AN INVESTIGATION INTO THE USE OF DICALCIUM PHOSPHATE AND COMPLEX AD_3E ON MINERAL STATUS AND HOMEOSTASIS OF FREE RANGING BEEF CATTLE DURING DRY SEASON

BY

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DECLARATION OF INDEPENDENT WORK

I , Cornellius Senyatso Morake, with Identity number and student number , do hereby declare that this research project submitted to the Central University of Technology, Free State for the Degree Master of Agriculture (Animal Science) is my own independent work; and complies with the code of Academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Central University of Technology, and has not been submitted before to any other institution by myself or by any other person in fulfilment (or partial fulfilment) for the attainment of any qualification.

Signature of Student:  **Date: December 2019**

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1 Peter 1:7 *These trials will show that your faith is genuine. It is being tested as fire test and purifies gold, when faith remains strong through trials it will bring praise and glory to the Lord.*

LIST OF ABBREVIATIONS

1.25-(OH) 2D3	= 1.25-dihydroxyvitamin D3
Ca	= Calcium
CaSR	= Calcium sensing receptor
DCAD	= Dietary Cation-Anion Difference
DM	= Dry Matter
FGF23	= Fibroblasts growth factor receptor
PTH	= Parathyroid hormone
P	= Phosphorus
RXR	= Retinoid X receptor
Na	= Sodium
VDR	= Vitamin D receptor
NRC	= National Research Council
DCP	= Dicalcium phosphate
DNA	= Deoxyribonucleic acid
ATP	= Adenosine triphosphate
SAVC	= South African Veterinary Council
ng	= nanogram
mM	= millimolar
MDCP	=Monodicalcium phosphate
CYP24AI	= Genes responsible for hydroxylation and degradation of Vitamin D sterols
TLRs	= Toll- like receptors
CD 14	= Marker molecule for monocytes and macrophages
TP	= Total protein
GLU	= Glucose
CREA	= Creatinine
BUN	= Blood urea nitrogen
HCC	= Hair coat condition
BSC	= Body condition scores
BW	= Body weights
BLP	= Blood phosphorus
BLCA	= Blood calcium
ALB	= Albumin

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ABSTRACT

In South African commonage beef production, revenue is adversely affected by low mineral concentration in pastures especially during the winter season. Therefore, this study was undertaken to accurately determine the mineral status of grasses, blood and the blood metabolite important for effective livestock production. Body weights, body condition score, hair coat condition, and blood mineral status and blood metabolites were physiological parameters used as indicators for growth and health. A total of thirty Bonsmara calves were blocked according to age and randomly assigned to 3 groups. In the three groups D1 was allowed maintenance ration only, D2 was given dicalcium phosphate lick adlib and D3 was fed dicalcium phosphate lick adlib and were also inoculated with a shot of vitamin AD3E plus minerals on top of the maintenance ration on monthly intervals after sample collections. Grass P concentrations ranged between 1.12 mg g^{-1} and 1.52mg g^{-1}, Ca from 6.18 mg g^{-1} to 13.03 mg g^{-1} and Mg from 1.50 mg g^{-1} to 2.48 mg g^{-1} and were lower than those recommended to meet animal mineral requirements. Highest mean ($P < 0.05$) values (245.13kg and 239.00kg) for BW were recorded in April and May, respectively, while the lowest mean value (204.20kg) was recorded in July. Body scoring condition (BCS) recorded highest ($P < 0.05$) mean value (5.87) in April with comparable mean values (5.70 and 5.67) in May and June, respectively, while the lowest mean value (5.50) was observed in July. Hair coat condition (HCC) was highest ($P < 0.05$) in animals offered D3 with comparable mean value (3.2) in animals treated with D2, while the lowest value (3.08) was recorded in animals receiving the D1 diet. Blood P and Mg were higher (1.74 mmol/L, 0.60 mmol/L and 1.85 mmol/L, 0.75 mmol/L) in animals receiving D2 and D3, respectively, than those on D1 diet (0.78 mmol/L and 0.44 mmol/L). Blood Ca increased across the treatments with highest value (3.70 mmol/L) in animals receiving D3, while the lowest value (1.57 mmol/L) was observed in animals fed with D1. Albumin, blood urea nitrogen, creatinine and total protein with highest ($P < 0.05$) mean values (45.33 G/L, 5.56 uMOL/L. 147.68 FLMOL/L and 73.83 g/l) were recorded in animals receiving D1, while lowest values were observed in animals receiving diets D2 and D3, respectively. Higher glucose mean values (3.25 and 3.36 mmol/L) were recorded in D2 and D3, respectively, compared to D1 (1.99 mmol/L). Faecal P (2.40 mg g^{-1}, 2.27 mg g^{-1} and 2.24 mg g^{-1}) and Ca (12.63 mg g^{-1}, 13.21 mg g^{-1} and 12.70 mg g^{-1}) were highest in May, June and July, respectively, while the lowest Faecal P value (1.06 mg g^{-1}) and Faecal Ca (10.13 mg g^{-1}) were recorded at the beginning of the experiment. Higher (5.10mg g^{-1}) Faecal Mg was documented in June with comparable mean values (1.91mg g^{-1} , 1.95 mg g^{-1} and 2.73 mg g^{-1}) recorded for April, May and July, respectively. The study indicates that minerals can be successfully utilized in the wintering of replacement calves on communal grazing systems.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background

In many parts of South Africa (SA), livestock farming is a viable agricultural activity with about 14, 1 million cattle. Beef production constitutes the bulk part of livestock farming, at about 80% of livestock production. Producers vary from commercial to communal subsistence farmers that rely on indigenous practices. These groups co-exist and own about 80% of the available herd, of which 60% is from commercial farmers and 40% is owned by emerging and communal farmers (DAFF, 2012). South Africa, like other countries, is experiencing climatic changes coupled with long, harsh and dry winter seasons, which leave our grazing areas deficient of the essential nutrients required for the physiological and metabolic needs of free ranging beef cattle (Bakunzi *et al*., 2012). This condition calls for the need to improve the low quality crop residues and forages with supplements like dicalcium phosphates and AD_3E complex plus minerals. Dicalcium phosphate is an ionic salt particle of calcium and phosphate, which is recommended by nutritionists during winter and summer (Viljoen, 2001[a]). Complex AD_3E plus minerals is a balanced micro-emulsion of minerals and vitamins used in the livestock industry for prevention and treatment of mineral and vitamin deficiencies.

Supplementation of calves on low quality crop residues and forages with dicalcium phosphate and AD_3E complex plus minerals will improve intake and digestibility of diets commonly used during winter (De Brouwer *et al.,* 2000). Increased costs of production have caused livestock producers to reduce feed costs whenever possible. Low quality crop residues which are relatively abundant, offer considerable potential for reducing feed costs for cattle farmers. The positive effects of dicalcium phosphate treatment on digestibility and intake of low-quality forages make them a viable option in cattle feeding programmes (Bakunzi *et al.,* 2012).

The trend of multivitamin and mineral injection is popular in the livestock industry due to the perception that it will replace mineral loss by forages during winter (Gadberry & Simon, 2012). The escalating cost of oral mineral supplementation has increased very much, exceeding R300/50kg bag, therefore feeding cost of an individual cow could approach costs higher than the economic thresholds. Winter supplementation improves animal weights, reproduction and health, whilst marginal mineral deficiencies may have direct adverse effects on the same production parameters (Spears, 1995).

Cattle require a proper balance of water, energy, protein, vitamins and minerals to achieve optimal production levels. However, it is not unusual for grasses to be deficient in one or more minerals. Phosphorus requirements for animals cannot be investigated in isolation as Ca and Vitamin D are also closely linked with those in many metabolic processes. Adequate phosphorus levels, calcium and phosphorus ratios, as well as suitable levels of vitamin D are critical for mineral homeostasis. Supplementation of phosphorus in a form that can be absorbed by the animal and can also be stored or used to support the physiological processes is essential if optimal livestock health and productivity are to be achieved (Horst *et al*., 1994).

Many experiments conducted in Southern Africa showed that phosphorus given as the only supplement during the dry season, when animals in the grass-veld areas are losing weight, has no beneficial effects on the economic performance of animals (Rautenbach *et al*., 2006). Lack of proper nutritional management has led to deficiencies that are usually manifested in the form of metabolic diseases like hypocalcaemia, hypomagnesia, and osteomyelitis (Bakunzi *et al*., 2012). Several studies on mineral homeostasis in beef cattle grazing natural pastures (MacDowell *et al*.; 1983: De Brouwer *et al*.; 2000: Bakunzi *et al*., 2012) revealed that Ca and P deficiencies resulted in loss of income due to metabolic disorders. A significant increase in weight, fertility and disease resistance was realized in cattle supplemented with dicalcium phosphate, but results from one experimental farm cannot be extrapolated directly to the other due to differences in limiting factors such as soil type, grass mineral content and other climatic conditions (De Brouwer *et al*., 2000).

1.2 Problem statement

Several studies have evaluated the mineral contents of the grasses on communal rangeland but it is still not known whether the results are applicable to all commonages and communal farms of the Republic of South Africa (De Waal *et al*., 1996; De Waal & Koekemoer, 1997; De Brouwer *et al*., 2000.; Mokolopi & Beighle, 2006). Poor grazing conditions as a result of low rainfalls, high temperatures, overgrazing, overstocking, erosion and poor mineral supplementation may adversely affect the outcomes and the true reflection of a study, hence, more trials should be done on mineral supplementation in cattle. Ruminants obtain most of their nutrients from natural grasses; consequently, they become susceptible to seasonal changes and subsequent mineral deficiencies. Also, the season has been shown to affect concentration of phosphorus and calcium in soils and plant parts, thus resulting in deficiencies, which cause clinical and subclinical losses due to metabolic diseases (Bakunzi

et al., 2012). During the dry season, calves may be unable to obtain enough dry matter to meet their nutritional requirements, which may result in poor weight gains. On the other hand, farmers are reluctant to give supplementary mineral licks to their animals because of the high cost of supplementation, coupled with a lack of knowledge on what, when and how to supplement (Mokolopi & Beighle, 2006).

