a high level compared with the rate of accretion (Benzie *et al,* 1957).

5.4.2. BONE RESORPTION

The concentrations of Hydroxyproline excreted in the urine have been shown to be a valuable index of bone matrix metabolism. Black and Carpen (1971) while studying urinary and plasma Hydroxyproline during pregnancy, parturition and lactation in cows with parturient hypocalcemia, found the results suggesting that bone resorption increases rapidly post-partum in cows since hydroxyproline values were greater 3 days following parturition than at weekly intervals for a month post-partum. But Beighle (1999) found no statistical significancy in cortical bone P concentrations in the rib bone during the lactation period, but calcium concentration 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 resorbs cortical bone during the middle of the lactation period and not during the peri-parturient period as previously thought (Beighle, 1999).

When blood P values were low the skeleton was severely resorbed, but the converse relationship was not always true (Benzie *et al*, 1957). Resorption of the skeletons of ewes fed on a low P ration was much greater than resorption of the skeletons of ewes fed on a low-calcium ration. The quantity of bone resorbed depends upon 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 (Benzie, 1957).

Resorption of the skeleton during pregnancy and lactation occurs also as a result of a low P intake and skeletal reserves are replenished when demands for P fall in late lactation (Benzie *et al,* 1959).

5.5. CATION:ANION BALANCES IN THE RUMINANT

5.5.1. CALCIUM

Block (1984) suggested that acid forming elements in the intestine allow for greater Ca solubility and earlier absorption. If the passive absorption of Ca is increased by excess anions via reduction in intestinal pH, then active absorption of Ca should decrease throughout the Parathyroid hormone (PTH) and 1 hydroxylase systems. As this system becomes inactive, bone resorption should decrease and plasma Hydroxyproline (OHPRO) should remain low. In his study, Block (1984) found that OHPRO increased in the anion group as parturition approached, indicating that bone was responsive. In the cation group, lower plasma Ca should have stimulated the PTH and 1-Hydroxylase systems to increase intestinal absorption of Ca and bone resorption.

A high positive dietary alkalinity reduced Ca absorption from the intestine and resulted in hypocalcemia, whereas a negative value apparently brought about the increased absorption needed to maintain a normal calcium balance (Dishington, 1975). Braithwaite (1975) studied the effect of absorption and retention of Ca and P on young and mature Ca deficient sheep and found that Ca retention was directly related to Ca absorption.

5.5.2. MILK FEVER

Milk fever (parturient paresis) is a metabolic disorder that occurs in aged dairy cows at parturition. Although the exact cause of milk fever is not known, there is an inter-play of the calcium, phosphorus, magnesium and vitamin D homeostatic mechanism which fails to maintain the concentration of calcium in plasma in the face of a sudden drain of calcium at the initiation of lactation (Braithwaite, 1976). Hypocalcaemic fetary results, and if not treated, 60 to 70% of affected cows die.

Dishington (1975), while assigning 14 cows to a basic diet supplemented with Na_2CO_3 during 4 weeks pre-partum and one week post partum found 12 of the 14 cows being susceptible to milk fever (hypocalcaemia paresis puerperalis), while 1 cow of the 14 cows, receiving the same basic diet supplemented with sulfates and chlorides, remained healthy. A mixture of $CaCl_2$, $Al_2(SO_4)_2$ and $MgSO_4$ was found to be a convenient prophylactic supplement.

While assigning cows randomly 45 days prepartum to one of the two diets, one diet contained an excess of anions, and the other one containing an excess of cations, Block (1984) found cows consuming the anionic diet had no milk fever, but cows consuming the cationic diets had a 47.4 % incidence of milk fever.

5.5.3. PLASMA RESPONCES

Suggestions have been made (Dishington, 1975) that diets containing acid forming ions cause greater Ca absorption in cows via decreasing interstinal

pH. But Block's (1984) trial indicates that blood of cows respond to anion-cation balanced diets as shown by the response of plasma hydroxyproline (OHPRO) by cows offered the anionic diets and lack of response by cows offered the cationic diets. It appears that the anion (acidiogenic) diet allowed for easier bone mobilization during Ca stress even though diets contained a high ratio of Ca:P (Block, 1984).

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

Anionic diets were also responsible for higher average daily gains, and higher concentration of serum P when compared to cationic diets. This was accomplished by the results reported by Beighle *et al*, (1988) that in his experiment, 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 as compared to the cationic diets.

Beighle *et al*, (1988), further observed 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.

5.5.4. INCREASED BONE MOBILIZATION

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 also occurs as a result of a low P intake and skeletal reserves, but are replenished when demands for P fall in late lactation (Benzie *et al*, 1959).

Only a small fraction of P and Ca of bone is rapidly exchangeable with the ionic Ca and P of blood and soft tissues. The size of the exchangeable Ca pool of sheep decreases markedly with increasing age and appears to be related to the rate of bone accretion (Braithwaite and Riazuddin, 1971). The exchangeable pool of P is utilized in times of need, and is depleated in sheep during periods of P deficiency (Young *et al,* 1966).

Low-serum Ca or blood P values are generally indicative of reduced skeletal reserves, but normal or near normal values are not always a reliable guide to the mineral status of the skeleton. When blood P values were low the skeleton was severely resorbed, but the converse relationship was not always true (Benzie *et al*, 1957).

Benzie *et al,* (1957), while experimenting on ewes fed low P, found that resorption was very severe, corresponding to a loss of 40% of skeletal ash, and involving the shafts of long bones as well as other parts of the skeleton. He also stated that the distribution of resorption throughout the skeleton is independent of the agent responsible for resorption.

5.5.5. ABSORPTION

Phosphorus is absorbed by young growing animals from both milk and forage-containing diets (Young *et al,* 1966) with a high availability (80 - 100%), but the availability is much lower (50 - 60%) in adult animals (ARC, 1965). Although inorganic P and phytate P are equally well absorbed, there is evidence that the organic P is less well retained and may become less available as the dietary Ca:P ratio is increased (Cohen,1974).

It has been suggested (Young *et al,* 1966) that the difference in ability of ruminants and non-ruminants to tolerate wide ratios of Ca to P is related to the pH at the site of absorption in the upper small intestine. Since the pH at this site is lower in the sheep than in man, rat, or pig, precipitation as insoluble tricalcium phosphate will presumably be decreased and the Ca and P availability increased.

CHAPTER 6

6. MATERIAL AND METHODS

6.1. OBJECTIVE

This experiment was aimed at finding a cheap, easily accessible, consumable lick for range cattle to be used by the small-scale farmers (who cannot afford expensive supplements) that will increase and improve the production.

6.2. PROCEDURES

6.2.1. HOUSING AND DIETS

Sixteen Bonsmara calves (males = 4; females = 12) were blocked according to sex, and randomly assigned to one of two groups, a group of eight for the first cluster (The 'A group'), and the other eight in another kraal (The 'B group'). The animals were confined in steel concrete floored experimental kraals measuring 15m by 15m, wide enough to allow freedom of movement, and the shade being provided. The feeding troughs were built with bricks and cement plastered. Water troughs were also built-in and automatic water pipes installed to allow regular flow of water.

Roughage was given *ad libitum* and was composed of 50% Cenchrus Ciliaris and 50% Medicago Sativa with separate troughs been provided for 50% bone meal mixed with 50% salt for the control animals, and the other feeding trough with bone meal and ammonium sulphate at 18%, 15%, 10%, 5%, 2.5% and 1.25% NH_4SO_4 for the experimental animals in the other kraal. Licks were fed once daily with weigh backs recorded each day.

6.2.2. COLLECTION OF SAMPLES

All samples (i.e. bone, blood and faeces) were taken on the same day to prevent variation between sampling periods and mineral contents. Calves were weighed on all sampling days and the lick weighbacks were recorded to note how much lick the animals consumed. Calves were also fed roughage ad lib.

6.2.2.1. BLOOD

Blood samples were taken at the beginning of the trial before the animals were given the lick and throughout the experiment at 10 day intervals. Calves were bled from the jugular vein after restraining to minimize variation in blood levels of minerals especially P due to handling stress. Anticoagulant free red stoppered vacutainer tubes were used when collecting blood and it was stored for 24 hours at a temperature of 4°C to allow clottting. The blood was centrifuged at 1000 rpm for 10 minutes and serum removed, and stored in clean plastic tubes frozen immediately at a temperature of –20 °C.

6.2.2.2. FAECES

Faecal samples were collected directly from the rectum and without any contamination from any surroundings, dried in the sun for two days and stored in clean plastic jars for later analysis.

6.2.2.3. BONE

Bone samples were taken by using a trephine to remove a ten millimetre circular core of bone which consisted of cortical and some trabecular bone.

A local anaesthic 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 scapel blade and a ±3 cm incision made in the skin over the bone 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 and a slow circular back and forth motion of the trephine was done until it could be felt that the trephine was well seated into the bone. With proper movement of the trephine the core was collected from the rib.

The muscles were then closed over the site using no. 1 chromic gut. The skin incision was closed using heavy vetafil.

WEEK	RIB NUMBER	LOCATION	SITE	RIB ABBREVIATION
0	9th	MIDDLE	LEFT	L9M
1	10th	MIDDLE	LEFT	L10M
2	11th	MIDDLE	LEFT	L11M
3	12th	MIDDLE	LEFT	L12M
4	9th	MIDDLE	RIGHT	R9M
5	10th	MIDDLE	RIGHT	R10M
6	11th	MIDDLE	RIGHT	R11M

Bone biopsies were taken as outlined below:

At the beginning of the trial, week 0, a biopsy was taken from the middle location of the left 9th (L9M) rib. At the end of week 1 a biopsy was taken from the middle location of the left 10th (L10M) rib, at the end of week 2 a biopsy was taken from the middle location of the left 11th (L11M) rib, at the end of week 3 a biopsy was taken from the middle location of the left 12th (L12M) rib.

At the end of week 4 a biopsy was taken from the middle location of the right 9th (R9M) rib, at the end of week 5 a biopsy was taken from the

middle location of the right 10th (R10M) rib and at the end of week 6 a biopsy was taken from the middle location of the right 11th (R11M) rib.

6.2.3. PREPARATION OF LABORATORY EQUIPMENT

All glassware laboratory equipment (crucibles, red-stoppered tubes, volumetric flasks, glass beakers, cylinders, pippets) were soaked in a 36 % pure HCl over night, rinsed with distilled water thrice and dried in a hot oven for 16 hours at a temperature of 60°C. Plastic containers and tubes were only rinsed thrice with distilled water and allowed to air dry. Rib biopsy equipment was washed and sterilised in an autoclave for 15 minutes and dried.

6.2.4. PREPARATION OF SAMPLES FOR ANALYSIS

6.2.4.1. BLOOD

Serum in the anticoagulant free red stoppered tubes was aspirated using pasteur pippetes. To precipitate the protein in serum, 0.7 ml of serum was added to 6.65 ml of stock Trichloracetic acid in clean test tubes which were covered, shaken individually on an electric stirrer and left to stand for 10 minutes. The samples were centrifuged at 2500 rpm for 10 minutes. From each sample 5 ml of the supernatant fluid was transfered to clean test tubes without unsettling the centrifuged material at the bottom. The sample solution was then mixed with 1.5 ml each of ammonium molybdate, hydroquinone and sodium sulphite and allowed to stand at room temperatue for 40 minutes and poured into the cuvettes and analysed.

6.2.4.2. FAECES

Faecal samples were collected into aluminium plates directly from the rectum and immediately dried by exposure to sunlight. Most of the samples were completely dry within a week. Once the faecal material was dry it was ground through a 2mm screen after which 1gram duplicate samples were weighed in dried, acid cleaned crucibles and placed in an oven at 106°C for 16 hours. The crucibles containing the dried faeces were then cooled in desiccators for 2 hours and then weighed to estimate the weight of oven dried faeces. The crucibles were then placed in a muffle furnace for ashing at 800°C for 16 hours and then 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. 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 5N HCl were added to each crucible and evaporated on very low heat until about 3ml was left in the crucible. The solution was then transferred to a 100ml 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 to the bottom. Thirty ml were removed from the flask without disturbing the sediment at the bottom and stored in McCartney bottles for future analysis.

6.2.4.3. BONE

Small crucibles were removed from a drying oven and allowed to cool for six hours. Bone samples were divided into half to provide for duplicate samples. Empty crucibles were weighed (A) and the weight was recorded.

Halved sample of bone was added and recorded its weight. Empty crucible was weighed (B) and its weight was recorded. The rest of halved sample of bone left was added to 'B' and the weight was recorded. The difference between the weight of the empty crucible and the crucible containing the bone was recorded as the fresh weight, crucibles with samples were put in a drying oven overnight, removed and allowed to cool for six hours. Samples were weighed and recorded as dry weight. Samples were ashed overnight for 16 hours at 600°C, removed and cooled in a desiccator, weighed and recorded as ash weight. Two milliliters of 5N Hydrochloric Acid was added to each crucible and the bone allowed to dissolve. The sample solution was then transfered to 100 ml volumetric flasks, mixed well and filled to the mark with distilled water. After the sediments had settled the supernatant fluid was transferred into McCartney bottle to be used in the analysis

6.2.5. METHODS OF ANALYSIS

SAMPLE PROCESSING

Samples included faecal, bone and blood were analysed through the Bran & Luebbe " Auto-Analyser II:Technicon Industrial Systems, Tarytown NY 10591.

Samples were analysed for P, Ca and Mg. All samples were analysed colorimetrically at 420 nm for P, 570 nm for Ca and 630 nm for Mg.

The quantitative analysis of concentrations of P, Ca and Mg in the unknown sample was obtained by relating to known references of standard solutions in mg%.

6.2.5.1. CALCIUM IN FEED SUPPLEMENTS

GENERAL DESCRIPTION

In this automated determination for calcium (Ca) in predigested calcium phosphate samples, the sample is buffered with potassium phosphate and 8-hydroxyquinoline is added to eliminate magnesium interference. Cresolphthalein was added and after the addition of diethylamine, a coloured complex was formed between calcium and cresolphthalein. The colored complex was then measured colorometrically at 570 nm.

The Auto-Analyser was operated at a net of 40 samples per hour, the sensitivity was done at 420 mg/l0.38 absorbance units, Coefficient of Variation at 240 mg/l0.36% and the detection limit at 4.2 mg/l

REAGENTS

All chemicals were ACS grade or equivalent.

LIST OF RAW MATERIALS

Hydrochloric Acid, conc. (HCL)

Potassium Phosphate, Monobasic (KH_2PO_4)

8-Hydroxyquinoline (C_9H_7NO)

Cresolphthalein Complexone (Metal Complexing Dye)

Diethylamine $(C_2H_5)_2NH$

Calcium Carbonate ($CaCO_3$)

Surfactants, Brij-35,* 30% solution (Technicon No. T21-0110)

Sodium Hydroxide, 50% solution (NaOH)

HYDROCHLORIC ACID, 1N

Releases the pròtein-bound Calcium

Preparation :

To 800 ml of distilled water, 82 ml of hydrochloric acid was carefully added. It was diluted to one liter with distilled water and mixed.

PHOSPHATE BUFFER, 1M

Preparation:

To 20ml of 1N hydrochloric acid, 2.5 g of 8-hydoxyquinoline was carefully added. Distilled water (800 ml) was added and the solution was mixed. Potassium Phosphate (136 g) was added and dissolved. It was diluted to one liter with distilled water, 1.0 ml of Brij-35 was added and mixed.

PHOSPHATE BUFFER, 0.2M

Preparation:

To about 800 ml of distilled water, 27.2 g Potassium Phosphate was added. It was diluted to volume with distilled water and 1.0 ml of Brij-35 was added and mixed.

CRESOLPHTHALEIN COMPLEXONE STOCK, 250 mg/l

Preparation :

Cresolphthalein complexone (250 mg) was dissolved in about 800 ml of distilled water. It was diluted to one liter with distilled water and mixed.

CRESOLPHTHALEIN COMPLEXONE WORKING, 0.2N HCL

Preparation :

To 200 ml of distilled water 16.5 ml of Hydrochloric Acid was added, 2.5 g of 8-hydoxyquinoline was also added and dissolved. Cresolphthalein complexone (100ml) was added and mixed. It was diluted to one liter with distilled water and mixed.

DIETHYLAMINE, 4%

Preparation:

Diethylamine (4.0 ml) was pipetted into a 100 ml volumetric flask and diluted to volume with distilled water. This solution was prepared daily.

PREPARATION OF CALCIUM STANDARDS

Stock standard 1000 ppm was used to make 50, 100,150,200 and 250 ppm calcium standards. To prepare 50 ppm standard, 5ml of stock standard (1000ppm) was mixed with 95ml of distilled water, 10ml stock standard with 90ml of distilled water to prepare 100 ppm, 15ml of stock standard with 85ml of distilled water to prepare 150ppm standard and 20ml of stock standard to 80ml of distilled water to prepare 200ppm standard.

SODIUM HYDROXIDE, 1N

Preparation :

About 80 g of sodium hydroxide was added to 800 ml of distilled water, mixed and diluted to one liter with distilled water.

SAMPLE PREPARATION

1. Digested sample (60 ml) was pipetted into a 100 ml beaker.
2. The pH was adjusted to about 3.0 with 50% sodium hydroxide. As the pH approaches 3.0, precipitation of iron and aluminium phosphate occured.

3. The pH adjustment was continued to 5.0 with 1N sodium hydroxide.

4. The entire sample was transferred, including the precipitate to a 100 ml volumetric flask and dilute to 100ml with distilled water.

5. The solution was filtered through a whatman GF/A glass fiber disc and was ready for analysis.

DESCRIPTION OF MANIFOLDS

Samples and standards were aspirated into the analytical cartridge where they are diluted with 1M phosphate buffer containing hydroxyquinoline. An aliquot of this dilution is resampled and further diluted with 0.2M phosphate buffer. This dilution is resampled and reacted first with cresolphthalein complexone reagent (with hydroxyquinoline added). Diethylamine is added for pH adjustment necessary for color development. The pink dye formed is measured in a colorimeter at 570 nm.

OPERATING NOTES

1. START-UP PROCEDURE

a. The levels of all reagents were checked to ensure an adequate supply.

b. The reagents lines were placed in their appropriate containers except the diethylamine (DEA).

c. The DEA line was placed in distilled water and the proportioning pump was started.

d. When the reagents have been pumping for at least 5 minutes, the DEA line was now placed in its container and the system was allowed to equilibrate.

2. SHUT-DOWN PROCEDURE

a. All the reagents lines were removed from their containers and placed in distilled water containing 1 ml/l of Brij-35.

b. The sample probe was removed from the wash receptacle and placed in the distilled water

c. After 15 minutes, the proportioning pump was stopped and the platen was removed.

6.2.5.2. INORGANIC PHOSPHORUS

TECHNICON AUTOANALYSER II INDUSTRIAL METHOD #339-04

HISTORY

The classical method of Bell and Doisy, cited by Bran+Luebbe "Auto-Analyser II: Technicon Industrial Method, for the determination of phosphate, based upon the reduction of phosphomolybdic acid by hydroquinone to molybdenum blue, has limitations because of an irregular and rapid fading of the colour in an alkaline solution.

Briggs (1922, 1924 cited by Bran+Luebbe "Auto-Analyser II: Technicon Industrial Method.) eliminated the problem by increasing the hydroquinone concentration and using an acidic molybdate reagent. However, this modification resulted in a brownish-yellow colour in the absence of phosphate which could be partially dissipated by the addition of sulfite.

Benedict and Theis (1924, cited by Bran+Luebbe "Auto-Analyser II: Technicon Industrial Method.) were able to intensify the blue colour of the reaction by incubation. However, this procedure is useful only where inorganic phosphate is present, as in serum or plasma filtrates. It cannot be

used on whole blood filtrates because any phosphate ester present may be hydrolysed by the heated acid and result in falsely evaluated values.

In 1925, Fiske and Subbarow (cited by Bran+Luebbe "Auto-Analyser II: Technicon Industrial Method.) pointed out that a small amount of hydroquinone is a slow reducing agent, but larger quantities generates a colour with molybdic acid. Ideally, the reducing agent should affect the complete reduction of phosphomolybdic acid within a reasonable period without generating a blank. Fiske and Subbarow (cited by Bran+Luebbe "Auto-Analyser II: Technicon Industrial Method.) suggested the use of 1,2,4-aminonaphtholsulfonic acid in lieu of hydroquinone. This procedure could be performed at room temperature and became popular for phosphate determination. Bartlett (1959, cited by Bran+Luebbe "Auto-Analyser II: Technicon Industrial Method.) modified the procedure by heating the reaction mixture at 100^0C for five minutes to intensify the blue color.

Kuttner and Cohen (1927, cited by Bran+Luebbe "Auto-Analyser II: Technicon Industrial Method.) revived the original suggestion of Deniges (1920, 1921; cited by Bran+Luebbe "Auto-Analyser II: Technicon Industrial Method.) which involved using stannous chloride as a reducing agent. Stannous chloride has an advantage over 1,2,4-aminonaphtholsulfonic acid in that the stock reagent is more stable. It also produces a more intense colour with phosphomolybdate, thereby permitting the measurement of smaller levels of phosphate. However, the working solution of stannous chloride tends to deteriorate, and fresh reagent must be prepared. Smith *et al.* (1939, cited by Bran+Luebbe "Auto-Analyser II: Technicon Industrial Method.) have reported that the oxidation of stannous chloride can be retarded by storing it in a hydrogen atmosphere.

Hurst (1964, cited by Bran+Luebbe "Auto-Analyser II: Technicon Industrial Method.) stabilised the stannous chloride in dilute solution by combining it with hydrazine sulphate. This modification permitted the rapid development of the molybdenum blue colour at room temperature, which was characteristic of the original stannous chloride method. As a result, phosphate levels as low as 1ug per liter can be determined accurately.

The inorganic phosphorus method used on the Technicon AutoAnalyser continuous-flow analytical instrument for the determination of inorganic P in serum is based upon the work of Hurst and Kraml (1964, cited by Bran+Luebbe "Auto-Analyser II: Technicon Industrial Method.).

In Technicon biochemical analyzers, the method has been further modified to replace the sulphuric acid in the ammonium molybdate reagent with hydrochloric acid. This modification has reduced carryover. The stannous chloride concentration also has been adjusted to insure linearity (Bran+Luebbe "Auto-Analyser II: Technicon Industrial Method).

EXPLANATION OF METHOD

The serum sample is added to an air segmented stream of 0.36N sulphuric acid and dialysed against an inorganic phosphorus recipient solution. The phosphate-containing dialysate is then mixed with an acid solution of ammonium molybdate to form phosphomolybdic acid. The stannous chloride-hydrazine reagent reduces the phosphomolybdic acid to molybdenum blue. The absorbance of the analytical stream is measured at 660 nm in a flowcell that has a 15-mm light path and an inside diameter of 1.5 mm (Bran+Luebbe "Auto-Analyser II: Technicon Industrial Method).

REAGENTS

AMMONIUM MOLYBDATE, 1% in 1.32N Hydrochloric Acid

(Technicon No. T01-0636)

PREPARATION:

Approximately 800 ml of distilled water was placed in a one liter volumetric flask. Ten grams of Ammonium molybdate was added and the solution was mixed until completely dissolved. To that solution, 108.9ml of concentrated hydrochloric acid was added and mixed thoroughly and diluted to one liter with distilled water.

INORGANIC PHOSPHORUS SAMPLE DILUENT , (DONOR STREAM- TOP OF BLOCK) (Technicon No. T01-0325)

Preparation :

To approximately 800 ml of distilled water, 10.1ml of Sulphuric Acid was slowly added and diluted to one litre with distilled water. One millilitre of Brij 35 was added and mixed thoroughly.

INORGANIC PHOSPHORUS RECIPIENT SOLUTION (0.36N) (Technicon No. T01-0330) (Stock)

Preparation

Approximately 800 ml of distilled water was placed in a one litre volumetric flask. Ten milliliters of concentrated sulphuric acid was added to the flask, mixed and diluted to volume with distilled water. One millilitre of Wetting Agent "A" was added and the solution was mixed thoroughly.

SULPHURIC ACID, 0.25N RECIPIENT SOLUTION (WORKING) TO BOTTOM OF BLOCK)

Preparation

Three hundred millilitres of distilled water was added to a one litre volumetric flask and filled to a one litre volume with stock sulphuric acid, 2

ml of Aerosol-22 was added and mixed. This reagent was prepared fresh every week.

STANNOUS CHLORIDE (STOCK)

Preparation:

Eight milliliters of concentrated hydrochloric acid was placed in a 10.0 ml volumetric flask and mixed with 400 mg of stannous chloride until completely dissolved and filled to volume with concentrated hydrochloric acid. The solution was refrigerated at a temperature of 4°C. The Stannous Chloride (Stock) when prepared and stored as directed, is stable for one week.

HYDRAZINE SULPHATE, 1% (Technicon No. T01-0327)

Preparation:

Approximately 800 ml of Distilled Water was placed in a one litre volumetric flask. Ten grams of Hydrazine Sulphate was added to 800 ml of Distilled Water and mixed until completely dissolved. It was diluted to volume with Distilled Water and mixed.

STANNOUS CHLORIDE-HYDRAZINE (WORKING)

Preparation :

Eighty milliliters of 1% hydrazine sulphate was placed in a 100 ml volumetric flask and 0.4 ml of stannous chloride (stock) was added and mixed. It was diluted to volume with 1% hydrazine sulphate and mixed. The Stannous Chloride-hydrazine was stored in a refrigerator between 4°C - 6°C. When prepared and stored as directed the stannous chloride-hydrazine (working) is stable for 48 hours.

6.2.5.3. NORMAL PHOSPHORUS (Range: 0-50ug/ml)

INDUSTRIAL METHOD No. 144-71A/PRELIMINARY

GENERAL DESCRIPTION

This automated procedure for the determination of phosphorus (P) utilizes the reaction between P and molybdovanadate to form phosphomolybdovanadate complex. The complex is measured colorimetrically at 420nm.

The Auto-Analyser was operated at a net of 60 samples per hour, sensitivity at 50ug/ml (0.31 absorbence units), Coefficient of Variation at 30ug/ml (0.54%) and detection limit at 1.0 μg/ml.

REAGENTS

MOLYBDOVANADATE REAGENT

Solution A was made using the following products, 2.53 g of Ammonium Metavanadate (NH_4VO_3), 1500ml of Distilled Water and 320ml of Nitric Acid, Conc. (HNO_3)

Preparations :

Ammonium Metavanadate (2.53 g) was dissolved in 200 ml of hot (90°C) distilled water, cooled and transfered to a four liter volumetric flask and mixed with 1500 ml of distilled water. It was mixed with 320 ml of concentrated nitric acid and mixed thoroughly.

Solution B was prepared by using 46.6g of Ammonium Molybdate [$(NH4)6Mo_7O_{24}.4H_2O$], 100ml of Distilled Water and 2ml of Levor IV* (Technicon No. T21-0332).

Preparation:

Ammonium molybdate (46.6 g) was dissolved in one litre of hot distilled water, cooled and mixed with 2 ml of Levor IV.

WORKING MOLYBDOVANADATE REAGENT

Solution B was added to the volumetric flask containing solution A and diluted to four litre volume with distilled water.

OPERATING NOTES

1. Before running the method, the controls of the Modular Printer were positioned as follows:

The mode switch was placed at the normal position, sampling rate switch at 60, range switch at 500 and the decimal switch at 00.0

2. Reagent baseline absorbance from water was 0.086.

6.3.5.4 MAGNESIUM IN SOIL EXTRACTS AND OTHER AQUEOUS SAMPLES.

METHOD NO. G-131-94

RANGE: 0-90mg/l as Mg

DESCRIPTION

The determination of magnesium is based on the reaction of magnesium (Mg) and magnesium blue dye in alkaline solution, to form a magnesium blue lake complex, the colour of which is proportional to the magnesium concentration.

REAGENTS

All chemicals were of ACS grade.

LIST OF RAW MATERIALS

Brij-35*, 30% solution (Bran+Luebbe No. T21-0110.06)

Calcium Carbonate ($CaCO_3$)

N,N-Dimethylformamide, ($HCON(CH_3)_2$)

Magnesium Blue

Magnesium Sulphate Heptahydrate ($MgSO_{4.7}H_2O$)

PVA-Polyvinyl Alcohol ($-CH_2CH(OH)-$)

Sodium Hydroxide. (NaOH)

REAGENT MAKE-UP

Di water refers to high quality water. Type I or Type II as defined in ASTM Standards. Part 31, D 1193-74.

SODIUM HYDROXIDE SOLUTION, 2N

Eighty grams of sodium hydroxide were added to about 600 ml of Di water and cooled to room temperature. It was diluted to one liter with Di water and mixed thoroughly.

STOCK PVA SOLUTION.0.2%

Two grams of polyvinyl alcohol were added to one liter of distilled water contained in a two liter Erlenmeyer flask. It was then heated to boiling, stired until PVA is dissolved and cooled to room temperature.

WORKING PVA SOLUTION, 0.05%

Two hundred and fifty milliliters of stock PVA solution, 0.2% and calcium standard solution, 7500 mg/l Ca. was added to about 600 ml of Di water and

diluted to one liter with Di water. One millilitre of Brij-35, 30% was added and mixed thoroughly.

STOCK MAGNESSIUM BLUE SOLUTION

Magnesium Blue (0.2 g) was dissolved in 200 ml of dimethylformamide and let to stay for one to two days, after that it was mixed thoroughly and diluted to one liter with Di water, mixed and filtered to remove any residue.