1.3 Research objectives

The main aim of the study is to investigate the use of dicalcium phosphate and complex AD_3E on the mineral status and homeostasis of free ranging beef cattle during dry seasons.

1.3.1 Specific objectives

1. To compare the recovery response time of animals treated orally to those treated with injectable minerals on Ca and P homeostasis in the dry season;
2. To determine the effects of DCP and co-AD_3E injection on serum minerals and chemistry and faecal mineral levels;
3. To investigate the effects of DCP and co-AD_3E on weight gains, body condition scoring and hair coat colour of animals grazing poor quality grass during the dry season;
4. The study will attempt to determine the relationship between hair coat score and calcium and phosphorus status in free ranging calves;

1.4 Hypothesis

The proposed dicalcium phosphate or complex AD_3E+mineral supplementation will improve the mineral status, blood chemistry, weight gains, body condition scoring and hair coat condition of calves grazing poor quality grass during the dry season.

CHAPTER TWO

LITERATURE REVIEW

2.1 The role of Ca and P in free ranging beef cattle

Calcium (Ca) is used in the formation and maintenance of bones and teeth. It also functions in the transmission of nerve impulses and contraction of muscle tissue. A dynamic system involving Ca, P and vitamin D exists to maintain a relative stable concentration of blood Ca and P homeostatic levels in cattle. Both minerals are stored in bone and mobilized into the circulatory system when there is a dietary calcium deficiency because the intake of the two minerals is in-adequate. As a result of the importance of Ca in bone structure, deficiency in young animals leads to skeletal deformities (rickets), while in older animals fragile bones can result from extended periods of dietary calcium deficiency (Hale & Olson, 2001).

According to Hale and Olson (2001), the critical times to ensure that diets contain adequate calcium are during pregnancy for proper bone growth of the foetus and during lactation periods to prevent excessive calcium mobilization from the bones of lactating cows and to maintain mineral homeostatic levels. Excessive mobilization of calcium from the skeletal system of lactating cows can lead to milk fever also known as peripurient paresis or hypocalcaemia with symptoms which include muscle stiffness and tremors, extreme weakness and loss of consciousness.

Phosphorus (P) functions in conjunction with calcium in bone formation. In addition, P is a component of deoxyribonucleic acid (DNA), the molecules which make up chromosomes and genetic inheritance. It is also involved in the chemical reactions of energy metabolism. Phosphorus, containing compounds like adenosine triphosphate (ATP) and monophosphate, is the body's major storage depot of readily available energy. Phosphorus deficiency is reported to be the most prevalent deficiency in free ranging beef cattle, because of its insufficiency in grass (Hale & Olson, 2001).

Other researchers (Mokolopi & Beighle, 2006; Peacock, 2010; Bakunzi *et al*., 2012) also documented that deficiency of P results in decreased animal performance, including reduced animal weight gains, poor reproductive efficiency and low milk production. For their role in bone metabolism, Ca and P supplementation are considered simultaneously, with the recommended ratio of 2:1 or 1.2:1. Any significant deviation from the ratio can result in abnormal bone formation and a condition known as water-belly (kidney stone).

The metabolic and physiological role of Ca and P is essential for the development and maintenance of skeletal tissue, maintenance of osmotic pressure and acid base balance, energy utilization and transfer, protein synthesis, transport of fatty acids, amino acid exchange and growth and cell differentiation (Fukumoto, 2004).

Vitamin D is also reported as an important regulator of calcium and phosphorus homeostasis in ruminants (Horst *et al*., 1993; Gibbens, 2012). Two major sources of vitamin D to ruminants are derived from photochemical conversion of 7-dehydrocholestrol to vitamin D3 in the skin or plates, and as a result of photochemical conversion of erogosterol to vitamin D2. A disruption of calcium and phosphorus homeostasis may not only lead to clinical and subclinical hypocalcaemia, but can also lead to numerous homeostatic disorders, including metritis, ketosis, retained placenta and abomasal displacement (Gibbens, 2012).

The primary function of vitamin D is to elevate plasma calcium and phosphorus to a level that will support normal mineralization of bones. It is also recorded that vitamin D plays a regulatory role in immune cell functions, as it regulates gene expression through its binding to tissue-specific receptors and subsequent interaction between the bond receptor and DNA. It is fundamental for the transcription regulation of a specific gene which typically includes 1, 25-(OH) 2D3 induced modulation in mRNA levels. Recent studies have identified a heterodimers of the vitamin D receptor (VDR) and a vitamin D receptor (RXR) within the nucleus of the cell as active complex for mediating positive transcriptional effects of 1,25-(OH)2D3 (Horst *et al*., 1994.

2.2 Effects of Mg on Ca and P availability

The normal plasma concentration for calcium, inorganic phosphorus and magnesium are 8.5-11.4, 3.1-6.0 and 1.8-3.2mg/100ml, respectively (Gibbens, 2012). To understand the interaction of these macro- nutrients we need to explore how they influence one another. Any deficiency or lack of them will lead to a homeostasis breakdown, which may result in hypocalcaemia, hypomagnesia and other metabolic disorders. Mg concentration below 0, 75 to 1,0mmol/L will reduce the secretion of PTH in response to hypocalcaemia, by reducing tissue sensitivity to PTH. Ca homeostasis is highly dependent on the Mg levels (Gibbens, 2012). For the PTH to mobilize Ca from bones, promote Ca absorption from the digestive tract and to stimulate the kidney to excrete excess phosphorus, while retaining Ca for reabsorption; it needs Mg to stimulate the PTH (Taylor *et al*., 2007). Mg is needed for Ca

absorption and without enough Mg, Ca collects in the soft tissues and causes one type of arthritis. Sufficient amounts of Mg determine this delicate and important balance. Without Mg, Ca may be not fully utilized, and under- absorption problems could lead to clinical and subclinical symptoms (Gibbens, 2012). Ca, P and Mg have a high diagnostic value in determining the nutritional status of animals due to their low variability in blood. Serum Mg levels reflect the current daily intake rather than reserves; thus, cattle are affected by low magnesium dietary content. Blood Mg levels below the critical threshold result in Grass tetany (Ndlovu *et al*., 2007).

2.3 Sources of minerals for ruminants

Ruminants grazing on pastures can receive a certain proportion of their minerals from water and soil ingestion (MacDowell, 1997; Rautenbach *et al*., 2006). However, plants are the main natural sources of minerals to ruminants. The mineral composition of plants depends on a number of factors, including soil type and composition, plant species, stage of maturity and dry matter yield of the plants, pasture management (fertilizer regimen) and climatic conditions. Most naturally occurring mineral deficiencies in ruminants are associated with specific regions and are directly related to soil characteristics. As plants mature, mineral concentrations decline due to a dilution process and translocation of nutrients to the root system (MacDowell, 1997).

Pasture management, forage yields and climate can influence the species of forage predominating and also change the leaf and stem ratio, thereby having a direct bearing on the mineral concentration of the pastures. Pastures show a decrease in leafiness and an increase in the stem and leaf ratio with age, which is usually realized during dry seasons (Rautenbach *et al*., 2006). Generally, the two sources of minerals include natural feeds (grasses and grains) and mineral supplements to balance the minerals in grasses (MacDowell, 1997).

2.4 Bioavailability of mineral for ruminants

Blezinger (2000) described mineral bioavailability as the amount of ingested nutrients absorbed and available to body physiological processes. Mineral bioavailability depends on several factors which include genetic make-up, trace mineral interaction, stage of production, reproduction response, microbial digestion, rumen pH levels, climatic factors and mineral supplementation strategies. Cattle have a compound or complex

gastrointestinal system that allows them to digest diets with high fibre contents, as well as a genetic make-up that influences its bioavailability of some fibrous rations and grasses. Certain trace minerals will either promote or hinder other minerals' absorption rates, for example excessive dietary zinc will negatively affect the availability of copper in ruminants. Hypomagnesia will hinder Ca absorption in the digestive tract due to low PTH levels in the soft tissues, leading to unavailability of minerals (Gibbens, 2012). Different stages of production, like the transition period will influence the bioavailability of other minerals differently, for examples of Ca. Pregnant and lactating cows have higher bioavailability than dry cows (Blezinger, 2000).

Immune response will definitely influence the bioavailability, as certain antibody tithers will either enhance or hinder mineral availability. Reproduction responses and maintenance of the new foetus will increase the bioavailability of minerals due to an increased intracellular exchange. Extreme pH levels will negatively affect the bioavailability of nutrients in the rumen. The optimum pH level value of the nutrient's absorption ranges between/from 6.3 to 7.2 for maximum absorption. Climatic factors which may lead to moist or hardened supplements will reduce the mineral intake, therefore reducing the absorption rate and bioavailability of supplemented minerals (Blezinger, 2000).

2.5 Mineral requirements of ruminants

Mineral requirements of livestock are dependent on the level of productivity. Increased growth rates and milk production will greatly increase mineral requirements of cattle. Improved management practices that lead to improved milk production and growth rates for cattle will necessitate more attention to mineral nutrition (MacDowell, 1996). A calcium requirement depends on the state of calcium metabolism, which is regulated by three mechanisms, intestinal absorption, renal absorption and bone turnover. These in turn are regulated by a set of interacting hormones, including parathyroid hormones (PTH), 1.25 dehydroxyvitamin D (1.25(OH) 2D), ionized calcium and their cross-ponding receptors in gut, kidney and bone (Peacock, 2010). The P requirements, as well as dietary P supply, can vary greatly during the year for a beef cow. Grazing during a period of good grass growth will provide considerably more P than winter pasture or corn stalks. Likewise, the requirement for P is considerably higher during peak production stages like lactation and calving (Karn, 2004). The beef mineral requirement of NRC (1996) suggests a range of 0.16 to 0.23% dietary P (DM basis) to cover the P needs of cows consuming a wide range diet.