SAMPLE PROCESSING

Samples that included faeces, bone and blood were analysed through the Bran % Luebbe " Auto-Analyser II : Technicon Industrial Systems, Tarytown NY 10591

6.2.6 ANALYSIS

6.2.6.1. FAECES

Analysis of P, Ca and Mg was done on the supernant fluid of the sample solution. To determine P, Ca and Mg fractions in faeces, approximately 5ml of standards (200ppm *2 including the initialising standard), 150ppm, 100 ppm and 50 ppm) was poured in cuvettes. In cuvette number six, a blank distilled water was poured, and from there onwards sample solutions were now poured in different cuvettes accordingly and proceeded like in 6.2.5.1\3 and 4.

6.3.6.2. BONE

Bone samples were analysed as in 6.2.5.1\3 and 4 above.

6.3.6.3. BLOOD

Blood samples were prepared as in 6.2.5.1 above and analysed as in 6.2.5.1\2 and 4 above.

6.3.7.CALCULATIONS

CALCULATION OF PHOSPHORUS, CALCIUM AND MAGNESIUM CONCENTRATIONS USING A STANDARD CURVE

The concentration of P in the samples was calculated by constructing a standard curve using the absorbance values of the known standards. The average of the absorbances of the replicates of the 3 known standards (5 mg%, 10 mg% and 20 mg%) were used. These standard concentrations were plotted on the X-axis of the graph paper while their corresponding absorbencies on the Y-axis. The 3 points were joined with a straight line which passed through zero intercept on the Y-axis since the blank tube had "zero" absorbence.

The equation for calculating the P concentration is given by:

$$Y = mx + c$$

Where Y = absorbence (proportional to concentrations in mg% or ppm)

M = slope (gradient) of the standard curve

X = standard dilutions concentrations

C = intercept value

Where $M = \frac{DY}{DX}$

and DY = change in absorbence value between two given points on the Y-axis

and DX = change in dilution standard concentration between two corresponding values on the X-axis.

The concentration of P in the samples was calculated as:

$$mg\% = \frac{\text{absorbence of the sample}}{\text{slope}}$$

If a solution containing the elements (i.e. Ca and Mg) is introduced into a hot flame, the elements present dissociate into " atomic vapour" in the " ground state". If the light beam from a Ca or Mg hollow cathode lamp is directed through the vapourised sample of the same elements, the Ca or Mg atoms will absorb some of the emitted light energy as they rise to the "excited state". This is done at a specific wavelength of each element and thus 422.7nm and 285.2 nm for Ca and Mg respectively. By measuring the amount of light energy absorbed, quantitative determination of the amount of an element in the sample can be made. The detector in the spectrophotometer measures the amount of light energy absorbed which is proportional to the concentration of Ca or Mg (Kaplan and Szabo, 1976).

After setting the atomic absorption spectrophotometer at a wavelength of 422.7nm (Ca) and 285.2 nm (Mg), a blank solution containing 10ml of distilled water and reagents was aspirated and the machine adjusted to zero reading. Then the solutions containing the three standards were aspirated and their absorbency recorded. These recorded absorbencies were used to draw a Ca and Mg standard curve from which the gradient (slope) was calculated and used to determine the concentration of the unknown Ca and Mg mg% in sample solutions. Standards were run with each batch of samples and a slope calculated for each.

To calculate the concentration of the minerals including P in mg/g the following formula was used:

$$Mg/g = \frac{mg\%}{Wt\ g}$$

Where: mg/g = milligrams per gram of the sample

Mg% = the absorbence of the sample divided by the slope of the standard solution.

Wt g = weight of the sample in fresh, dry and ash weight basis.

CHAPTER 7

7. EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

7.1. OBJECTIVE

Percentage P, Ca and Mg were measured because the aim of improving the efficiency of the utilization of P, Ca and Mg is to improve the growth rate and P status of cattle fed a diet low in phosphorus.

7.2. EXPERIMENTAL DESIGN

Samples were taken on a ten day interval from week 0 until week 6 throughout the supplementation. These samples were analysed for P, Ca, and Mg.

A randomised block design analysis of variance was used. Regression analyses was used to compare relationships between blood, bone and faeces and to compare the effects of dietary anion on bone blood and faecal P, Ca and Mg, were non-linear and linear regression was used. In comparing the weeks, the repeated measures analysis of variance was used that also dealt with comparisons among treatments.

The factors to be studied were the effect of sulphur anion as an acidiogenic agent on P, Ca, Mg in and out of the bones and blood and throughout the faeces in weeks of supplementation.

7.3. STATISTICAL ANALYSIS-

Statistical analysis was done on a Windows 98 Microsoft Excel tool menu, two samples assuming equal variences. Analysis of Variance (ANOVA) was done to determine whether the application of ammonium sulphate anions as an acidiogenic agent in the diet can have a significant effect on the total P, Ca and Mg utilization and movements in and out of the bone, blood and the faeces. To compare the treatment means the least significant differences (LSD) will be calculated by the MS Excel tool menu with the following formulas:

A. Read $Q_{0.05}FK, V$

Where $Q_{0.05}F$ = The standard values in a P0.05, 0.01 table for factor F

K = Number of means to be compared

V = Error degrees of freedom (EDF)

B. Least Significance Ratio $= Q * \frac{MSE}{n}$

where SEM $= \sqrt{\frac{\text{error mean square}}{n}}$

n = number of observations in each treatment

7.3.1. DESIGN

The experiment on the two sides was a completely randomised design with two replicates/treatments. Samples were taken on a ten day interval from week 0 until week 6 throughout the supplementation. The samples were analysed for P, Ca, and Mg.

7.3.2. DATA

Data was collected for:

- P, Ca and Mg concentrations in mg% for blood and mg/g for faeces and bone
- Bone thickness
- Liveweight
- Feed consumption of all 16 calves.

CHAPTER 8

8.RESULTS / DISCUSSION AND CONCLUSION

8.1. THE OVERALL EFFECT OF ANIONS (AMMONIUM SULPHATE) ON P HOMEOSTASIS

When the effects of the anionic diet were observed on the parameters measured it was found that the diet which contained a high level of anions, 18% NH_4SO_4, increased the bone thickness, increased the P content of the bone, increased the lick intake by the calves, increased the faecal P when expressed as ash weight, increased the P content of the blood and increased the weight gained by the calves compared to the animals on the control diet as shown in Table 1. Note that the Standard Error Means of all the data will be found at the end of the results (appendix 1, 2 and 3).

8.1.1. SUM OF MEAN TOTALS

8.1.1.1. BLOOD P

In Table 1, the effect on the serum inorganic P concentration of the blood was exerted by the anionic diet. The calves on the six anionic diets had a mean concentration of 4.91mg% P in the serum as compared with a serum inorganic P concentration of 3.19 mg% in the calves on the control diet. The serum inorganic P concentration at week 0 (4.84mg% P) was significantly increased to 5.60 mg% (wk1) and then significantly decreased to about 1.90 mg% at week 3 and followed by a significant increase to 7.76 mg% at the end of the experiment (Table 1).

TABLE 1: EFFECT OF ANION LEVEL ON P CONTENT OF ALL PARAMETERS

ANIONIC LEVEL	BONE THICKNESS (mm)	WEIGHT GAIN Kg/calf/day	Bone P(AW) Mg/g	Lick Intake per day Kg	Faecal P Ash Mg/g	Blood P Mg%
18%	2.14^{z}	0.33	210.58^{z}	0.25	32.96^{y}	7.76^{yz}
15%	1.51^{x}	0.08	199.38^{z}	0.5	29.17^{y}	7.03^{yz}
10%	1.56^{y}	0.02	168.39^{y}	0.25	10.77^{x}	4.27^{x}
5%	1.46^{x}	0	154.36^{y}	0	9.21^{x}	1.90^{w}
2.5%	1.58^{y}	0.34	176.31^{y}	0.75	10.87^{x}	2.94^{w}
1.25%	1.36^{w}	-0.236	206.47^{y}	1.5	18.62^{z}	5.60^{y}
0	1.57^{y}	-	113.29^{x}	-	19.84^{z}	4.84^{x}
SUM OF MEANS	1.60	0.534	185.91	0.54	18.60	4.915
CONTROL	1.43	0.514	137.89	0.325	14.22	3.19

$^{w, x, y,z}$ means with the same letters in a column are not significantly different within treatment groups (P>0.05)

8.1.1.2. FAECAL P

In Table 1, the effect on the mean faecal P concentration of the treated group compared with the control group was exerted by the anionic diet. The calves on the six anionic diets had a mean concentration of 18.60 mg/g P in the faeces as compared with a mean faecal P concentration of 14.22 mg/g in the calves on the control diet. Faecal P was significantly (P>0.05) decreased from 18.62 mg/g (wk1) to 9.21mg/g (wk3) and then significantly increased

to 32.96 mg/g (wk6). The individual treatment means in animals fed 18%, 15% and 1.25% NH_4SO_4 in the treated group were higher than the overall mean P concentration of the control group that was 14.22 mg\g on the ash weight basis (Table 1).

8.1.1.3. BONE THICKNESS

As can be seen in Table 1, the acidogenic diet was responsible for an increase in the bone thickness of the calves receiving a high anionic diet. The mean bone thickness in the calves fed the six anionic diets was 1.60 mm compared with 1.43 mm for those calves on the control (Table 1).

8.1.1.4. BONE P

Table 1 clearly shows that bone P concentration increased steadily due to the anionic concentration of the diet. The calves on the anionic diet had a mean concentration of 185.91mg/g bone P ash weight as compared with the P content of 137.89 mg/g in the calves on the control diet. The individual means were only significantly ($P<0.05$) different from the controls at weeks 1, 2 and 5 (Table 7). Bone P was significantly increased from 113.29 mg/g (wk0) to 206.47mg/g (wk1) and then non significantly decreased to 154.36 mg/g (wk3) and thereafter significantly ($P<0.05$) increased to 210.58mg/g (wk6). All the means on the treated group fed different levels of ammonium sulphate were higher than the mean P concentration of the control group that was 137.89 mg\g on the ash weight basis (Table 1).

8.1.1.5. LICK INTAKE

The amount of lick consumed at all steps of the experiment is given in Table 1. The mean intake per day for the group of calves receiving the anionic lick was 0.54kg compared to 0.33kg for those receiving the control lick. During the 3rd sampling

period (WK3) the calves offered the anionic lick refused to consume the bone meal containing 5% ammonium sulphate. That is the reason why the lick intake is 0 for week 3. During the subsequent weeks molasses was added to the anionic lick to encourage the calves to consume it. Dried molasses was added at 10% of the lick at weeks 4, 5 and 6.

8.2. FAECES/BONE/BLOOD

8.2.1. FRESH WEIGHT

8.2.1.1. FAECAL P

At weeks 1, 4 and 6 in the experiment the anionic lick was responsible for a significant increase ($P<0.05$) in faecal P concentration compared with the control lick and at weeks 2, 3 and 5 the animals on the anionic diet had more P in the faeces than those on the control diet though it was not significant ($P>0.05$) in the P content of the faeces when expressed as a function of the fresh weight (Table 2, Fig1).

TABLE 2. FAECAL P CONCENTRATION OF TREATED AND UNTREATED GROUPS OF ANIMALS GIVEN DIFFERENT LEVELS OF AMMONIUM SULPHATE (FRESH WEIGHT BASIS)

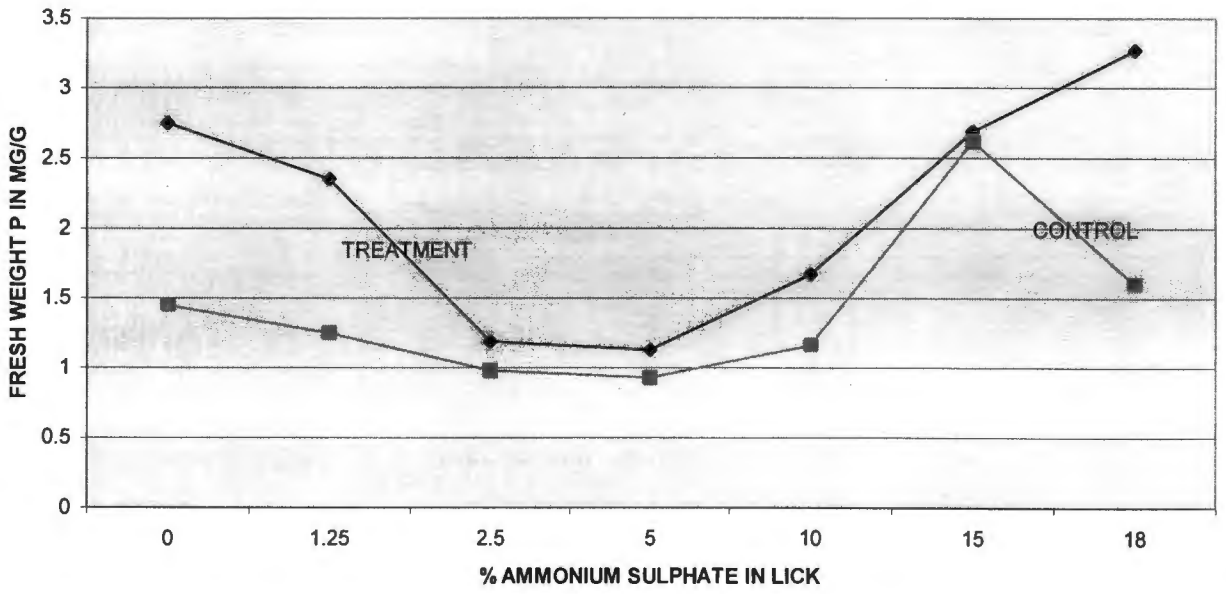
DIETARY LEVEL	WK0	WK1	WK2	WK3	WK4	WK5	WK6
	0	1.25%AS	2.5%AS	5%AS	10%AS	15%AS	18%AS
FAECAL P	Mg/g	Mg/g	Mg/g	Mg/g	Mg/g	Mg/g	Mg/g
ANION	2.75^{ax}	2.35^{ax}	1.19^{ay}	1.13^{ay}	1.67^{ay}	2.69^{ax}	3.27^{az}
CONTROL	1.45^{b}	1.25^{b}	0.89^{a}	0.93^{a}	1.16^{b}	2.62^{a}	1.60^{b}

$^{a, b}$ 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 ($p<0.05$)

The acidogenic diet produced an overall mean for the entire experimental period of 2.05 mg/g of P in the faeces compared to 1.4 mg/g in the faeces of the calves under the control diet on the fresh weight basis. The P concentration significantly ($P<0.05$) decreased from 2.75 mg/g (week0) to 1.13 mg/g (week3) and then significantly ($P<0.05$) increased to 3.27 mg/g (week6) at the end of the experiment. This shows that the anionic diet was responsible for increasing the P content of the faecal sample on the fresh weight basis as seen in Figure 1 and Table 2. This effect is seen in the faeces where the high anionic diet (18% ammonium sulphate) resulted in significantly ($P<0.05$) more P being lost in the faeces than at all other sampling periods.