2.6 Mineral supplementation

Mineral supplementation is increasingly becoming a challenge as farmers are trying to optimize the production levels of their animals. Hybrids and genetically improved animals result in higher production performance, which leads to increased mineral needs and alternative supplementation regimes. Indiscriminate supplementation of feed with minerals may be harmful as excess levels may be toxic to animals. Excess mineral supplements or mineral imbalances may have an antagonistic effect on other essential nutrients, thus resulting in deficiencies which may be manifested in clinical and subclinical disorders. Assessment of nutritional status of animals and their needs must be established prior to feeding self-prepared mixes and other premix formulas (Herdt, 2016).

2.6.1 Evaluation of mineral requirements

Gadberry & Simon, (2012), an extension livestock specialist at Arkansas University of Agriculture, reported that pasture tests and water tests provide a gross indication of nutrients available to the animal. It does not indicate the biological availability or how much is actually available for the animal physiological processes. Blood and tissue analysis are more expensive compared to a pasture test. Good indicators of deficiencies in the herd involve multiple screening of animals and having their values compared with averages observed at laboratories and reported in literature to provide a benchmark to determine deficiencies. It is important to ensure that a correct sample and correct sample packaging measures are undertaken for proper mineral analysis procedures. If deficiency is suspected, expert advice should be sought and if the deficiency is confirmed, the suspected mineral can be added to the diet taking consideration of the factors which affect bioavailability and toxicity (Herdt, 2016).

The approach to mineral feeding to avoid overfeeding and underfeeding requires a well-planned mineral supplementation programme guided by a proper mineral analysis of nutrient sources and needs of animals. This should improve cattle performance and reduce costs of production. For proper balancing of rations, a number of factors must be considered. Water mineral content, mineral requirements of a particular class of animals, mineral content of pastures (feed) available, results of mineral status of feed and pastures and the estimated feed intake are all supposed to be considered to get a clear understanding of the mineral status of animals (Gadberry & Simon, 2012).

2.6.2 Methods of mineral supplementation

Blackwood & Clayton (2007) refer to mineral supplementation as measures of supplying deficient nutrients for the purpose of survival or production, especially during the periods of drought and peak mineral requirements. Several conflicting reports have been published by (Blackwood & Clayton, 2007; Gadberry & Simon, 2012) on the use and benefits of oral and injectable methods of mineral supplementation to animals. The advantages and disadvantages of this method rely on the principles which include, identifying the most limiting component, selection of the limiting component, balancing of supplements, ensuring efficient rumen function, and choosing feeding techniques which are cost -effective (Blackwood & Clayton, 2007). There are six common ways of supplementing cattle with minerals, namely offering free choice individual mineral sources in a cafeteria system, offering a mixture of minerals as lick, supplementing with minerals mixed with concentrates, feeding a mixture of minerals individually to cows, offering injection inoculations or oral dosing and low-releasing bullets or soluble impregnated glass or bolus (MacDowell,1983).

2.6.3 Pathway of mineral supplements

Subcutaneously administered components go under the skin via tissues into the bloodstream and later into target cells, while topical enters the body through the skin into the tissues through the blood to target cells. Oral route involves ingestion through the gastrointestinal system, and later absorbed in the small intestines into the blood system and target cells (Gadberry & Simon, 2012).

2.7 Mineral homeostasis

Mineral homeostasis refers to a state of mineral equilibrium which is controlled by the intra- and extra-cellular levels of two ions Ca and P with three hormones, parathyroid (PTH), the active metabolite of vitamin D 125 dihydrovitamin D (1.25(OH)2D3), and fibroblast growth factor 23(FGF23) acting on three target tissues (intestines, bones and kidney). Other ions involved in normal homeostasis are pH, sodium, potassium, magnesium, chlorine, bicarbonate, and sulphur, which all alter the cellular handling of Ca and P (Bikle, 2008). Likewise, other hormones including calcitonin, prolactin, glucocorticoid hormones, growth hormones, insulin, insulin-like growth factors (IGFs), and a large number of cytokines contribute in important ways to the regulation of homeostasis. A large number of tissues other than bone, intestines, and the kidneys are target tissues for the calciotropic hormones in ways that contribute to bone mineral homeostasis (Bikle, 2008).

Macro- and micronutrients are inorganic substances essential to maintain the normal functions and the living status in domestic and wild animals, including humans. These nutrients play a critical role in the physiological processes related to health, growth, and reproduction, and adequate function of the immune and endocrine system (Djokovic *et al*., 2014). Blood levels of calcium and inorganic phosphorus in dairy cows during the peripartum period and lactation reflect their metabolism or supply of these macronutrients through feeding and their utilization by peripheral tissues, the mammary gland in particular. Any decline in their blood levels below the physiological limit and any deficiency of these nutrients and their unfavourable ratio in lactating cows generally lead to subclinical and clinical manifestations which can adversely affect animal health and fertility (Djokovic *et al*., 2014).

2.7.1 Calcium and Phosphorus homeostasis

The ingested calcium and phosphorus are absorbed by different segments of the small intestines. The active absorption of sodium (Na+) throughout the entire course of the intestines results in large net water adsorption. This mainly occurs in the small intestines, where calcium and phosphorus are concomitantly taken up in passive, paracellular manner and down their concentration gradient. The active transcellular adsorption of phosphorus occurs predominantly from the ileum, whereas active calcium, transportation takes place largely from the duodenum (Renkena *et al*., 2008).

Calcium and phosphorus homeostasis are a coordinated interaction of intestinal uptake, reabsorption in the kidney and bone demineralization establishes the maintenance of normal calcium and phosphorus balance. The calcium sensing receptor (CaSR), present in the thyroid glands, senses blood calcium levels and triggers the secretion of the calcitropic hormones, parathyroid hormone and calcitonin. Similar hormones are involved in the regulation of phosphorus balance. The blood phosphorus levels are controlled by PTH, 1,25(OH) 2D3, klotho and fibroblast growth factor member 23(FGF23). A negative feedback mechanism prevents the accumulation of FGF23 and klotho since 1,25 (OH) 2D3 productions are inhibited by FGF23 and klotho (Renkena *et al*., 2008).

Any mineral imbalances can cause metabolic disturbances and, in some cases, they can produce specific deficiency diseases like ketosis and hypocalcaemia. These mineral deficiencies will affect mineral homeostasis and subsequently productivity, fertility, conception rate and calving percentage (Herdt, 2016). Several authors have reported that indiscriminate mineral supplementation have a negative impact on mineral homeostasis

(McGrath *et al*., 2012: Gibbens, 2012) and more recently, Herdt, 2016).This supplementation may lead to poor mineral interactions and the suppression of other essential elements, thus resulting in mineral imbalances (Blezinger, 2000).

2.7.2 Effects of Ca and P homeostasis on body condition scoring

Calcium and phosphorus homeostasis is essential for optimum physiological functions in beef cattle production. Beef cattle have nutrient requirements in priority order for maintenance, foetal development, lactation, growth and breeding. Ca and P have a high diagnostic value in determining the nutritional status due to their variability in blood plasma (Rossi & Wilson, 2006). Body condition scoring is an accurate method commonly used to assess the nutritional status of free ranging beef cattle (Ndlovu *et al*., 2007). This visual evaluation method can be employed and used easily by emerging and subsistence farmers. Productivity is adversely affected during the dry seasons and consequently the body condition scores; as adverse body conditions are associated with poor nutritional status, poor weight gains and mineral imbalances.

Poor body condition scores are associated with reduced income per cow, increased post-partum intervals, weak calves at birth, low quality and quantity colostrum and lower weaning weights (Rossi & Wilson 2006; Ndlovu *et al*., 2007). Adequate mineral supplementation during peak nutritional requirement times during the dry season will have a positive effect on condition scores (Rossi & Wilson, 2006).

2.7.3 Effects of mineral homeostasis on body weights

Growth is measured as an increase in body weight. It includes not only cell multiplication (hyperplasia), but also cell enlargements (hypertrophy) and the incorporation of specific components from the environment. Body weights are commonly used because it is easier and quicker to perform, and usually employed by several researchers as indicators of production (Ndlovu *et al*., 2007). Body weight should be used concurrently with body condition scores as it is a subject of variation. They are also influenced by different factors, including stage of pregnancy and lactation, gut fill, breed and size and more importantly, by nutritional or mineral supplement. Body weights can also be used to inform livestock owners and nutritionists of their individual mineral requirements (Ndlovu *et al*., 2007: Mishra *et al*., 2016). As attributed by De Waal *et al*. (1996), mineral supplementation proved to have a significant effect on animal weight gains; a fact attested by Horst *et al*. (1994) that anionic

diets influence metabolism, homeostasis, cell development and cell proliferation. Mineral supplementation stimulates bone mineral deposition, skeleton development and anabolic body growth, which results in animal mass gains (Gunther & Tekin, 1987).

2.8 Effects of mineral homeostasis on hair coat score

Hair coat condition score has become popular and may be useful in assessing mineral deficiencies and imbalances. The hair coat condition in animals is an indicator of general animal health. Cattle with a healthy hair coat are more likely to grow and perform to their genetic potential, while cattle with dull, off- coloured hair are associated with prolonged nutritional deficiencies or experiencing some level of poor mineral homeostasis (Gill *et al*., 2004). Protein, minerals and vitamins are constantly required to keep the hair follicle healthy (Gill *et al*., 2004: Fisher *et al*., 2003). Lack of nourishment from any of these nutrients can lead to lowered hair health. Hair coat scores can be used as a parameter to evaluate the mineral status and general health of cattle. This method, if correctly practised, can be used as a parameter for early detection of adverse mineral imbalances in free ranging beef cattle (Fisher *et al*., 2003).