FIG 1. THE FAECAL P CONCENTRATION OF TREATED AND UNTREATED ANIMALS GIVEN DIFFERENT LEVELS OF AMMONIUM SULPHATE (FRESH WEIGHT)



The anionic diet resulted in more P being lost in the faeces than the untreated group on the fresh weight basis (Table 2). As can be seen in Figure 1 the faecal P remained higher in all the treated group than in the control group. It should be noted that as the concentration of ammonium sulphate increase in the lick there was a curvilinear effect were the

concentration of P linearly decreased and then was followed by an increase after the 5 % NH_4SO_4 was given.

8.2.1.2. BONE AND BLOOD P

TABLE 3: THE EFFECT OF LICK ANIONIC CONCENTRATION ON BONE P FRESH WEIGHT AND BLOOD PARAMETERS

DIETARY LEVEL	WK0	WK1	WK2	WK3	WK4	WK5	WK6
	0	1.25%AS	2.5%AS	5%AS	10%AS	15%AS	18%AS
BONE P	Mg/g	Mg/g	Mg/g	Mg/g	Mg/g	Mg/g	Mg/g
ANION	64.72^{aw}	81.12^{aw}	105.43^{ax}	60.18^{ay}	91.21^{ay}	121.27^{az}	125.68
CONTROL	105.71^{b}	65.33^{b}	88.71^{b}	54.78^{a}	62.93^{b}	101.87^{b}	92.945
BLOOD P	Mg%	Mg%	Mg%	Mg%	Mg%	Mg%	Mg%
ANION	4.84^{ax}	5.60^{ay}	2.94^{aw}	1.90^{aw}	4.27^{ax}	7.02^{az}	7.76^{az}
CONTROL	2.87^{b}	2.28^{b}	2.53^{a}	2.17^{a}	3.59^{a}	4.51^{b}	4.08^{b}

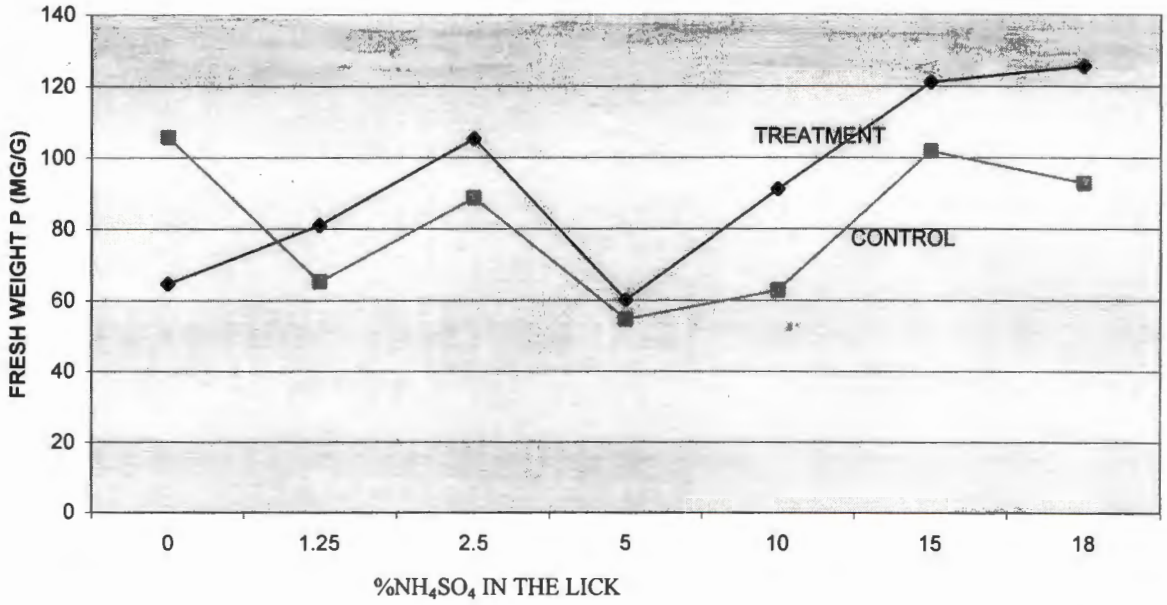
^{a,b} means with different letters in a column are significantly different between treatment groups (bone $P<0.05$, blood $P<0.01$).

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

The highest concentration of P in the bone was 125.68 mg/g and the lowest being 60.18 mg/g P in the calves on the anionic diet compared with the calves on the control diet with the highest mean being 101.87 mg/g and the lowest mean being 54.78 mg/g on the fresh weight basis. The concentration of P in the blood was significantly ($P<0.01$) higher in the calves on the anionic diet (5.60 mg%, 7.02 mg% and 7.76mg% at weeks 1, 5 and 6 when the concentration of ammonium sulphate was 1.25 %, 15 % and 18 % in the lick) than in the calves on the control diet (2.28 mg%, 4.51 mg% and 4.08

mg% at the same period) as seen in Table 3, Fig. 2 and Fig. 4b.

FIG 2. THE EFFECT OF ANIONIC LEVEL AND P INTERACTION ON BONE PARAMETERS (FRESH WEIGHT)



At weeks1, 5 and 6 (Table 3.) the animals receiving an acidiogenic diet had significantly ($P<0.01$) higher serum inorganic P concentrations (5.60, 7.02 and 7.76 mg%) compared to those receiving the control diet (2.28, 4.51 and 4.08 mg%). At the same time periods, the concentration of P in the bone of calves on the anionic diet was significantly higher ($P>0.05$) with the means 81.12, 121.27 and 125.68 mg/g wereas the bone from the calves on the control diet contained 65.33, 101.87 and 92.945 mg/g as seen in Table 3 and Fig 2. These means are higher than those reported by Little and Minson (1977) and Beighle *et al,*(1993) when they reported a mean bone P content of 102.93 mg/g of fresh weight . There was no significant ($P>0.05$) difference at week 3 because the animals on the anionic lick did not consume any lick at that time period. Had the animals on the anionic lick consumed the lick at 5% ammonium sulphate, we would have expected the bone P to have shown a continuous increase as the trial progressed and the

ammonium sulphate in the lick increased, which would have given us a more or less straight line in Figure 2.

8.2.2. DRY WEIGHT

8.2.2.1. FAECAL PHOSPHORUS

TABLE 4: FAECAL P CONCENTRATION (DRY WEIGHT BASIS) OF TREATED AND UNTREATED GROUPS OF ANIMALS GIVEN DIFFERENT LEVELS OF AMMONIUM SULPHATE

DIETARY LEVEL	WK0	WK1	WK2	WK3	WK4	WK5	WK6
	0	1.25%AS	2.5%AS	5%AS	10%AS	15%AS	18%AS
FAECAL P	Mg/g	Mg/g	Mg/g	Mg/g	Mg/g	Mg/g	Mg/g
ANION	2.92^{ax}	2.42^{ax}	2.24^{ay}	2.05^{ay}	1.77^{ay}	2.99^{ax}	3.64^{az}
CONTROL	1.53^{a}	1.33^{b}	1.94^{a}	1.71^{b}	1.23^{b}	2.85^{a}	1.71^{b}

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

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

The acidogenic lick produced an overall mean concentration of 2.52 mg/g of P in the faeces on a dry weight basis compared to 1.79 mg/g in the faeces of the calves given the control lick. All the means from the treated group remained higher than those from the control lick. This clearly demonstrates that the anionic lick increased faecal P concentration in all weeks of supplementation on the dry weight basis. At weeks 1, 3, 4 and 6 those animals receiving the anionic lick had significantly ($P<0.05$) more P in their faeces compared to the animals receiving the control lick.

FIG 3. THE RELATIONSHIP BETWEEN FAECAL P CONCENTRATION OF THE TREATED AND NONTREATED GROUPS OF ANIMALS GIVEN DIFFERENT LEVELS OF AMMONIUM SULPHATE (DRY WEIGHT)

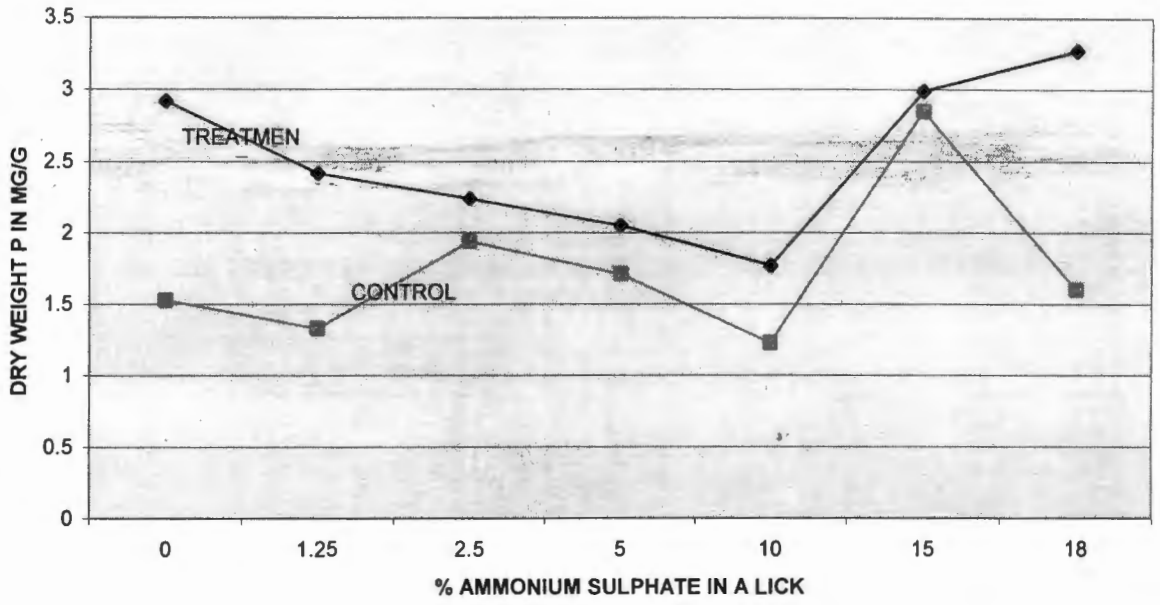


Figure 3 shows the effect in the faeces where the anionic lick resulted in more P being lost in the faeces of the treatment group in all weeks of the experiment compared to the control group (Table 4). The anionic diet resulted in more P being lost in the faeces than the untreated group on the Dry Weight basis (Table 4 and Fig 3).

There was more faecal P in the animals on the anionic diet compared to the control diet throughout the entire experimental period. That was significant ($P>0.05$) at weeks 1, 3, 4, and 6 when compared with the control group. The faecal P significantly ($P<0.05$) decreased from week 0 (2.92 mg/g) to week 2, 3 and 4 (2.24, 2.05 and 1.77 mg/g) when the animals received 2.5, 5 and 10% ammonium sulphate then significantly ($P>0.05$) increased at week 6 (3.64 mg/g) when the animals were given a lick containing 18%ammonium sulphate (Table4). The control group mean faecal content ranged from 1.23 to 2.85 mg/g (Dry Weight). Beighle *et al,* (1997) found the

faecal P concentration of the animals receiving the anionic diet being higher than those of animals receiving cationic and the control diet.

8.2.2.2. BONE AND BLOOD PHOSPHORUS

TABLE 5: EFFECT OF DIETARY ANIONIC LEVEL ON P INTERACTION ON BONE AND BLOOD PARAMETERS (DRY WEIGHT BASIS)

DIETARY LEVEL		WK0	WK1	WK2	WK3	WK4	WK5	WK6
		0	1.25%AS	2.5%AS	5%AS	10%AS	15%AS	18%AS
BONE P		Mg/g	Mg/g	Mg/g	Mg/g	Mg/g	Mg/g	Mg/g
ANION		70.53^{ax}	131.05^{ay}	114.81^{ay}	94.53^{ay}	103.67^{ay}	131.94^{az}	157.07^{az}
CONTROL		115.09^{b}	107.96^{b}	90.77^{b}	79.85^{b}	68.45^{b}	105.44^{b}	124.27^{b}
BONE THICK (mm)	**TR**	1.57^{a}	1.36^{a}	1.58^{a}	1.46^{a}	1.56^{a}	1.51^{a}	2.14^{a}
	CN	1.45^{b}	1.54^{b}	1.44^{b}	1.57^{b}	1.37^{b}	1.21^{b}	1.45^{b}
BLOOD P		Mg%	Mg%	Mg%	Mg%	Mg%	Mg%	Mg%
ANION		4.84^{ax}	5.60^{ay}	2.94^{aw}	1.90^{aw}	4.27^{ax}	7.02^{az}	7.76^{az}
CONTROL		2.87^{b}	2.28^{b}	2.53^{a}	2.17^{a}	3.59^{a}	4.51^{b}	4.08^{b}

^{a,b} means with different letters in a column are significantly different between treatment groups (Bone $P<0.05$, blood $P<0.01$)

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

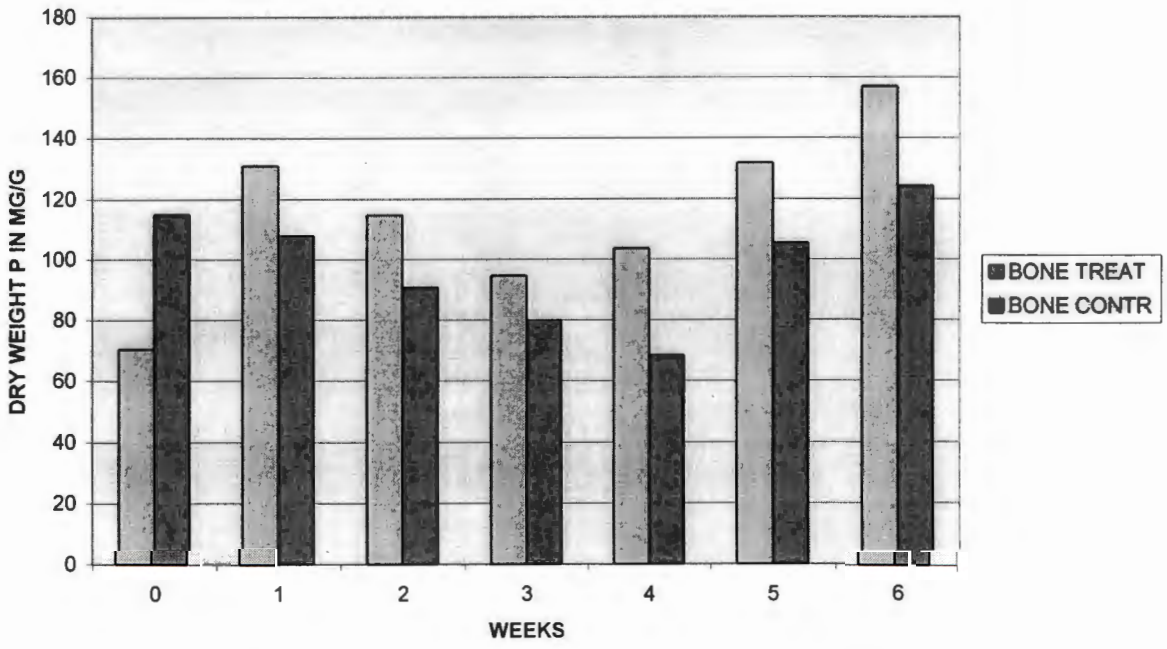
CN=CONTROL, TR=TREATMENT, BONE THICK=BONE THICKNESS

The highest concentration of P in the bone on a dry weight basis was 157.07 mg/g when 18% ammonium sulphate was given in the lick and the lowest was 94.53 mg/g P when 5% ammonium sulphate was given in the lick in the

calves on the anionic diet, but in the calves on the control diet the highest mean was 124.27 mg/g and the lowest mean being 68.45 mg/g. These means are within the range of those reported by Cohen (1973b) and Beighle *et al*, (1993) when they reported an overall mean of 107.88 mg/g of P dry weight in the rib bone samples of animals in serial sampling for mineral analysis without any anionic supplementation.

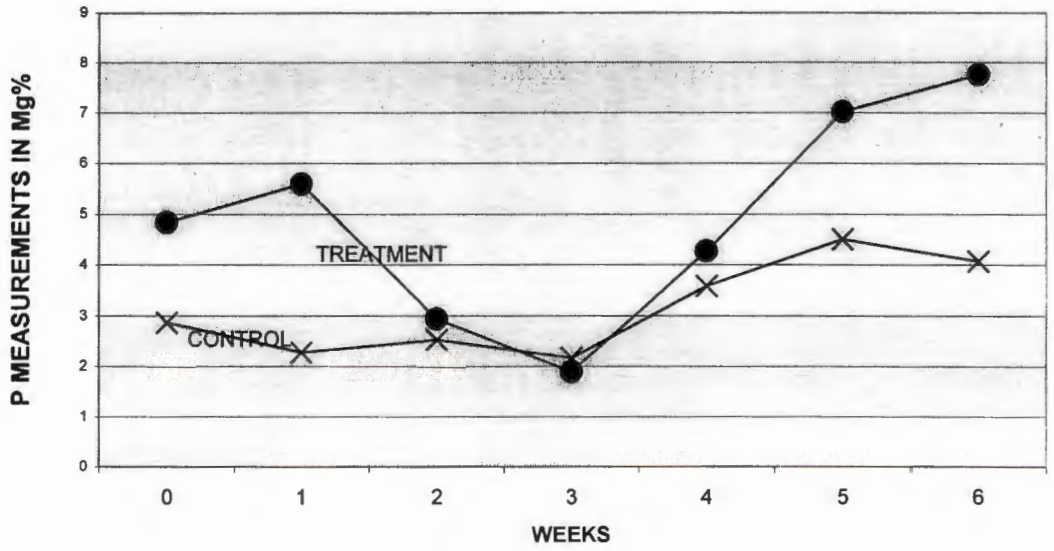
The total mean concentration of P in the blood was much higher in the calves on the anionic diet than in the calves on the control diet. The overall mean P content of blood of calves fed the acidogenic lick was significantly ($P<0.01$) higher (serum inorganic P content 4.91 mg%) than those on the control diet (3.19 mg%). With the exception of week 3, animals on the anionic lick had higher blood P than those animals on the control lick (Table 5) while the average blood concentration of P was highest in the calves fed the 18, 15, 10, 2.5 and 1.25 % ammonium sulphate supplements.

FIG 4a. THE EFFECT OF DIETARY ANIONS ON BONE (DRY WEIGHT)



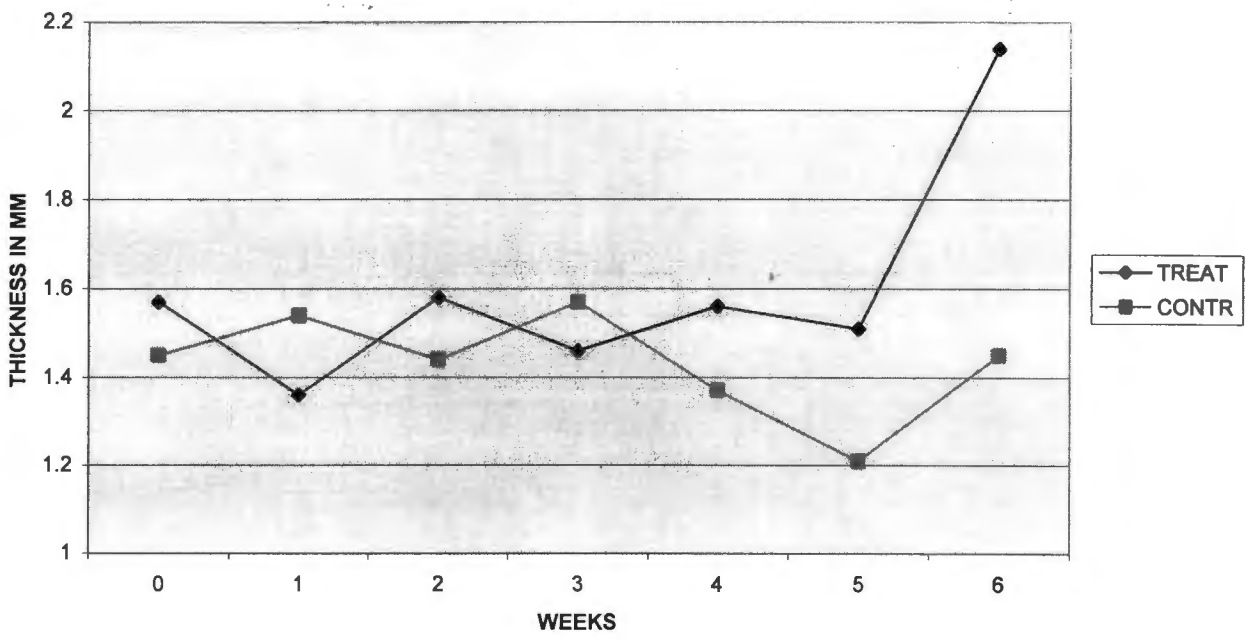
At weeks 1, 5 and 6 (Table 5 and fig.4b) the animals that received an acidiogenic lick had significantly ($P<0.01$) higher serum inorganic P concentrations (5.60, 7.02 and 7.76 mg%) compared to those receiving the control diet (2.28, 4.08 and 4.51 mg%) (Fig 4b). The concentration of P on a dry weight basis in the bone of calves on the anionic diet was 131.05, 114.81, 94.53, 103.67, 131.94 and 157.07 mg/g were as the bone from the calves on the control diet contained 107.96, 90.77, 79.85, 68.45, 105.44 and 124.27 mg/g P (Table 5). The anionic diet was responsible for significantly ($P>0.05$) more bone P in animals within the treatment group as the concentration of ammonium sulphate increased. Bone P significantly ($P<0.05$) increased from 70.53 mg/g P (wk0) to 131.05 mg/g P (wk1) and then again significantly ($P<0.05$) increased to a higher concentration of 131.95 mg/g (wk 5) and 157.07 mg/g P (wk6). Analysis of the data by weeks of supplementation (Table 5 and Fig 4a) shows a significant effect of dietary anions on bone P concentrations of animals fed anions in the lick compared with those receiving the control lick on the dry weight basis.

FIG 4B. THE RELATIONSHIP BETWEEN BLOOD P CONCENTRATION OF THE TREATED AND THE UNTREATED ANIMALS



At weeks 1, 2, 5 and 6 ($P<0.01$) and at weeks 4 and 3 ($P<0.05$) a significant increase in P content of the bone was observed when compared with the control group (Table 5).

FIG 4C. THE DIFFERENCE BETWEEN BONE THICKNESS OF THE TREATED AND THE UNTREATED ANIMALS



The bone thickness was greater in the calves fed the anionic diet except at weeks 1 and 3 compared to those fed the control diet (Table 5). At week 3 where the animals consumed no lick the bone thickness decreased from 1.58 mm to 1.46mm and the blood concentrations also significantly ($P<0.01$) decreased from 2.94 mg% to 1.90 mg% and the faecal P decreased from 2.24 mg/g to 2.05 mg/g but bone P remained significantly ($P<0.05$) above control values on the Dry Weight Basis (Tables 4 and 5; Fig3 and 4b and 4c). Bone thickness responded to a decrease in dietary P faster than bone P! Calves on the anionic lick were not receiving any P during wk3 but bone P remained above values in the control group probably from the effect of the previous treatment but bone thickness decreased. It was expected that because animals on the anionic lick were not consuming any bone meal, blood, bone and faecal P would all decrease to where there were no longer

any significant (P>0.05) differences from the control values, but bone P remained above control values and bone thickness decreased to where it was significantly (P<0.05) less than control bone thickness. This would indicate that bone thickness is a more sensitive measure of bone mineral status than previously thought.

8.2.3. ASH WEIGHT

8.2.3.1. FAECAL PHOSPHORUS

TABLE 6: FAECAL P CONCENTRATION (ASH WEIGHT BASIS) OF TREATED AND UNTREATED GROUPS OF ANIMALS GIVEN DIFFERENT LEVELS OF AMMONIUM SULPHATE

DIETARY LEVEL	Wk0	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6
	0	1.25%AS	2.5%AS	5%AS	10%AS	15%AS	18%AS
FAECAL P	Mg/g	Mg/g	Mg/g	Mg/g	Mg/g	Mg/g	Mg/g
ANION	19.84^{ay}	18.62^{ay}	10.87^{ax}	9.21^{ax}	10.77^{ax}	29.17^{az}	32.96^{az}
CONTROL	16.18^{a}	15.04^{b}	8.78^{a}	8.00^{b}	9.13^{b}	26.58^{a}	17.78^{b}

$^{a,\ b}$means with the same letters in a column are not significantly different between treatment groups (p<0.05)

$^{x,\ y,z}$ means with different letters in a row are significantly different between weeks (p<0.05)

The acidogenic lick produced an overall mean concentration of 18.60 mg/g of P in the faeces (ash weight) compared to 14.22 mg/g in the faeces of the calves under the control lick. This effect is seen in the faeces where the high anionic diet resulted in more P being lost in the faeces at all weeks throughout the entire experiment (Table 6). It is evident that the anionic diet

resulted in more P being lost in the faeces compared to the control group on the ash weight basis (Table 6).

8.2.3.2. BONE AND BLOOD PHOSPHORUS

TABLE 7: THE EFFECT OF ANIONIC LICK ON BLOOD AND BONE PARAMETERS (ASH WEIGHT BASIS)

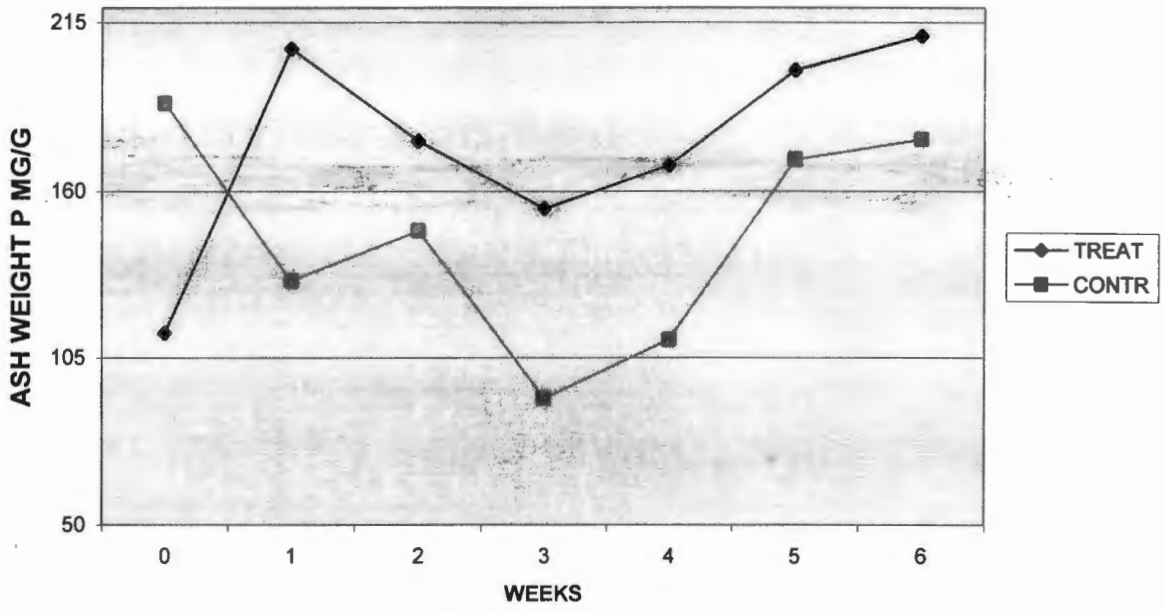
DIETARY LEVEL	WK0	WK1	WK2	WK3	WK4	WK5	WK6
	0	1.25%AS	2.5%AS	5%AS	10%AS	15%AS	18%A
BONE P	Mg/g	Mg/g	Mg/g	Mg/g	Mg/g	Mg/g	Mg/g
ANION	113.29^{ax}	206.47^{ay}	176.31^{ay}	154.36^{ay}	168.39^{ay}	199.38^{az}	210.5
CONTROL	188.64^{a}	130.54^{b}	146.98^{b}	91.71^{a}	110.99^{a}	170.34^{b}	176.7

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

$^{w, x, y,z}$ means with different letters in a row are significantly different between weeks ($p<0.05$)

The highest concentration of P in the bone was 210.58 mg/g ash weight and the lowest being 154.36 mg/g P ash weight in the calves on the anionic diet, compared to the calves on the control diet with the highest mean being 176.76 mg/g and the lowest mean being 91.71mg/g.

FIG 5. THE RELATIONSHIP BETWEEN BONE P OF TREATED AND UNTREATED ANIMALS (ASH WEIGHT)



Animals given the anionic lick had more bone P than those given the control lick throughout the trial significant (P>0.05) at weeks 1, 2, and 5. The concentration of P in the bone of calves on the anionic diet was 206.46, 176.31, 154.36, 168.39, 199.38 and 210.58 mg/g (ash weight) where as the bone from the calves on the control diet contained 130.54, 146.98, 91.71, 110.99, 170.34 and 176.76 mg/g P (ash weight) (Table 7, Fig.5).