2.8.1 Effects of mineral homeostasis on conception rates

Mineral deficiencies have been reported to have adverse effects on conception rates in most countries in the world, and South Africa is not an exception (MacDowell *et al*., 1983; Kawas *et al*., 1993; Mokolopi & Beighle, 2006; Bakunzi *et al*., 2012;). A conception rate of 20-100% was reported in the tropical regions when free ranging cattle were supplemented with minerals. The study also revealed a growth rate of 10-20% and a significant decrease in the mortality rate. Conception rate is important for early detection and identification of infertility as a result of poor mineral homeostasis (MacDowell *et al*., 1983). Mineral imbalances during the breeding season may result in hormonal disorders, irregular oestrus cycle and lower conception rate of free ranging beef cattle (Lippolis, 2011).

2.8.2 Blood mineral contents

Blood levels of Calcium and inorganic phosphorus in cows during the peripheral periods of lactation reflect the metabolism or supply of these nutrients through feed and their utilization by the peripheral tissues. Any decline in Ca and P concentration in the blood below the physiological limits and ratio in ruminants generally lead to subclinical and clinical

manifestations that adversely affect animal production, health and fertility (Djokovic *et al*., 2014). Phosphorus concentrations in blood plasma are normally 1.3 to 2.6mg/dl, 6 to 8mg/dl for growing cattle and 4 to 6mg/dl for adults (NRC, 2001).

About 1 to 2g circulates as inorganic phosphate in blood of a 600kg animal. Due to the greater concentration of erythrocytes in blood, whole blood contains 6 to 8 times more phosphorus than plasma. Approximately 5 to 8g are present in the extracellular pool of a 600kg cow. The intracellular concentration of phosphorus is about 25mmol/l (78mg/dl), and total intracellular phosphorus is about 155g in a 600kg cow. Phosphorus is also required by ruminal microorganisms for digestion of cellulose and the synthesis of microbial protein. Phosphorus from the dietary sources and salivary recycling within the rumen should be at least 5g/kg of organic matter digested to optimize degradation of cell walls from feeds by microbes (NRC, 2001).

Plasma calcium concentration constitutes about 98% of the calcium in the body and is found within the skeleton, where calcium along with phosphate ions serves to provide structural strength and hardness to bone. The other 2% in the body is found primarily in the extracellular fluids of the body. Normal calcium concentration in blood plasma is about 2.2 to 2.5mM (9 to 10mg/dl) in the adult cow, with slightly higher values in calves. Approximately 40 to 45% of the total calcium in plasma is bound to plasma proteins, primarily albumin and the other 5% is bound to organic components of the blood such as citrate or inorganic elements (NRC, 2001).

2.9 Blood metabolites

The blood metabolites concentration represents an integrated index of the adequacy of nutrient supply and utilization. The blood biochemical profile is important for accurate diagnosis and prognosis of disease associated with nutritional deficiencies. Seasonal and physiological variation may affect their concentrations in the animal blood serum. Blood metabolites are very important indicators of animal nutritional status, as their insufficiency is highly correlated to certain physiological parameters like body weights and body condition scores. They have recently been accepted as accurate indicators of nutrient availability and metabolism (Ndlovu *et al*., 2007; Mapiye *et al*., 2010). Blood biochemical parameters such as glucose, urea, total protein creatinine and albumin are becoming the most important parameters for the diagnosis, prevention, treatment and prognosis of animal's metabolic diseases (Mapiye *et al*., 2010: ISSI *et al*., 2016).

2.10 Feacal minerals excretion

Phosphorus excretion occurs in faeces and urine of ruminants, but normally it is of little significance. The excess of phosphates during resorption of bone tissue is excreted from the body via the kidneys in young ruminants, while in adults it is eliminated through the gastrointestinal tract. Cows excrete 88.2% of phosphorus consumed on a daily basis, out of which 66.6% in faeces, while the rest is either excreted via the urine, milk, saliva and sweat (Iqbal *et al*., 2005). Phosphorus in faeces is either exogenous, which is unabsorbed dietary P, and also P from intestinal cell walls and digestive secretions. The P excreted endogenously is crucial for the maintenance of mineral homeostasis (NRC, 2001). Faecal excretion of P may be divided into three fractions, viz P of dietary origin unavailable for absorption, P of endogenous origin that inevitably has to be excreted under actual nutritional and physiological conditions, and P of the endogenous origin which is excreted to maintain homeostasis. The total endogenous faecal P may constitute more than 2/3 of the total faecal P in cattle and sheep (Iqbal *et al*., 2005).

The extent of faecal mineral excretion differs for different minerals and depends on the feed intake of the cow. Faecal mineral output that is not of endogenous origin comes from dietary minerals that are not absorbed in the gastrointestinal tract. If dietary Ca is limited, the production of calcitriol increases, and this may result in the more efficient uptake of Ca in the intestines and thus decreases faecal excretion of Ca. The digestibility and solubility of minerals from different sources differs. The solubility of magnesium oxide which is mostly used in mineral supplements has a high influence on the absorption and faecal losses of magnesium (Kronqvist, 2011).

2.11 Grass mineral content

The phosphorus levels of our grasses are notoriously low in pastures, due to poor P in most of the soils. Therefore, the mineral content of most grazing areas is significantly too low to support optimum productivity of cattle sufficiently. As a result of this, fertilization of pastures and mineral supplementation become a general practice to livestock farmers. The area's micro-climate has a direct bearing on the mineral content of pastures. To optimise range livestock production, veld minerals must be evaluated regularly depending on the average rainfall of the area (De Brouwer *et al*., 2000).

There is a positive correlation between grass mineral content and blood mineral content of free ranging beef cattle. Their level is also positively correlated to fertility, health and weight

gains. Grass mineral status escalates with the stages of plant maturity and seasonal changes. Grasses are the primary source of essential minerals like Ca, P, Mg, K, S, Fe, Mn and Cu for cattle and has a direct bearing on the metabolic and physiological functions (MacDowell, 1997).

2.12 Dicalcium phosphate treatment

It is a nutritional challenge to achieve both calcium and phosphorus homeostasis in cattle, as such a diet must supply significant absorbable inorganic calcium required to stimulate PTH secretion (Gibbens, 2012). It is apparent that for optimum performance, animals in natural pastures should have regular access to carefully balanced mineral supplements. The animal body contains less than 5% by mass of mineral elements; some of which are in minute content only, but their functional importance in the metabolism of the animals is of high importance for fertility, health and growth rate (Groenewald & Boyazoglu, 1980).

In a country like South Africa where there are so many deposits of minerals in soils, herbage is frequently found to be lacking mineral elements, this is especially true to phosphorus. This is generally attributed to the seasonal climatic variations in the large stock farming areas, where long, dry winters occur, when veld grass leaches and becomes white tough and unpalatable, and low in nutritional value (Groenewald & Boyazoglu, 1980).

2.13 Overview of dicalcium phosphate

A nutritionist at Kynoch feeds defines dicalcium phosphate as a calcium phosphate with the chemical formula $CaHOP^4$ and it is a dehydrate. The “di” prefix is the common name that arises, because the formation of the $HPO^2{}_4$ anion involves the removal of two protons from phosphoric acid, H_3PO_4. It is also known as dibasic calcium phosphate or mono hydrogen phosphate. Dicalcium phosphate is an ionic salt, meaning it is made up of charged particles of calcium and phosphate, both of which human and animals need in the diets (Viljoen, 2001).

Dicalcium phosphate has a chemical formula CaHPO4; it is a combination of positively charged particles of calcium and negatively charged particles of hydrogen phosphate, which is interchangeable with phosphate in the body. As such it is a source of both the mineral calcium phosphate which may be used for a variety of purposes, including producing bone matrix and synthesis of DNA, which is responsible for genetic material (Viljoen, 2000).

2.13.1 Bioavailability of dicalcium phosphate

Feedstuffs of plant origin do not contain enough digestible phosphates to meet the requirements for animal production. For this reason, additional inorganic P is added to animal diets in the form of dicalcium phosphates. A fraction of the feed ingested is inevitably lost in the normal digestion and metabolic process, meaning that no element is completely absorbed or available for the biochemical functions of the animal body (Viljoen, 2001b). He further revealed that variation in the bioavailability within sources with same generic description can be enormous, so the most accurate method to evaluate the bioavailability figures for a specific product is for a reputable manufacturer to provide bioavailability figures tested by a reputable institution, which employs sound techniques (Viljoen, 2001b).

To substantiate the above-mentioned statement, we refer to Kynofos 18 Grande, a dicalcium phosphate, dihydrate, feed grade (V3255), and produced by Yara animal nutrition, a member of the animal feed manufacturers association (AFMA), the official industrial body of the formal animal feed manufacturing industry. According to the manufacturer, Kynofos, 18 Grande was analysed for quality using the AOAC Official methods of analysis; Petermann method (AOAC, 1991; AOAC, 1990_65).

2.14 Kynofos product description

2.14.1 Nutritive value

Dicalcium phosphate is produced from the only high quality defluorinated phosphoric acid-based on the high-quality phosphate ore found in the country. Together with good quality slaked lime used in the closely controlled manufacturing process, it ensures a high bioavailability and minimum undesirable elements below those stipulated by the European Union (EU). The dihydrous form makes the product more biologically available than the anhydrous products. It is widely used as a versatile P supplement for all type of feeds, and it is recommended for use in concentrate, compound feeds, mineral feeds and free-choice supplements for ruminants and monogastricts (Viljoen, 2001b).