THE EFFECT OF LICK ANIONS ON ALL THREE PARAMETERS

TABLE 8. EFFECT OF LICK ANIONS ON BONE, FAECAL AND BLOOD P CONCENTRATION.

WEEKS	BONE		FAECAL		BLOOD	
	MG/G Dry Weight		MG/G Dry Weight		MG%	
	CONTROL	TREAT	CONTROL	TREAT	CONTROL	TREAT
0	115.09^{b}	70.53^{ax}	1.53^{a}	2.92^{ax}	2.87^{b}	4.84^{ax}
1	107.96^{b}	131.05^{ay}	1.33^{b}	2.42^{ax}	2.28^{b}	5.6^{ay}
2	90.77^{b}	114.81^{ay}	1.94^{a}	2.24^{ay}	2.53^{a}	2.94^{aw}
3	79.85^{b}	94.53^{ay}	1.71^{b}	2.05^{ay}	2.17^{a}	1.9^{aw}
4	68.45^{b}	103.67^{ay}	1.23^{b}	1.77^{ay}	3.59^{a}	4.27^{ax}
5	105.44^{b}	131.94^{az}	2.85^{a}	2.99^{ax}	4.51^{b}	7.02^{az}
6	124.27^{b}	157.07^{az}	1.71^{b}	3.64^{az}	4.08^{b}	7.76^{az}

$^{a, b}$ means with different letters in a row are significantly different between treatment groups (bone and faeces $P<0.05$, blood $P<0.01$)

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

Throughout the experiment animals receiving the anionic lick consistently had a higher concentration of P in the bone, faeces and blood than the animals receiving the convential lick (Table 8). All these measurements followed the same trend where the parameters in the treatment animals, especially bone P and serum inorganic P increased from week 0 to week 1 and then decreased until week 3 thereafter, a rapid increase beginning at week 4 sometimes nearly twice as much as the mean on the previous treatment. Bone P was significantly ($P<0.05$) increased from 70.53mg/g

(wk0) to 131.05mg/g (wk1) and then decreased to 94.53 mg/g (wk3) and thereafter significantly ($P<0.05$) increased to 157.07mg/g (wk6) on the dry weight basis. At all sampling periods the anionic diet was responsible for significantly ($P<0.05$) more bone P compared to the control group. This would indicate that the anionic diet improved the P status of the animals based on significant ($P<0.05$) improvement in bone P concentration. Only at the highest percentage of NH_4SO_4 did we see the significant increase in the faecal P so that the loss of P in the faeces was less at 2.5, 5 and 10% NH_4SO_4.

FIG 6. THE EFFECT OF ANIONIC LICK ON THE CONCENTRATION OF BONE P OF EXPERIMENTAL ANIMALS (DRY WEIGHT)

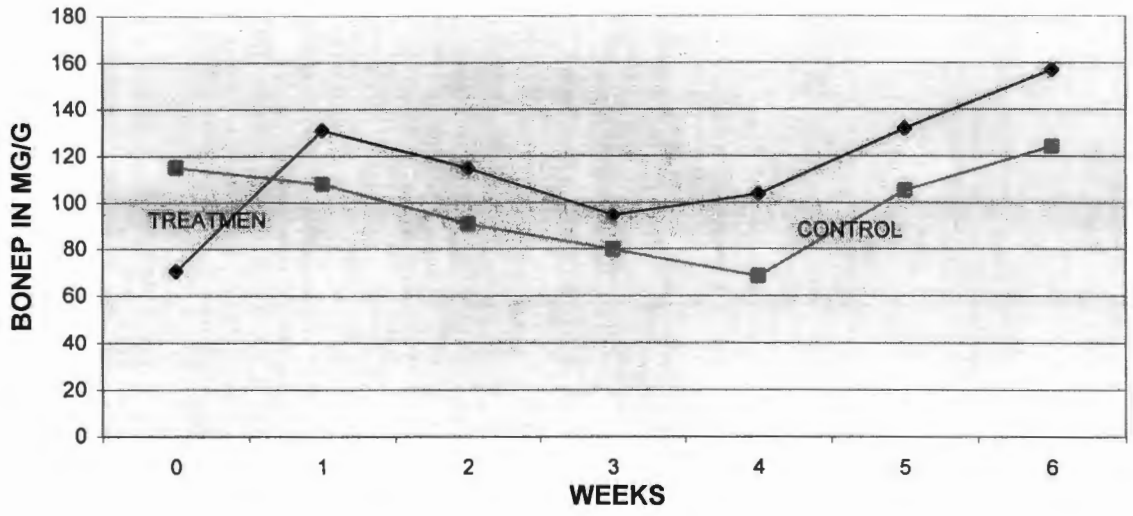
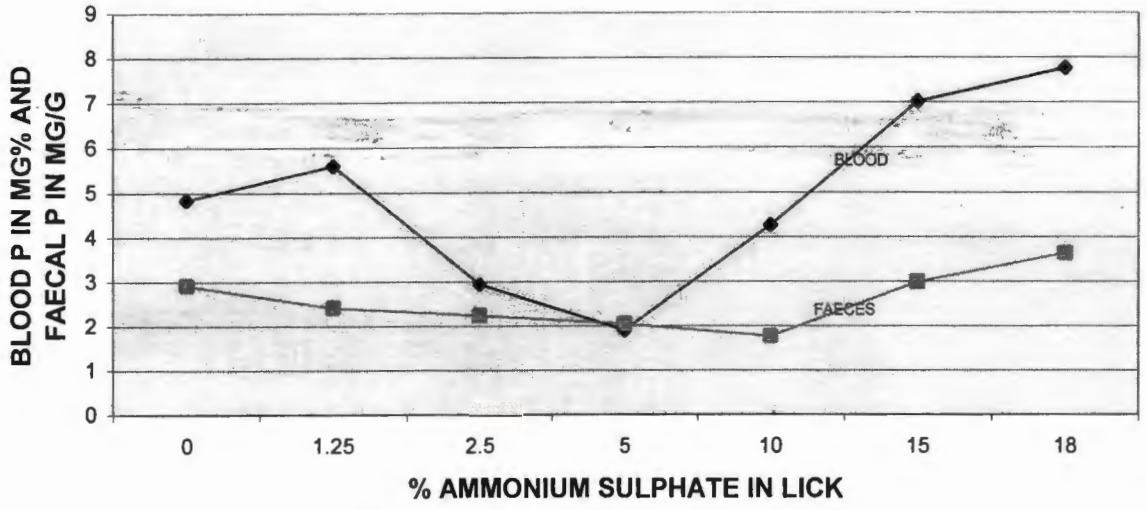


FIG 7. THE DIAGRAMATIC REPRESENTATION OF FAECAL AND BLOOD P CONCENTRATION OF THE TREATED GROUP FED DIFFERENT LEVELS OF AMMONIUM SULPHATE



The means of inorganic blood P of the treated group on week 0 (4.84mg% P) was significantly ($P<0.01$) increased to 5.60 mg% (wk1) and then significantly ($P<0.01$) decreased to 1.90 mg% at week 3 and followed by a significant ($P<0.01$) increase to 7.76 mg% at the end of the experiment (Fig 7, Table 8). This agrees with research conducted by Beighle *et a,* (1997), where blood P remained above pre-treatment values throughout the trial and was significant ($P<0.02$) at weeks 2, 3 and 8. The faecal P significantly ($P<0.05$) decreased from week 0 (2.92 mg/g) to week 4 (1.77 mg/g) then significantly ($P>0.05$) increased at week 6 (3.64 mg/g).

Simultaneous blood, bone and faecal P concentrations that were higher than those of the control at weeks 1 and 6 due to the anionic diet are an indication of a P sparing effect of the anionic lick in the animal body. This is in agreement with Beighle *et al,* (1994) who also found simultaneous increases in the bone, blood and faecal P concentrations in the anionic treatment group compared with the cationic treatment group.

The effect of the lick anions on the amount of P in the faeces was consistent in the parameters measured and the dietary anions led to more P being lost in the faeces and in addition the P concentration of the bone and blood concurrently increased in the treated group compared with the control group. These results agree with what Beighle *et al,* (1997) found when comparing blood, bone and faecal P concentration in the animals receiving an acidiogenic diet and the animals receiving a basiogenic diet. When compared with pre-treatment values (week 0), blood, bone and faecal concentrations of P increased simultaneously during the first two weeks among the anionic treatment group (Beighle *et al,* 1997).

Unlike the measurement of the energy requirements of animals, there is a substantial difference between the apparent and true absorption of P from the intestine into the blood and from all other P sources from the body secretions (saliva, hormones e.t.c) and there is no predictable relationship between these facts (Ternouth, 2001). Due to the fact that the faecal P concentration of the treated group remained higher throughout most of the experiment compared to the controls, then the ever increasing inorganic P in the blood and bone caused by the addition of anions in the diet, came from 'somewhere in the animal body' and not from the diet itself.

8.3. CALCIUM AND MAGNESSIUM

8.3.1 FAECAL

TABLE 9: MEAN CALCIUM CONTENT OF BOVINE FAECES ACCORDING TO THE PERCENTAGE OF ANIONIC DIET PROVIDED, MEASURED AS FRESH, DRY AND ASH WEIGHT.

	FRESH WEIGHT		*DRY WEIGHT*		*ASH WEIGHT*	
% Ammonium Sulphate	TREAT	CONTR	TREAT	CONTR	TREAT	CONTR
0	1.28[a]	1.02[a]	1.32[a]	1.05[a]	3.72[a]	2.76[b]
1.25	2.78[a]	2.47[b]	2.87[a]	2.54[b]	8.38[a]	4.71[b]
2.5	4.87[a]	4.31[b]	5.02[a]	4.44[b]	14.36[a]	11.28[b]
5	4.62[a]	4.70[a]	4.69[a]	4.77[b]	6.09[a]	6.14[a]
10	2.65[a]	2.40[a]	2.70[a]	4.45[b]	3.14[a]	2.32[b]
15	2.80[a]	1.75[b]	2.88[a]	1.61[b]	7.05[a]	2.90[b]
18	3.77[a]	3.39[b]	3.88[a]	3.49[b]	11.57[a]	9.05[b]

[a, b] means with different letters in a row are significantly different between treatment groups ($P<0.05$)

The concentration of calcium (Ca) in the faeces of the anionic treatment group was significantly ($P<0.05$) greater at 1.25, 2.5, 15 and 18% NH_4SO_4 compared to the control group (Table 9). At 5% and 10% NH_4SO_4 the faecal Ca of the treated was significantly ($P<0.05$) less on a dry weight basis than that of the control group because they consumed less lick at those percentage levels of the anionic diet. The trend was an overall decrease in bone Ca concentrations with increasing concentrations of ammonium sulphate in the lick on the dry weight basis (Table 9). This

clearly shows the overall resorption of calcium from bone and its subsequent loss in the faeces as a result of the acidogenic diet. This is in agreement with Block (1984) who suggested that acid forming elements in the interstine allow for greater Ca solubility and easier absorption. If passive absorption of Ca is increased by excess anions via reduction in interstinal pH, then active absorption of Ca should decrease through the parathyroid hormone (PTH) and 1- hydroxylase system. As these systems become inactive, bone resorption should decrease and plasma hydroxyproline (OHPRO) should remain low. OHPRO increased in the anionic group on Block's (1984) experiment and that indicated that bone was responsive. Fresh and ashed faeces also followed the same trend of increased faecal Ca concentrations that was significantly ($P<0.05$) greater in the treatment animals compared to control animals at weeks 1, 2, 5 and 6 on a fresh weight basis and 1, 2, 4, 5 and 6 on an ash weight basis.

The dried faeces Ca means on the treated group were significantly ($P<0.05$) higher than those of the control group with the means 2.87 vs 2.54 mg/g (wk 1), 5.02 vs 4.44 mg/g (wk 2), 2.88 vs 1.61 mg/g (wk 5) and 3.88 vs 3.49 mg/g (wk 6) and significantly ($P<0.05$) lower at weeks 3 and 4 (Table 9).

Animals in the anionic treatment group had significantly ($P<0.05$) more faecal magnesium (Mg) than those in the control group at all weeks of the experiment except at week 3 (wk 3) on the fresh, dry and ash weight basis. At week 3 ash weight the means were not significantly different between treatment groups but treatment means were significantly ($P<0.05$) less than control means on a fresh and dry weight basis (Table 10).

Faecal Mg decreased from week 1 to week 3 and then increased to week 4 and 5 then decreased again to week 6 and the means were 0.54 mg/g (wk1) to 0.31mg/g (wk3) and then increased to 0.58 mg/g (wk5) and then decreased to 0.40 mg/g at wk 6 on the fresh weight basis (Table 10).

TABLE 10: MEAN MAGNESIUM CONTENT OF BOVINE FAECES ACCORDING TO THE PERCENTAGE OF ANIONIC DIET PROVIDED, MEASURED AS FRESH, DRY AND ASH WERIGHT.

	FRESH WEIGHT		***DRY WEIGHT***		***ASH WEIGHT***	
% Ammonium Sulphate	TREAT	CONTR	TREAT	CONTR	TREAT	CONTR
0	0.17[a]	0.17[a]	0.18[a]	0.18[a]	5.26[a]	4.74[a]
1.25	0.54[a]	0.48[b]	0.55[a]	0.50[b]	16.41[a]	9.51[b]
2.5	0.45[a]	0.35[b]	0.46[a]	0.36[b]	13.79[a]	9.46[b]
5	0.31[a]	0.35[b]	0.31[a]	0.36[b]	3.87[a]	4.54[a]
10	0.57[a]	0.42[b]	0.59[a]	0.43[b]	6.86[a]	4.08[b]
15	0.58[a]	0.52[b]	0.59[a]	0.54[b]	14.48[a]	9.54[b]
18	0.40[a]	0.33[b]	0.41[a]	0.34[b]	12.29[a]	8.59[b]

[a, b] means with different letters in a row are significantly different between treatment groups ($P<0.05$)

On a Dry weight basis faecal Mg decreased from 0.55 mg/g when the ammonium sulphate concentration was 1.25% in the lick to 0.31 mg/g when the ammonium sulphate concentration was 5% in the lick and then increased to 0.59 mg/g when the ammonium sulphate concentration was 10 and 15% in the lick and then decreased to 0.41 mg/g when 18% ammonium sulphate was given in the lick. On an ash weight basis, faecal

Mg decreased from 16.41 mg/g when the ammonium sulphate concentration was 1.25% in the lick to 3.87 mg/g when the ammonium sulphate concentration was 5% in the lick and then increased to 14.48 mg/g when the ammonium sulphate concentration was 15% in the lick and then decreased to 12.29 mg/g when the ammonium sulphate concentration was 18% in the lick. Along with the decrease in faecal Mg came increases in bone Mg when animals were consuming the lick with 18%NH_4SO_4. This would indicate that the anion in the lick had an effect of conserving Mg in the body by increasing bone Mg and decreasing faecal Mg.