2.14.2 Effects of dicalcium phosphate on animal weights

The results of the study by (Gunther & Tekin, 1987) on the effectiveness of dicalcium phosphate as mineral supplement during pig fattening are in complete agreement with the findings revealed by (Bakunzi *et al*., 2012). The experiment yielded increased body weight

and carcass weight (Gunther & Tekin, 1987). Several independent studies in South Africa determined that dicalcium phosphate not only corrects mineral deficiencies, but it also plays a very significant role in the mass gain of free ranging beef cattle during winter dry seasons (De Waal *et al*., 1996: De Brouwer *et al*., 2000: Bakunzi *et al*., 2012).

2.14.3 Effects of dicalcium phosphate on body condition scores

Body condition scoring describes the systematic process of assessing the degree of fatness of an animal, and several authors have documented the association between body scoring condition and weight gain, a parameter usually related with increased productivity (Rossi & Wilson, 2006 Ndlovu *et al*., 2007). A linear increase in body condition scoring was observed in the dairy cows fed on Napier grass, supplemented with dicalcium phosphate and a linear decrease in non- supplemented animals in the same trial (Rahman *et al*., 2014). Optimum body condition scores are usually used as visual parameters to evaluate animal productivity and can also be used as an essential management tool for fertility and nutritional status of free ranging beef cattle (Ndlovu *et al*., 2007). Significant body condition scores are attributed to the high percentages of Ca and P in the dicalcium phosphate supplement (Rahman *et al*., 2014).

2.14.4 Effects of dicalcium phosphate on conception rate

A conception rate of 88% was reported in a trial at Potchefstroom on heifers in natural pastures supplemented with dicalcium phosphate (De Brouwer *et al*., 2000), and similar results were observed at Armoedsvlakte (De Waal & Koekemoer, 1997: De Waal *et al*., 1996). Weight gains and optimum body condition scores influenced positive conception rates in animals supplemented with dicalcium phosphate rations and these further prove the effectiveness of minerals in diets (Rahman *et al*., 2014). The findings by Kawas *et al*. (1993) revealed that an increase of 20-100% in conception rate was achieved in animals supplemented with dicalcium phosphate in the tropical regions. Iqbal (2005) and Rahman *et al*. (2014) reported that inadequate supplementation of dicalcium phosphate is usually associated with unthriftiness, in appetence decreased feed intake, poor growth and poor reproduction. They further stated that in heifers with P deficiency, incidences of depressed fertility and low conception rates are frequently experienced. The imbalance of calcium and inorganic phosphorus induces the disturbance of locomotors system and slowed down evolution of the uterus, which may result in irregular cycles, abortions, endometritis, abnormal heat signs and eventually decreased conception rates (Hadzimusic & Krnic, 2012).

2.14.5 Effects of dicalcium phosphate on immunity

The effects of dicalcium phosphate on its own on immunity has not been not fairly reported by local and international authors, but in a study at Potchefstroom (De Brouwer *et al*., 2000) the control and low treatment group, which did not receive P supplementation during winter showed that the P status of this group decreased from sufficient to an acute deficiency within 175 days. The results from this study in Potchefstroom also showed that from a group of 24 cows, three cows died of emaciation and other complications brought about by aphosphorosis, while others were killed as a result of fractured pelvises which occurred during subsequent mating seasons. The remainder of the group displayed classical symptoms of aphosphorosis, which included stiff gait, emaciation, coarse hair coat, and accelerated hoof growth (De Brouwer *et al*., 2000).

2.14.6 Effects of dicalcium phosphate on production

Phosphorus deficiency is intimately associated with depressed growth rates and generalized impairment of bodily functions. It is not surprising, therefore that effects of P deficiency which include unthriftiness, decreased feed intake, worm infestation, pica, poor growth and reproduction (Iqbal, 2005). Cows fed a diet deficient in calcium and phosphorus may develop an insidious and complex syndrome characterized by weight loss, rough hair coat, and abnormal stance, and lameness, spontaneous fracture in vertebrae, pelvis and ribs. This effect is particularly prevalent during winter dry seasons when the quality of pastures is very low and in soils which are notoriously deficient in phosphorus (De Waal *et al*., 1996). Apart from the above indicated roles, mineral homeostasis is important for high yielding milk production in dairy cows (Hadzimusic & Krnic, 2012).

2.15 Interaction of vitamin D on Ca and P availability

Vitamin D was originally discovered nearly more than a century ago as a factor in butterfat that prevented rickets. In years that followed, it was also found to be synthesized in the skin exposed to sunlight and critically involved in calcium homeostasis (Nelson & Merriman, 2017). The physiological role of the vitamin D system continues to evolve beyond Ca and skeletal mineral homeostasis, to include significant roles in modulating innate and adaptive immune functions. It has long been recognized that vitamin D deficiency, as reflected in serum 25(OH) D3 concentrations, causes decreased resistance to infection, but this action was generally thought to be secondary to endocrine effects of vitamin D and Ca metabolism (Lippolis, 2011). Vitamin D from the diet is absorbed from the intestinal tract, due to longer

retention time of feed in the intestinal tract. It is absorbed from the intestinal tract in association with fats, as are all fat- soluble vitamins. Like the others it requires the presence of bile salts to be absorbed and is absorbed with other neutral lipids via chylomicron into the lymphatic system of the animals. As a result of this, only 50% of the oral dose is absorbed; however sufficient amounts are produced daily from the exposure to sunlight (Nelson& Merriman, 2017).

Vitamin D metabolism in ruminants begins prior to absorption. Degradability of vitamin D to active metabolites occurs in the rumen by microbes, which explains the higher vitamin D requirements in ruminants (Horst *et al.,* 1994). The vitamin D metabolic pathway begins with the photo conversion of 7-dehydrocholosterol, or through dietary supplementation. Vitamin D3 is readily converted to 25-hydroxyvitamin D3 by 1α-Hydroxylase, a tightly regulated enzyme expressed in the kidney and macrophages in cattle. The 1,25(OH) 2D3 activates the vitamin D receptors (VDR) and also induces its own catabolism via the 24-hydroxylase. The 24-hydroxylated vitamin D metabolized is further excreted in the bile (Nelson & Merriman 2017).

2.15.1 The role of vitamin D in Ca and P metabolism

Vitamin D and its metabolites can improve the calcium balance and facilitate mineral deposition in bone matrix largely without direct effects on bone cells, although some beneficial effects may occur via mature osteoblast, as demonstrated in mice osteoblast-specific overexpression of VDR and 1α hydroxylase (Nelson & Merriman, 2017). Bone mineralization and resorption are mediated by two types of cells: Osteoblasts, responsible for calcification and bone formation, whilst Osteoclasts are responsible for demineralization and adsorption. The process is regulated by a tight balance between activators and inhibitors (Goselink *et al*., 2015). Osteoblasts are presumed to have extracellular Pi sensing mechanism, as specific genes are more expressed when Pi influx is increased at high extracellular Pi levels. In response to increasing extracellular Pi levels the expression of FGF23 inhibits mineralization by stimulating the production of organic pyrophosphate (PPi). A low Pi to PPi ratio (low Pi availability) inhibits hydroxyapatite formation, while a high ratio promotes mineralization (Goselink *et al*., 2015).

2.15.2 Effects of Vitamin D on immunity

Vitamin D has been shown to play a role in regulating gene expression in immune cells and their ability to kill pathogens. Serum levels of the major form of vitamin D, 25(OH) D3, have

been correlated with the efficacy of human macrophages to kill mycobacterium tuberculosis in culture (Nelson *et al*., 2010). Several studies (Nelson *et al.,* 2010: Lippolis, 2011; Nelson & Merriman, 2017), concur that vitamin D consists of two pathways viz: endocrine and autocrine. The endocrine pathway affects Ca homeostasis, whereas the autocrine pathway affects immune cell functions. Bacterial- associated molecules stimulate 1α-OHase activity in macrophages. The 1,25D produced in macrophages stimulates production of defence proteins that kill bacteria (Nelson & Merriman, 2017). The active vitamin D hormone also contributes to immune, reproductive and mammary physiology and in cattle, 1, 25$(OH)_2D_3$ strongly enhances production of nitric oxide and β-defensin antimicrobial peptides molecules that are toxic to bacteria (Nelson *et al*., 2010).

2.15.3 Vitamin D and autoimmune in animals

Autoimmune diseases are characterized by an underlying inherited predisposition, but there is much current interest in environmental factors such as vitamin D that may contribute to the manifestation of these disorders (Hewison, 2010). Autoimmune diseases occur when the immune system is confused or overly stressed and begins to attack their own tissues instead of outside pathogens. Vitamin D insufficiency is incriminated in a number of physiological malfunctions, including autoimmune diseases. The transition period is known to exacerbate human autoimmune disease and ameliorate other diseases (Lippolis, 2011).

Vitamin D prevents autoimmune diseases by promoting regulatory T-cells, which are responsible for accurately differentiating between outside pathogens and self-cell destruction. Immune cells (B cells, T cells, monocytes, dendrite cells) from multiple autoimmune diseases appear to respond to the immunomodulatory effects of vitamin D (Mostafa & Hegazy, 2015). Vitamin D directly and indirectly regulates the differentiation and activation of the CD4+ T- lymphocytes and can prevent the development of autoimmune processes. Dendric cells (DC) are the professional antigen presenting cells (APC) that play an essential role in the initiation and maintenance of T- cell dependent immune responses. In-vitro 1.25 $(OH)_2D_3$ vitamin inhibits the differentiation of monocytes to DC, therefore it reduces the number of these professional APC to stimulate T- cells (Szodoray *et al*., 2008).

2.15.4 Vitamin D as a supplement in animals grazing natural pastures

Vitamin D is typically recognized as being necessary for proper bone formation and maintaining Ca and P in the body. It is also discovered that vitamin D is required for the activation of critical innate immune defences of cattle against microbial pathogens. In cases

of vitamin D insufficiency, cattle may be vulnerable to infectious diseases; therefore, it is critical to ensure animals are getting enough vitamin D in supplements (Nelson & Merriman, 2017). Vitamin D_2 and vitamin D_3 are used in supplementation of animals and human diets throughout the world. Vitamin D_3 is a form of vitamin D that is synthesized by vertebrates and Vitamin D_2 is the major naturally occurring form in plants. Vitamin D_2 occurs naturally in plants and may constitute as much as 1% of the total vitamin D in alfalfa (Horst *et al*., 1994). The bulk of vitamins are lost during harvesting and oxidation, those which are not available to animals. The entire vitamin D requirement for cattle during winter depends on the feeding programme (NRC, 2001).

2.15.5 Vitamin D requirements in cattle

Vitamin D is provided by sunshine over the summer but can be limited in the winter with shorter daylight hours. It is needed to prevent rickets, and reduces the incidence of having weak, deformed or dead calves during the calving season. Vitamin requirements are influenced by age, size of the animal, stage of production and health status. Generally, stress increases vitamin requirements. Vitamins can be supplemented by injections, free choice supplementation or orally with feed mixtures (Horst *et al*., 1994). Vitamin D is present in plasma at the concentration of 20 to 50ng/ml. Concentrations of <5ng/ml would be indicative of a vitamin D deficiency, and a concentration of 200 to 300ng/ml would indicate vitamin D toxicities (Horst *et al*., 1994).

The NRC recommendations for beef cattle are 275IU/kg of diet, however, beef cattle, may require addition supplementation to keep serum 25(OH) D3 above 20ng/ml during winter months with limited sun. Beef cows require 40.000IU to 60.000IU of vitamin A daily prior to calving, and after calving it increases to 60.000 to 70.000IU daily. As milk production increases, the requirements also increase. Vitamin D requirements are 10 per cent of the vitamin A levels, and most commercial products supply them in appropriate ratios. Vitamin E requirements are 200 to 300IU/ day pre-calving and 300 to 500IU/day post post-calving to beef cows and first-calving heifers (NRC, 2001).

2.15.6 Effects of vitamin D on production

Even though vitamin D levels have not yet been specifically determined for production, optimal serum 25(OH)D_3 concentration should be kept at ranges of 40-80ng/ml/ However, beef cattle in winter months or conditions with limited sunlight may require additional vitamin D supplementation to keep serum 25(OH)D_3 at levels above 20ng/ml (Nelson, 2011). Ca

and P homeostasis initiated by vitamin D is essential for growth, reproduction, gestation and lactation (Horst *et al*., 1994). High yielding dairy cows require additional Ca and P in rations, but equal vitamin D requirements is essential for increased production needs. Vitamin D deficiencies are incriminated for poor body scoring, poor production and poor weight gains and poor feed conversion rates, and most importantly poor absorption rates of Ca and P (Ndlovu *et al.,* 2007: Yaremico & Kreplin, 2015).

The imbalance in Ca: P ratio renders phosphorus unavailable and since this is exacerbated by a vitamin D deficiency, the problem may be manifested in conditions which may include poor production in animals (Yaremico & Kreplin, 2015). Phosphorus deficiency is intimately associated with generalized impairment of most physiological functions in the animal body which include unthriftiness, in appetence, decreased feed intake, worm infestation, poor growth, all which are incriminated for poor animal production (Iqbal, 2005).

2.15.7 Effects of Vitamin D on fertility

Reproductive efficiency of cows is greatly affected by optimal serum $25(OH)_2$ D_3 concentrations and proper levels of vitamins which are very important for successful reproduction (Horst *et al*., 1994). Vitamin D deficiency on reproduction indicated that a much smaller number of calves were born if a deficiency existed and that some calves were born dead, others were lacking vitality; while some cows did not even conceive. Vitamin D deficiencies may also result in suppression of the signs of oestrus and delayed puberty, uterine evolution and delayed first oestrus after calving in cattle (Nelson *et al.,* 2011).
The spectrum of vitamin D target organs has expanded, and the reproductive role of vitamin D is highlighted by expression of the vitamin D receptor (VDR) and enzyme that metabolize vitamin D in testes, reproductive tracts and spermatozoa. The expression levels of VDR and CYP24A1 in spermatozoa serve as positive predictive markers of semen quality. VDR mediates a nongenomic increase in intracellular calcium concentration that induces sperm motility (Jensen, 2014).

2.15.8 Effects of vitamin D on hair coat condition

Vitamin D was primarily acknowledged for its importance in bone formation; however, increasing evidence of its interference with proper function of nearly every tissue in the body including brain, heart, muscles, immune cells, skin and hair have proven to be of equal importance in the wellbeing of almost all livestock. Therefore, its deficiency has been incriminated in a long panel of physiological dysfunctions (Mostafa & Hegazy, 2015). An

optimum concentration of vitamin D is necessary to delay aging phenomena, including hair loss. Active vitamin D 1, 25 $(OH)_2$ D/VDR promotes the ability of β- catenin to stimulate hair follicle differentiation. VDR activation plays an important role in the hair follicle cycle, especially angen initiation (Mostafa & Hegazy, 2015).

Limited studies have been done in humans and animals to determine the role of vitamin D in the hair cycle. In vitro studies have supported the concept that VDR may play a vital role in the postnatal maintenance of the hair follicle. Mesodermal papilla cells and the outer root sheath epidermal keratinocytes express VDR in varied degrees in correlation with the stages of the hair cycle. In both the late anage and catagen stages there is an increase in VDR, which is associated with decreased proliferation and increased differentiation of the keratinocytes. The changes are thought to promote the progression of the hair cycle (Mostafa & Hegazy, 2015).

Vitamin D deficient cattle may exhibit hair coats that are coarse, rough, dull and off- colour e.g. black may become greyish (Foster *et al*., 2016). Cattle may present depigmentation, rusty or yellow tinges and often the hair will become brittle and easy to break. Vitamin D supplementation should be recommended towards achieving normal serum levels, thereby avoiding the deleterious effects accompanied by its deficiencies (Mostafa & Hegazy, 2015).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

The investigation was carried out during the period May–July 2018, which is a dry season in South Africa. The objective was to investigate the use of dicalcium phosphate and complex AD3 E on mineral status and homeostasis of free ranging beef cattle during the dry winter season. In this study, animal serum inorganic P (Pi), Ca and Mg, faecal P, Ca and Mg, grass P Ca and Mg, body condition scoring and hair coat condition were used as indicators of mineral nutrition status.

3.1.1 Study location

The study was carried out on the farm Slanghuewel (26°54'15.6" S 27 ° 57'11.5''E) in the Sasolburg area in the Northern Free State of South Africa. The grass population of the area consists mostly of the climax, sub-climax and pioneer grass species namely, *Eragrostis spp*, *Cynadon Dactylon* and *Themeda Triandra*. The area mostly consists of sandy to loam soil with an average rainfall ranging from 450 – 650 mm per annum and temperatures ranging from 17ºC to 27ºC (ACCU Weather Forecast, 2018). The study location was chosen because the farmers in this area generally feed roughages; crop residues and common salts in winter. The rates of metabolic disease accounted for are related to mineral deficiencies and are more prevalent during winter and spring.

3.1.2 Study animals

A total of 30 crossbreed Bonsmara type calves with a mean weight of 175 - 200 kg were used in the study. Animals were balanced on age, size, condition and randomly allocated into three experimental groups. The groups were allowed to graze on similar available pastures throughout the trial. The first control group (D1) was grassed on natural pastures and other plant residues throughout the experiment diets so they meet minimum requirements for calf's maintenance ration as stated by NRC (2001). The second group (D2) was allowed maintenance ration and dicalcium phosphate (DCP) lick, whilst the third group D3 was also allowed maintenance ration and dicalcium phosphate but was further injected with complex vitamin AD_3E+minerals monthly throughout the experiment from April to July. Group D2 and D3 were given DCP ad lib whilst complex AD_3E+minerals were inoculated

subcutaneously to D3 once during sample collections. The animal monitoring sheets were completed on a daily basis to monitor the animal health status during the experiment.

3.2 Sample collection

Grass, blood and faecal samples were collected on the study area (Slanghuewel farm). The first samples were collected at the end of April as representative of basic sample also known as day zero for trail. All animals were kept under the same conditions while the samples were collected. Animals were handled in a calm manner to prevent mineral variations due to stress. All groups were sampled on the same day to avoid time variations because of time lapses between sampling times. All samples were collected by competent officials from the Department of Agriculture (veterinary services), who are fully registered members of the South African Veterinary Council (SAVC).

3.2.1 Blood Collection

Blood samples from each animal were collected by jugular venepuncture into sterile red stoppered anticoagulant VAC-U-Test tubes for three months. Care was taken to ensure that animals are not stressed, as even a little stress may cause mineral variations. A minimum of 10 ml blood sample was collected per animal and immediately stored on ice. Two samples were collected per animal as backup. Collected samples were stored at 4^{o}C for 24 hours to allow clotting before centrifuging. Clotted blood samples were centrifuged at 1000rpm for 10 minutes to separate serum. Serum was transferred into clean sterile tubes using a sterile pipette and stored immediately at a temperature of -20^{o}C for later analysis. Mineral analysis was done by the North West University's Department of Animal Science.

3.2.2 Faecal Collection

Faecal samples were collected directly from the rectum using arm-length plastic gloves and placed into a sterile cap sample bottle without any contamination from the environment. Samples were sun dried and grounded through a 2 mm screen for later analysis.

3.2.3 Grass Collection

Grass samples were taken around the sampling area where the animals were grazing per single period of collection to prevent time and mineral variation between sampling periods. During grass sample collection, a 5m x 5m homogenous vegetation unit (HVU) was marked

in different directions to be used as replicates. In each HVU, three $1m^2$ quadrants were randomly put to sample grass species. All the species found were harvested, dried, ground and bulked together and placed in a tight container pending analysis.

3.3 Weighing of Animals

Individual animals were weighed during sampling intervals and used as an indicator of growth in the experiment. A Tal -Tec LS4 electronic digital scale was used for more accuracy.