8.3.2. BONE AND BLOOD

Mean Ca content of fresh rib bone ranged from 188.47 mg/g to 348.09 mg/g. Table 11 clearly shows that bone Ca decreased from week 1 with the mean 288.73 mg/g to week 2 (233.43 mg/g) then steadily increased to weeks 3 (328.94 mg/g) and week 4 (348.09 mg/g) but again decreased until week 6 with a mean of 188.47 mg/g.

The treatment group bone calcium content remained lower than that of the controls except at week 3 were the treated group was slightly higher than the controls with the means 328.94 mg/g vs 325.13 mg/g for the control animals (Table 11). This was probably due to the lack of lick intake at wk 3 by the treatment group.

TABLE 11: MEAN CALCIUM CONTENT OF BOVINE BONE ACCORDING TO THE PERCENTAGE OF ANIONIC DIET PROVIDED, MEASURED AS FRESH, DRY AND ASH WERIGHT.

	FRESH WEIGHT		***DRY WEIGHT***		***ASH WEIGHT***	
% Ammonium Sulphate	TREAT	CONTR	TREAT	CONTR	TREAT	CONTR
18	188.47[a]	283.42[b]	249.00[a]	411.04[b]	355.13[a]	576.07[b]
15	208.21[a]	294.28[b]	310.41[a]	485.92[b]	466.25[a]	584.59[b]
10	348.09[a]	378.88[a]	442.52[a]	411.38[a]	574.06[a]	642.36[a]
5	328.94[a]	325.13[a]	317.45[a]	353.01[b]	555.29[a]	559.54[a]
2.5	233.43[a]	310.79[b]	243.39[a]	350.54[b]	394.57[a]	565.16[b]
1.25	288.73[a]	218.23[b]	293.92[a]	344.80[b]	491.28[a]	425.95[a]
0	180.12[a]	135.10[a]	196.14[a]	152.83[a]	320.68[a]	244.90[a]

[a, b] means with different letters in a row are significantly different between treatment groups $P<0.05$

On a dry weight basis, the concentration of Ca in the bone of the anionic treatment group was significantly ($P<0.05$) lower as compared to the control treatment group at all weeks of supplementation except week 4. The treated group mean bone Ca level ranged between 243.39 mg/g to 442.52 mg/g on a dry weight basis. Bone Ca decreased from 293.92 mg/g at wk 1 to 243.39(wk 2) and then heavily increased to 442.52 mg/g (wk 4) and then decreased to 249.00 mg/g (wk 6) on a dry weight basis (Table 11).

In the ashed bone, the concentration of Ca in the bone of the anionic treatment group was lower (significantly, $P<0.05$, at weeks 2, 5 and 6) as compared to the control treatment group at all weeks of supplementation except at week 1 where the treated group Ca content was not significantly ($P>0.05$) higher than that of the control but no significant ($P<0.05$) with the mean 491.28 mg/g vs 425.95 mg/g (Table 11). This was probably because the anionic lick contained only 1.25% NH_4SO_4 and there was not enough anions to pull the Ca out of the bone.

The mean bone Ca content ranged between 320.68 mg/g to 574.06 mg/g on an ash weight basis. Ashed bone Ca of the treated group decreased from 491.28 (wk1) to 394.57 mg/g (wk2) and then increased to 574.06 mg/g (wk 4) and then decreased to 355.13 mg/g Ca (wk6) on the ash weight basis (Table11).

TABLE 12: MEAN MAGNESIUM CONTENT OF BOVINE BONE ACCORDING TO THE PERCENTAGE OF ANIONS IN THE LICK, MEASURED AS FRESH, DRY AND ASH WERIGHT.

	FRESH WEIGHT		***DRY WEIGHT***		***ASH WEIGHT***	
% Ammonium Sulphate	TREAT	CONTR	TREAT	CONTR	TREAT	CONTR
18	16.90[a]	18.79[b]	20.56[a]	23.90[b]	26.15[a]	26.82[a]
15	14.47[a]	12.46[b]	16.25[a]	13.28[b]	19.07[a]	14.69[b]
10	13.44[a]	15.43[b]	13.75[a]	16.07[b]	16.07[a]	19.81[b]
5	14.28[a]	13.98[a]	14.65[a]	14.05[a]	17.18[a]	16.79[a]
2.5	12.27[a]	13.64[a]	12.48[a]	13.44[a]	13.85[a]	16.06[b]
1.25	15.19[a]	13.25[b]	18.27[a]	15.22[b]	22.61[a]	16.32[b]
0	8.39[a]	8.22[a]	9.13[a]	9.22[a]	14.85[a]	14.82[a]

[a, b] means with different letters in a row are significantly different between treatment groups $P<0.05$

The mean Mg content of the bone from the treatment group was higher than that of the controls at wk 1, wk 3 and wk5 and lower than that of the controls at weeks 2, 4 and 6. The mean Mg content of the bone ranged from 12.27 mg/g to 16.90 mg/g Mg on a fresh weight basis (Table 12). The same results were observed on a Dry and Ash weight basis. The mean Mg content of Dry bone of the treated group ranged from 12.48 mg/g to 20.56 mg/g Mg and that of the ashed bone in the treated group ranged from 13.85 mg/g to 26.15 mg/g Mg.

8.4. ALL MINERALS IN FRESH RIB BONES OF CALVES FED DIFFERENT LEVELS OF AMMONIUM SULPHATE

TABLE 13: EFFECT OF ANIONIC LEVEL OF THE DIET ON BONE CALCIUM, MAGNESIUM AND PHOSPHORUS AND BONE THICKNESS ON A FRESH WEIGHT BASIS.

WEEKS	*NH_4SO_4 %*	*BONE P*	*BONE Ca*	*RATIO Ca:P*	*BONE Mg*	*BONE THICKNESS*
0	0	64.72^{w}	180.12^{w}	2.8:1	8.39^{w}	1.57^{y}
1	1.25	81.12^{w}	288.73^{y}	3.6:1	15.19^{z}	1.36^{w}
2	2.5	105.43^{x}	233.43^{x}	2.2:1	12.27^{x}	1.58^{y}
3	5	60.18^{y}	328.94^{z}	5.5:1	14.28^{y}	1.46^{x}
4	10	91.21^{y}	348.09^{z}	3.8:1	13.44^{y}	1.56^{y}
5	15	121.27^{z}	208.21^{x}	1.7:1	14.47^{y}	1.51^{x}
6	18	125.68^{z}	188.47^{w}	1.5:1	16.90^{z}	2.14^{z}

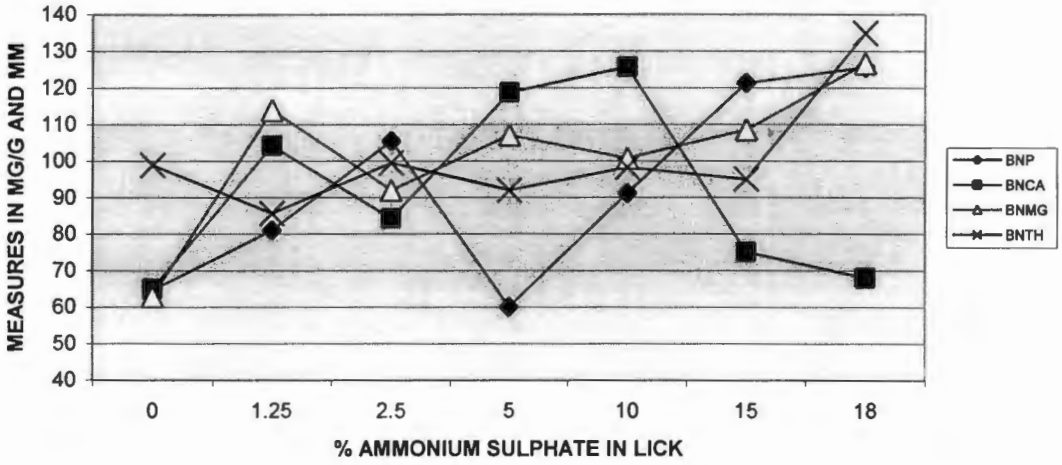
$^{w, x, y, z}$ means with different letters in a column are significantly different within a treatment group between % ammonium sulphate or weeks ($P<0.05$)

When concentrations of bone Ca and P from the anionic treatment group were compared, it was found that only at weeks 1, and 4 did Ca and P respond in the same direction of either increasing or decreasing concentrations from the previous mean content. During the other weeks of supplementation these 2 minerals responded in opposite directions that is, when P content of the bone increase then the calcium content decreases (Table 13).

The above findings are in agreement with Beighle *et al.* (1995, 1997); Belonje (1978) and Block (1984) when they found that the movement of Ca and P in and out of the bone in the animals in the anionic treatment group were in opposite directions for most weeks of supplementation. When Ca values decrease the P value increases and the other way round.

They also found a substantial effect of the anionic diet on the ability of animals to maintain a constant Ca:P ratio in the bone with a wide variation in the bone. In the current results the Ca:P ratio also showed a wide variation varying from 1.5:1 to 5:1. It should also be noted that the bone thickness followed bone P and not Ca (Fig. 8)

FIG 8. THE RELATIONSHIP BETWEEN BONE P, CA, MG AND BONE THICKNESS IN MG/G AND MM



BNP = BONE P X 1; BNCA = BONE CALCIUM / 2.77; BNMG = BONE MAGNESIUM X 7.5;

BNTH = BONE THICKNESS X 63

An increase in the concentrations of faecal (wk 5 and 6; Table 13) and bone P at wk1, 5 and 6 (Table13) and blood from wk1, 5 and 6 in the anionic treatment group, are all evidence of a P-sparing effects as previously reported by Beighle *et al*, (1997) when they found that the anionic treatment group demonstrated simultaneous increases in bone, faecal and blood P concentrations at various stages of the experiment compared to the control treatment group.

Bone thickness also responded to the anionic diet. When bone P increased from 81.12 mg/g (wk1) to 105.43 mg/g (wk2), bone thickness also

increased from 1.36mm to 1.58mm (wk1 to wk2) but when the bone P decreased from 105.12mg/g to 60.18 mg/g (wk 3) bone thickness also decreases from 1.58mm to 1.46mm (wk3). That relationship is observed until week 6. Bone P increased from 121.27 mg/g to 125.68 mg/g (wk 6) and the bone thickness also increased from 1.51mm to 2.14mm (wk6) (Table 13, Fig.8)

Bone Mg fluctuated and it is difficult to build a relationship between bone Mg, Ca and P. When bone Ca increased, bone Mg also increased but only at 1.25% NH_4SO_4 (wk1) and 5% NH_4SO_4 (wk3) and 2.5% NH_4SO_4 (wk2) were bone Ca decreased with a decrease in bone Mg. At the other weeks of supplementation wk4, 5, and 6 (10%, 15% and 18% NH_4SO_4) these two minerals responded in an opposite direction (Table 13, Fig.8).

Bone Mg and P both responded in the same direction at 1.25,15 and 18% NH_4SO_4 and at 2.5, 5 and 10% NH_4SO_4 they responded in opposite directions (Table 13). This is also in agreement with Beighle et al.,(1995) who also failed to find any relationship between Mg and Ca+P in bone (Fig. 8).

8.5. BODY MASS

TABLE 14: EFFECT OF LICK ANIONS ON WEIGHT GAIN AND LICK CONSUMED BY THE CALVES.

DIETARY ANION	**LICK CONSUMED**			**WEIGHT GAIN KG**	
%NH_4SO_4 IN BONE MEAL LICK	TREAT	CONT **KG**	NH_4SO_4 CONSUMED /AU/DAY IN THE LICK (G)	TREAT **Kg per group or kg/au/day**	CONTR **Kg per group or kg/au/day**
18	0.25^a	0.25^a	4.5	26.25^a/0.33	14.37^b/0.18
15	0.5^a	0.320^b	7.5	6.25^a/0.08	3.75^b/0.046
10	0.25^a	0.25^b	2.5	1.875^a/0.02	-2.5^b/-0.03
5	0^a	0^a	0	0^a/0	8.75^b/0.11
2.5	0.75^a	0.325^b	18.75	27.5^a/0.34	15.62^b/0.20
1.25	1.5^a	0.325^b	1.88	-18.87^a/-0.236	0.625^b/0.008
0	0	0	0	0	0

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

As can be seen the acidogenic diet was responsible for a significant ($P<0.05$) increase in the body weight gained by the calves. The mean weight gained by the calves fed 18% Ammonium Sulphate anionic diet was 26.25 kg/group and 0.33kg/au/day at the end of the trial compared with 14.37 kg and 0.18 kg/au/day for those calves on the control diet. While the average blood concentrations of P was highest in the calves fed 18%, 15 and 2.5% of

ammonium sulphate, the weight gained by the calves on the treated group was also greater at these percentage levels of the lick than the control group. In each case the highest level of ammonium sulphate in the lick supplement resulted in a better weight gained by the calves (Table 14.)

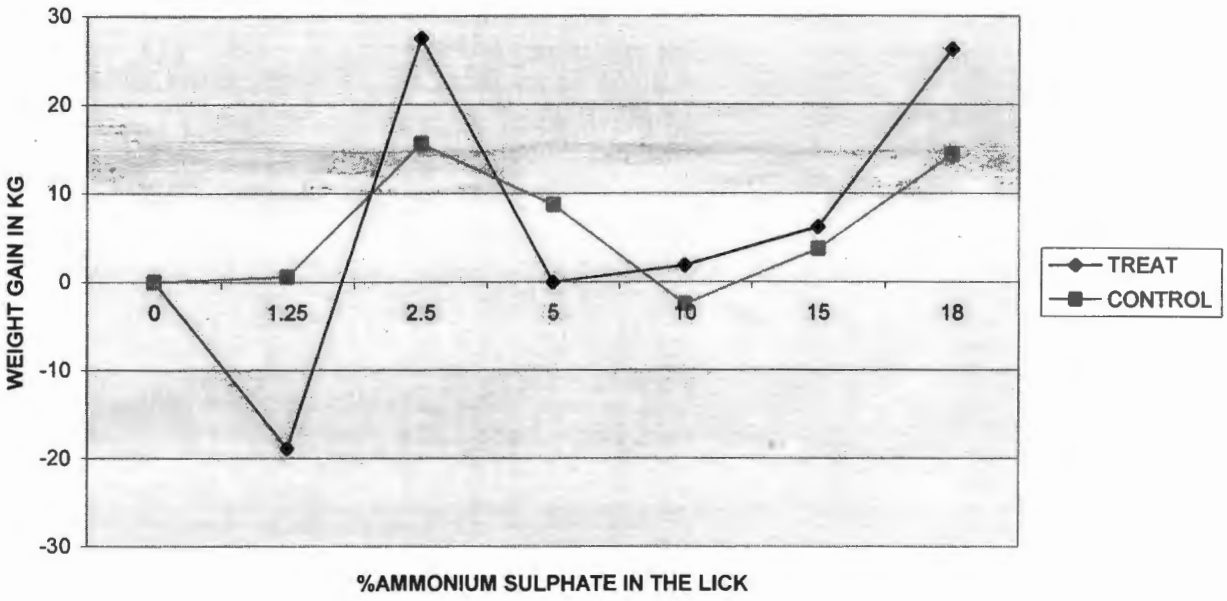
8.6. LICK CONSUMPTION

The mean weight gained by the calves at 18% Ammonium Sulphate and 2.5% Ammonium Sulphate in the lick was significantly higher (26.25 kg and 27.5 kg) than those on the control lick and the calves fed these licks consumed more lick, that is 0.25 kg equals the controls lick consumption and 0.75 kg more than that of the control animals (Table 14).