3.4 Body Conditions Score

Body condition scoring was assessed and recorded during the sampling times. Condition scores were used as indicators of production. Mineral homeostasis and adequate supplementary feeding are essential for optimum body condition scoring with positive results in cattle production. The evaluation and recording of scores were done according to the criteria described by Mishra *et al.,* (2016).

Table 1: Body scoring condition adopted from Mishra *et al.*, 2016.

Score	Condition	Appearance
1	Emaciated	Shoulder, rib and back are visible
2	Very thin	Some muscle, no fat deposits
3	Thin	Some fat deposits, ribs visible
4	Borderline	Fore ribs not visible
5	Moderate	12^{th} and 13^{th} ribs not visible
6	Good	Ribs covered, sponginess
7	Very good	Abundant fat on tail head
8	Fat	Fat cover thick and spongy
9	Obese	Extremely fat throughout

3.5 Hair Coats Scoring

Hair coat scores were evaluated and recorded according to a five-point system (1=100% shed, 2=75%, 3=50%, 4=25% and 5 full hair), consistent with those described by Turner

and Schleger (1960) as cited by (Foster *et al.*, 2016). The length and the depth of the coat will be used as principal criterion of classification (Fisher et al., 2003).

3.5.1 Description of hair coat score

Score description 1: No detectable problem, healthy, shiny, slicking hair coat.
Score description 2: Slight shed and slick hair coat.
Score description 3: Halfway shed and hair coat exhibits off- colour.
Score description 4: There is enough dead hair to cover a significant percentage of the body.
Score description 5: Full hair which appears dead, brittle with no slick and is dull in colour.

3.6 Laboratory Analysis

3.6.1 Blood Analysis

Serum samples were prepared for a simple dilution procedure in 15ml disposable centrifuge tubes. In the centrifuge tube 0.6 ml of serum and 2% nitric acid were added. The samples were diluted to 6 ml with deionised water. QC samples were also prepared in an identical way using the calibration standard and blank solutions. The samples were centrifuged at 4000rpm for 10 minutes to separate solids that might have been left. The supernatant fluid was used to determine the micro- and macro mineral content of the blood, using the PerkinElmer ICP-MS instrument, AAS Flame spectrophotometer and a UV-VIS Spectrophotometer.

3.6.2 Faecal and Grass Analysis

Milled grass and faecal samples were analysed for laboratory dry matter (DM), Ash content and minerals (calcium (Ca), phosphorus (P), and magnesium (Mg).To determine the dry matter (DM) content, approximately 1g sample of grass and faeces were placed into pre-weighed crucibles and placed in an oven set at a temperature of 105°C for 12 hours, removed from the oven and placed in a desiccator to cool and weighed for dry matter content (DM). The loss in weight was determined as the moisture content and DM was calculated as the difference between initial sample weight and moisture weight. Organic matter content (OM) was determined by ashing the dried samples in a muffle furnace set at 600 °C for 16 hours. After ashing, crucibles were removed and placed in a desiccator to

cool. Samples were weighed and the loss in weight was measured as organic matter (OM) content and the residue as ash.

3.6.3 Mineral Analysis

The mineral content of all samples was determined using the dry ashing macro and trace minerals methods following the guidelines provided by the Agri-Laboratory Association of Southern Africa (AgriLASA, 1998). Phosphorus was analysed by using the Ultra Violet Spectrophotometer (Chemicallab instruments, method no. 075-01, Bavaria, Germany). Calcium, Mg were analysed by flame photometer on atomic absorption spectrophotometer (Perkin-Elmer, 1982).

3.7 Animal Management

Animals were kept under the same conditions that the farmer used. All farmers in the commonage practise a system known as night kraaling, where animals are grazed during the day and kraaled at night. The herd health management programme designed by the local Veterinary officials was also adhered to. Animal disease vaccination and routine parasite control were followed throughout the trial. The farm manager and farm workers were trained on the research protocol and animal monitoring sheets. The farm workers completed the monitoring sheet on a daily basis as they did their visual inspections and reported to the prime investigator in an effort to achieve early diagnosis of any problem that might have arisen during the trial. Animal ethics committee of the University of the Free State approved the student project number UFS-AED 2017/0049 under strict recommendation on animal welfare and methodology.

3.8 Statistical Analysis

Data collected during the trial was subjected to a general two-factor analysis of variances using the procedure of SAS (1996) to determine whether mineral supplementation and month of collection had an effect on blood, faecal mineral levels, hair coat condition and body condition scoring. The probability of variance was considered significant at 5% or less.

$Y_{ijk} = u + M_i + D_j + (M_i \times D_j) + e_{ijk}$

Where =

Y_{ijk} = dependent variable,

μ = population mean,

M_i = Effect of the i^{th} month

D_j = Effect of the j^{th} diet,

$(M_i \times D_j)$ = Interaction effect between the i^{th} month and the j^{th} Diet

E_{ijK} = residual error, assumed to be independently distributed

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

Table: 2 Mineral content of grass harvested from the study area in winter season

Parameters	APRIL	MAY	JUNE	JULY	MEAN
P	1.31±0.02^{a}	1.12±0.05^{a}	1.19±0.03^{a}	1.52±0.08^{a}	1.29±0.03
Ca	8.23±1.03^{ab}	6.19±0.54^{b}	9.47±1.52^{ab}	13.03±1.85^{c}	9.23±1.46
Mg	2.02±0.22^{a}	2.48±0.40^{a}	1.50±0.03^{a}	1.71±0.06^{a}	1.93±0.07

^{abc}: Means along the same row with different superscripts are significantly different (P <0.05)

Grass P concentrations ranged between 1.12mg g^{-1} to 1.52mg g^{-1}, Ca from 6.19mg g^{-1} to 13.03 mg g^{-1} and Mg from 1.50 mg g^{-1} to 2.48mg g^{-1}. No significant (P>0.05) differences between grass P and Mg were recorded between months except for grass Ca, where a significant decrease was seen from April to May and then an increase in June and July with means ranging from 6.19mg g^{-1} to 13.03 mg g^{-1}. In general, the grass minerals levels were too low to meet the animal maintenance requirements for minerals, as stated in the NRC, (1996). Most farmers also give mineral licks with vitamin and minerals injectable, not taking into consideration the loss of excess minerals thought the faeces. Grass mineral concentrations reported here are in agreement with findings reported by De Brouwer et al. (2000); Mokolopi & Beighle (2006); Bakunzi *et al.*, (2012) and, more recently, Mokolopi (2019).

Table 3: Growth parameters and blood minerals as affected by diet in the season

Parameters	APRIL	MAY	JUNE	JULY	SEM
BW	245.13^{a}	239.00^{a}	223.90^{b}	204.20^{c}	5.08
HCC	2.50^{c}	2.87^{b}	3.60^{a}	3.87^{a}	0.11
BCS	5.87^{a}	5.70^{ab}	5.67^{ab}	5.50^{b}	0.10
Blood P	1.05^{c}	2.03^{a}	1.41^{b}	1.33^{b}	0.66
Blood Ca	1.88^{d}	3.55^{a}	3.22^{b}	2.47^{c}	0.11
Blood Mg	0.35^{b}	0.47^{ab}	0.61^{ab}	0.95^{a}	0.06

^{abc}: Means along the same row with different superscripts are significantly different (P <0.05)

Highest mean (P <0.05) values (245.13kg and 239.00kg) for BW were recorded in April and May, respectively, while the lowest mean value (204.20kg) was recorded in July. BLP and BLCA had highest (P <0.05) mean values (1.41 and 3.55) in May while the lowest mean values (1.05 and 2.27) were recorded in April and July, respectively. Blood Mg was highest (P <0.05) in July with comparable mean values (0.47mmol/L and 0.61mmol/L) in May and June, respectively, while the lowest value (0.35mmol/L) was observed in April. HCC had highest (P <0.05) mean values (3.60 and 3.87) in June and July, respectively, while the lowest value (2.50) was recorded in April. BCS recorded highest (P <0.05) mean value (5.87) in April with comparable mean values (5.70 and 5.67) in May and June, respectively, while the lowest mean value (5.50) was observed in July (Table 7).

Table 4: Serum minerals as affected by experimental duration

Parameters	APRIL	MAY	JUNE	JULY	SEM
ALB (G/L)	43.67^{a}	40.33^{b}	37.53^{c}	36.43^{c}	1.00
BUN (uMOL/L)	6.01^{a}	4.08^{c}	4.83^{b}	4.46^{ab}	0.19
CREA (FLMOL/L)	147.63^{a}	105.83^{b}	108.27^{b}	98.60^{c}	2.46
GLU (MMOL/L)	2.31^{c}	2.73^{b}	3.02^{ab}	3.41^{a}	0.14
TP (G/L)	72.67^{a}	68.90^{b}	66.10^{ab}	65.27^{c}	1.07

^{abc}: Means along the same row with different superscripts are significantly different (P <0.05)

Albumin was highest (P <0.05) at the onset of the experiment while the lowest values (37.53G/L and 36.43G/L) were recorded in June and July, respectively. Blood Urea Nitrogen had the highest (P <0.05) mean value (6.01) in April with comparable mean (4.46uMOL/L) in July, while the lowest value (4.08 uMOL/L) was documented in May. Creatinine had the highest (P <0.05) mean value (147.63FLMOL/L) in April while the lowest mean value (98.60 FLMOL/L) was recorded at the culmination of the experiment. Glucose values increased (P <0.05) from the onset to the end of the experiment. Total Protein was highest (P <0.05) in April with the comparable mean value (66.10G/L) in June, while the lowest mean value (65.27G/L) was recorded in July.