At week 5, the calves given 15% Ammonium Sulphate consumed 0.5 kg of the lick but gained less weight as compared to the calves receiving the 2.5% and 18% ammonium sulphate lick. The calves that were fed 5% Ammonium Sulphate in the lick consumed no lick, gained no weight and the serum inorganic P was also lower at that time period (Table 14). With the exception of what happened at 15% Ammonium Sulphate level were the calves consumed 0.5kg of the lick but gained 6.25kg of weight, it is clear that the more the calves consume Ammonium Sulphate lick the more they gained weight. This further supports the argument that the anionic supplements had a beneficial effect on the calves.

These means are not only significantly different but show a definite trend towards the very positive effects of the anionic diet on the ability of the calves to gain weight during the experiment and the inability of the calves on the control diet to gain as much weight as their counter parts.

FIG 9. THE RELATIONSHIP BETWEEN WEIGHT GAINED BY ANIMALS FED A CERTAIN PERCENTAGE OF AMMONIUM SULPHATE IN THE LICK BETWEEN THE TREATED AND THE NONTREATED GROUP.



The 2.5 kg loss of weight in week 4 of the calves receiving the control diet is far below all calves receiving the control diet on the other weeks of supplementation (Table 14, Fig.9).

The treated group gained significantly more weight on weeks 2, 4, 5 and 6 with the highest mean of all being 27.5 kg at week 6 and the nearest being 26.5 kg on week 2. At week 3 the animals on the treated group did not gain weight. This in turn points out the positive effect of an anionic diet in increasing the weight gained by animals than those receiving the control diet (Fig 9).

During week1 there were heavy rains that spoiled the lick and damaged the roughage given to the calves. This was responsible for the effect that led to the loss of weight especially by the treatment groups.

8.7. CONCLUSION

- The anionic lick was responsible for an increase in the faecal phosphorus concentration of animals on a fresh, dry and ash weight basis.
- The anionic lick was capable of increasing bone, blood and faecal phosphorus simultaneously when expressed in fresh, dry and ash weight.
- The acidiogenic lick was able to increase blood inorganic phosphorus with the exception of what happened in week 3 when the animals on the treated group did not consume any lick and the mean blood inorganic P was far less than that of the control group.
- The faecal P concentration of the treated group remained high throughout the experiment. At the same time there was increasing inorganic P in the blood and bone as a result of the addition of anions in the diet. This additional P must have come from 'somewhere else in the animal's body' or from the diet itself, rather than the bone or blood.
- The anionic lick was responsible for a significant ($P<0.05$) increase in rib bone thickness from 1.36 to 2.14 mm.
- The anionic lick showed a definite trend towards the very positive effect on the ability of the calves to gain more weight during the experiment compared to their counterparts on the control lick.
- The anionic lick led to more P being lost in the faeces and on the other hand, P concentration of the bone and blood were concurrently increased.
- The results of this research indicate that the best lick combination is a 2.5% or 18% Ammonium Sulphate + Bone Meal Lick.

- The suggestion is that acid-forming elements in the intestine allow for greater P solubility and easier absorption.
- Further research is needed to investigate the formulation of an anionic lick that prevents the increasing faecal P content, but at the same time allow for increasing bone and blood P advantageously.
- Faecal Ca in the faeces of the anionic treatment group was higher than that of the control in most weeks of supplementation.
- Bone Ca of the treated group remained lower than that of the controls except at 1.25% NH_4SO_4 when the treated group mean Ca concentration was higher than the control group.
- Bone thickness responded in the same way as bone P at most sampling periods. When bone P increased, bone thickness also increased and when bone P decreased bone thickness also decreased.
- Bone Ca and P absorption and resorption by bone was in opposite directions in most weeks of supplementation. When bone Ca increased bone P decreased and when bone Ca decreased bone P increased.
- Bone Mg was unpredictable when compared to the other two mineral parameters.
- Animals on the anionic diet had increasing bone P and decreasing bone Ca for most weeks.
- Results clearly show that bone P responds more positively to the dietary anions than bone Ca and it acted independently of Ca, indicating that bone P responds to an anionic diet differently than bone Ca.
- In this trial it was demonstrated that the anionic diet was responsible not only for a decrease in the bone and blood P at certain percentage levels of the diet in the experiment, but also for increased concentrations of bone and blood P at other percentage levels of the diet (i.e. 1.25, 10, 15 and 18% NH_4SO_4).

- The addition of anions, preferably at a higher anionic diet from 10% to 18% and a lower anionic diet of 1.25% NH_4SO_4 have a beneficial effect, sparing P in the body. This could have very beneficial effects in animals forced to graze on P-deficient veld.

APPENDIX 1: FAECAL PHOSPHORUS MEANS AND THEIR RESPECTIVE STANDARD ERROR MEANS

All these standard error means were calculated by using the Microsoft Excell statistical software on the tools menu-Data Analysis (Two sample assuming equal variances).

WEEKS		FRESH WEIGHT Mg/g	DRY WEIGHT Mg/g	ASH WEIGHT Mg/g
0	T	2.75 ± 0.18	2.92 ± 0.18	19.84 ± 1.98
	C	1.45 ± 0.23	1.53 ± 0.25	16.18 ± 3.28
1	T	2.35 ± 0.16	2.42 ± 0.17	18.62 ± 1.78
	C	1.25 ± 0.24	1.33 ± 0.22	15.04 ± 1.22
2	T	1.19 ± 0.20	2.24 ± 0.39	10.87 ± 1.87
	C	0.98 ± 0.19	1.94 ± 0.37	8.78 ± 1.58
3	T	1.13 ± 0.37	2.049 ± 0.67	9.21 ± 3.15
	C	0.93 ± 0.20	1.71 ± 0.39	8.001 ± 1.83
4	T	1.67 ± 0.36	1.77 ± 0.36	10.77 ± 2.76
	C	1.16 ± 0.34	1.23 ± 0.36	9.13 ± 2.24
5	T	2.69 ± 0.22	2.99 ± 0.37	29.17 ± 2.64
	C	2.62 ± 0.17	2.85 ± 0.24	26.58 ± 2.12
6	T	327 ± 0.17	3.64 ± 0.35	32.96 ± 2.55
	C	1.60 ± 0.24	1.71 ± 0.36	17.78 ± 3.93

APPENDIX 2:BONE PHOSPHORUS MEANS AND THEIR RESPECTIVE STANDARD ERROR MEANS

All these standard error means were calculated by using the Microsoft Excell statistical software on the tools menu-Data Analysis (Two sample assuming equal variances).

WEEKS		FRESH WEIGHT Mg/g	DRY WEIGHT Mg/g	ASH WEIGHT Mg/g
0	T	64.72 ± 21.12	70.53 ± 23.20	131.29± 37.42
	C	105.71± 37.70	115.09± 38.03	188.64± 64.45
1	T	81.12 ± 20.15	131.05± 46.26	206.47± 70.26
	C	65.33 ± 17.63	107.96± 32.72	130.54± 38.56
2	T	105.43± 20.54	114.81± 22.37	176.31± 33.58
	C	88.71 ± 19.66	90.77 ± 21.18	146.98± 21.81
3	T	60.18 ± 14.75	94.53 ± 28.75	154.36± 49.04
	C	54.78 ± 20.33	79.85 ± 34.87	91.71 ± 41.68
4	T	91.21 ± 31.80	103.67± 36.44	168.39± 60.01
	C	62.93 ± 16.95	68.45 ± 18.39	110.99± 29.82
5	T	121.27± 26.55	131.94± 28.85	199.38± 40.44
	C	101.87± 14.53	105.44± 15.66	170.34± 16.97
6	T	125.68± 30.07	157.07± 50.29	210.58± 62.54
	C	92.945± 10.78	124.27± 16.75	176.76± 21.83

APPENDIX 3: BLOOD PHOSPHORUS, BONE THICKNESS AND WEIGHT GAIN MEANS AND THEIR RESPECTIVE STANDARD ERROR MEANS

All these standard error means were calculated by using the Microsoft Excell statistical software on the tools menu-Data Analysis (Two sample assuming equal variances).

WEEKS		BLOOD (Mg%)	BONE THICK-NESS (mm)	WEIGHT GAIN (KG)
0	T	4.84 ± 0.94	1.57 ± 0.18	-
	C	2.87 ± 0.48	1.45 ± 0.17	-
1	T	5.60 ± 0.83	1.36 ± 0.16	-18.875
	C	2.28 ± 0.41	1.54 ± 0.13	0.625
2	T	2.94 ± 0.39	1.58 ± 0.08	27.5
	C	2.53 ± 0.42	1.44 ± 0.19	15.62
3	T	1.90 ± 0.45	1.46 ± 0.17	0
	C	2.17 ± 0.27	1.57 ± 0.18	8.75
4	T	4.27 ± 0.76	1.56 ± 0.17	1.875
	C	3.59 ± 0.32	1.37 ± 0.18	-2.5
5	T	7.02 ± 0.93	1.51 ± 0.18	6.25
	C	4.51 ± 0.69	1.21 ± 0.14	3.75
6	T	7.76 ± 1.12	2.14 ± 0.15	26.25
	C	4.08 ± 0.72	1.45 ± 0.23	14.37

APPENDIX 4: FAECAL CALCIUM AND MAGNESIUM MEANS WITH THEIR RESPECTIVE STANDARD ERROR MEANS

All these standard error means were calculated by using the Microsoft Excel statistical software on the tools menu-Data Analysis (Two sample assuming equal variances).

CALCIUM FAECES		WK0	WK1	WK2	WK3	WK4	WK5	WK6
FRESH	T	1.28± 0.23	2.78± 0.45	4.87± 0.13	4.62± 0.28	2.65± 0.24	2.80± 0.35	3.77± 0.50
	C	1.02± 0.22	2.47± 0.56	4.31± 0.33	4.70± 0.13	2.40± 0.35	1.57± 0.30	3.39± 0.59
DRY	T	1.32± 0.32	2.87± 0.55	5.02± 0.52	4.69± 0.65	2.70± 0.54	2.88± 0.63	3.88± 0.70
	C	1.05± 0.32	2.54± 0.68	4.44± 0.56	4.77± 0.55	2.45± 0.75	1.61± 0.54	3.49± 0.65
ASH	T	3.72± 0.35	8.38± 1.23	14.36± 1.23	6.09± 1.67	3.14± 1.87	7.05± 1.97	11.57± 2.80
	C	2.76± 1.22	4.71± 1.65	11.28± 0.26	6.14± 1.87	2.32± 1.13	2.90± 1.23	9.05± 2.53
MAGNESSIUM FAECAL		WK0	WK1	WK2	WK3	WK4	WK5	WK6
FRESH	T	0.173± 0.011	0.54± 0.015	0.45± 0.012	0.31± 0.01	0.57± 0.16	0.58± 0.19	0.40± 0.13
	C	0.173± 0.011	0.48± 0.10	0.35± 0.04	0.35± 0.05	0.42± 0.02	0.52± 0.01	0.33± 0.002
DRY	T	0.179± 0.012	0.55± 0.03	0.46± 0.03	0.31± 0.02	0.59± 0.03	0.59± 0.03	0.41±. 0.03
	C	0.178± 0.03	0.50± 0.021	0.36± 0.10	0.36± 0.012	0.43± 0.032	0.54± 0.02	0.34± 0.03
ASH	T	5.259± 5.1	16.41± 4.2	13.79± 6.545	3.87± 0.3	6.86± 0.12	14.48± 7.336	12.29± 3.55
	C	4.74± 0.006	9.51± 2.34	9.46± 2.33	4.54± 0.005	4.08± 0.003	9.54± 2.32	8.59± 1.22

APPENDIX 5: BONE CALCIUM AND MAGNESIUM MEANS WITH THEIR RESPECTIVE STANDARD ERROR MEANS

All these standard error means were calculated by using the Microsoft Excel statistical software on the tools menu-Data Analysis (Two sample assuming equal variances).

BONE SAMPLE

CALCIUM	BONE	WK0	WK1	WK2	WK3	WK4	WK5	WK6
FRESH	T	180.12± 6.55	288.73± 5.65	233.43± 4.65	328.94± 3.87	348.09± 6.66	208.21± 5.36	188.47± 5.23
	C	135.10± 3.55	218.23± 7.00	310.79± 7.66	325.13± 7.95	378.88± 8.99	294.28± 6.54	283.42± 6.44
DRY	T	196.14± 0.22	293.92± 0.32	243.39± 0.15	317.45± 0.32	442.52± 0.42	310.41± 0.44	249.00± 0.30
	C	152.83± 0.01	344.80± 0.75	350.54± 0.87	353.01± 0.85	411.38± 0.88	485.92± 0.90	411.04± 0.88
ASH	T	320.68± 10.23	491.28± 11.36	394.57± 11.16	555.29± 12.21	574.06± 12.33	466.25± 11.35	355.13± 11.20
	C	244.90± 8.23	425.95± 10.88	565.16± 12.98	559.54± 15.13	642.36± 17.17	584.59± 13.56	576.07± 13.83

MAG	BONE	WK0	WK1	WK2	WK3	WK4	WK5	WK6
FRESH	T	8.39± 0.02	15.19± 0.51	12.27± 0.62	14.28± 0.70	13.44± 0.65	14.47± 0.71	16.90± 0.88
	C	8.22± 0.03	13.25± 0.64	13.64± 0.67	13.98± 0.69	15.43± 0.55	12.46± 0.63	18.79± 0.93
DRY	T	9.13± 0.92	18.27± 1.83	12.48± 1.12	14.65± 1.34	13.75± 1.25	16.45± 1.75	20.56± 2.56
	C	9.22± 0.98	15.22± 1.68	13.44± 1.23	14.05± 1.31	16.07± 1.77	13.28± 1.27	23.90± 2.99
ASH	T	14.85± 8.23	22.61± 13.58	13.85± 6.46	17.18± 11.25	16.07± 11.00	19.07± 12.32	26.15± 16.32
	C	14.82± 8.20	16.32± 10.58	16.06± 10.34	16.79± 10.67	19.81± 12.19	14.69± 8.16	26.82± 16.55

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