Table 5: Monthly main effects of faecal mineral

Parameters	APRIL	MAY	JUNE	JULY	SEM
Faecal P	1.06[b]	2.40[a]	2.27[a]	2.24[a]	0.18
Faecal Ca	10.13[b]	12.63[a]	13.21[a]	12.70[a]	0.66
Faecal Mg	1.91[b]	1.95[b]	5.10[a]	2.73[b]	0.56

[ab]: Means along the same row with different superscripts are significantly different (P <0.05)

Faecal P values (2.40 mg g^{-1}, 2.27 mg g^{-1} and 2.24 mg g^{-1}) and Faecal Ca values (12.63mg g^{-1}, 13,21mg g^{-1} and 12.70mg g^{-1}) were highest in May, June and July, respectively, while the lowest Faecal P value (1.06mg g^{-1}) and Faecal Ca (10.13mg g^{-1}) were recorded at the beginning of the experiment. Higher (5.10mg g^{-1}) Faecal Mg was documented in June with comparable mean values (1.91mg g^{-1}, 1.95mg g^{-1} and 2.73mg g^{-1}) recorded for April, May and July, respectively (Table 9).

Table 6: Influence of supplemented diets on growth parameters and blood minerals of cattle

Parameters	D1	D2	D3	SEM
BW	212.43[c]	225.53[b]	246.23[a]	4.402
HCC	3.08[b]	3.2[ab]	3.35[a]	0.091
BCS	5.6	5.7	5.75	0.085
Blood P	0.78[b]	1.74[a]	1.85[a]	0.057
Blood Ca	1.57[c]	3.08[b]	3.70[a]	0.097
Blood Mg	0.44[b]	0.60[a]	0.75[a]	0.054

[abc]: Means along the same row with different superscripts are significantly different (P <0.05)

All the parameters considered were significantly (P <0.05) influenced by the dietary treatments except for BCS. BW and Blood Ca increased across the treatments with highest values (246.23kg and 3.70mmol/L, respectively) in animals receiving D3, while the lowest means (212.43kg and 1.57mmol/L, respectively) were documented in animals fed with D1. HCC was highest (P <0.05) in animals offered D3 with comparable mean value (3.2) in animals treated with D2, while the lowest value (3.08) was recorded in animals receiving the D1 diet. Blood P and Blood Mg were higher (1.74mmol/L, 0.60mmol/L and 1.85mmol/L, 0.75mmol/L) in animals receiving D2 and D3, respectively, while the least values (0.78 mmol/L and 0.44mmol/L) were documented in animals receiving the D1 diet (Table 10).

Table 7: Influence of supplemented diets on growth blood metabolites

Parameters	D1	D2	D3	SEM
ALB (G/L)	45.33[a]	35.65[b]	37.50[b]	0.86
BUN (uMOL/L)	5.56[a]	4.59[b]	4.39[b]	0.17
CREA (FLMOL/L)	147.68[a]	97.15[b]	100.43[b]	2.13
GLU (MMOL/L)	1.99[b]	3.25[a]	3.36[a]	0.12
TP (G/L)	73.83[a]	66.53[b]	64.35[b]	0.92

[ab]: Means along the same row with different superscripts are significantly different (P <0.05)

The dietary treatments significantly (P <0.05) influenced all the parameters considered. Albumin, Blood Urea Nitrogen, Creatinine and Total Protein followed the same trend with highest (P <0.05) mean values (45.33G/L, 5.56uMOL/L. 147.68FLMOL/L and 73.83G/L) were recorded in animals receiving D1, while lowest values were observed in animals receiving diets D2 and D3, respectively. Higher glucose mean values (3.25G/L and 3.36G/L MMOL/L) were recorded in D2 and D3 respectively, with lowest value (1.99MMOL/L) recorded in D1 (Table 11).

Table 8: Effects of diet on faecal parameters

Parameters	D1	D2	D3	SEM
Faecal P	1.43[c]	1.90[b]	2.65[a]	0.15
Faecal Ca	8.58[b]	13.97[a]	13.95[a]	0.57
Faecal Mg	3.44[a]	1.89[b]	3.45[a]	0.49

[abc]: Means along the same row with different superscripts are significantly different (P <0.05)

The diets significantly (P <0.05) influenced the selected faecal minerals. FCP was highest (P <0.05) in animals offered D3 and lowest mean value (1.43mg g-1) in those receiving D1 diet. FCCA increased in those fed D2 and D3 diets, respectively, while lowest value (8.58mg g-1) was recorded in those on the D1 diet. Higher FCMG were documented in groups offered D1 and D3 diets (3.44mg g-1 and 3.45mg g-1, respectively) while the lowest value (1.89 mg g-1) was documented in animals receiving the D2 diet.

BW had highest (P <0.05) means (261.9kg and 258.4kg) documented for cattle fed D3 in April and D3 in May, which is comparable to those on D1 and D2 in April, D2 in May and D3

in June, while the lowest means (196.5kg and 196.5kg) was recorded for cattle in groups D1 and D2 in July. Highest (P <0.05) HCC mean values (4 and 4) were observed in animals assigned to D1 and D2 in the month of July, while the lowest mean values were recorded in animals receiving D1 in May, D1 in June. BCS was highest (P <0.05) in animals treated in D3 for the month of May while the lowest values (5.0 and 5.0) were recorded in animals assigned to D1 and D2 in July. Blood P had the highest mean value (3.08mmol/L) in cattle fed D3 in May with the lowest values (0.59mmol/L and 0.65mmol/L) recorded in animals receiving D1 in June and D1 in July respectively.

Blood Ca recorded the highest mean value (5.06mmol/L and 5.13mmol/L) in animals receiving D3 in May and June, respectively, while the lowest values (1.22mmol/L) were recorded in those receiving the control diet in July. Blood Mg had the highest (P <0.05) mean value (1.60mmol/L) in animals treated with D3 in July, while the lowest value (0.31mmol/L) was recorded in animals receiving D2 in April; which is comparable with animals receiving D1 and D3 in April; D1, D2 and D3 in May; D1 and D3 in June and D1 in July.

Table 9: Interaction effect of the month × diet for physiological parameters

	APRIL			MAY			JUNE			JULY			
Parameters	D1	D2	D3	D1	D2	D3	D1	D2	D3	D1	D2	D3	SEM
BW	234,7^{abc}	238,8^{abc}	261,9^{a}	216,4	242,2^{abc}	258,4^{a}	202,1^{de}	224,6^{bc}	245^{ab}	196,5^{e}	196,5^{e}	219,6^{bcde}	8,05
HCC	2,4^{d}	2,7^{d}	2,4^{d}	2,4^{d}	3,3^{bc}	2,9^{cd}	4^{a}	3,4^{bc}	3,4^{bc}	2^{d}	2^{d}	3,6^{ab}	0,181
BCS	5,8^{ab}	5,8^{ab}	5,9^{ab}	5,5^{bcd}	6,0^{c}	6,8^{a}	5,2^{bc}	5,7^{ab}	6,4^{b}	5,0^{cd}	5.0^{cd}	5,8^{bcd}	0,17
Blood P	1,03^{fg}	0,89^{gh}	1,23^{ef}	0,85^{gh}	2,16^{b}	3,08^{a}	0,59	2,04^{b}	1,61^{cd}	0,65^{h}	1,87^{bc}	1,46^{de}	0,11
Blood Ca	1,93^{e}	1,82^{e}	1,89^{e}	1,66^{ef}	3,94^{b}	5,06^{a}	1,46^{ef}	3,08^{cd}	5,13^{a}	1,22^{f}	3,48^{bc}	2,70^{d}	0,19
Blood Mg	0,38^{cd}	0,31^{d}	0,37^{cd}	0,37^{cd}	0,5^{ecd}	0,52^{cd}	0,44^{cd}	0,88^{b}	0,50^{cd}	0,57^{bcd}	0,68^{bc}	1,60^{a}	0,11

^{abcdefgh}: Means along the same row with different superscripts are significantly different (P <0.05)

Table 15: shows the interaction effect of months and diets on blood metabolites. Albumin recorded comparable highest (P <0.05) mean values (45.72, 42.7, 42.6; 44.7, 41.4; 45.2 and 45.7) in cattle receiving D1, D2, D3 in April; D1, D3 in May, D1 in June and D1 in July, respectively, while comparable lowest values (34.9; 31.9; 33.1 and 30.5) were recorded in animals offered D2 in May; D2 in June; D2 and D3 in July, respectively. BUN was highest (P <0.05) in those assigned the D3 diet in April, while the lowest mean value (3.02) was recorded in animals receiving D3 in May. Creatinine recorded highest values (154.7 and 153.3) in animals receiving D2 in April and D1 in June while the lowest values (72.8 and 77.3) were in animals receiving D2 and D3 in July. Glucose mean value (4.4) was highest in animals receiving D2 in July but lowest (2.87) in animals receiving D3 in May. Total protein highest mean values (74.7, 75; 74.2 and 74.5) were recorded in animals offered D1, D2 in April; D1 in June and D1 in July.

Table 11 shows the interactive effect of months and diets on faecal mineral excretion. All the parameters considered were significantly (P <0.05) influenced. Faecal P was recorded in animals offered D3 in May which is comparable to animals receiving D3 in June, while the lowest value (0.61) was recorded in animals receiving D1 in April. Faecal Ca had the highest value (17.78) in animals receiving D3 in July, while the lowest value (7.02) was recorded in animals on the D1 diet in July. Faecal Mg had the highest mean value (8.64) in animals treated with D3 in July, while the lowest mean values (1.50, 2.22, 2.01; 1.71, 1.49; 1.39; 1.27 and 1.64) were recorded in animals receiving D1, D2, D3 in April; D1, D3 in May; D2 in June; D2 and D3 in July.

Table 10: Interaction effects of months x diets on blood metabolites

	APRIL			MAY			JUNE			JULY			
Parameters	D1	D2	D3	D1	D2	D3	D1	D2	D3	D1	D2	D3	SEM
ALB (G/L)	45.72^{a}	42.7^{a}	42.6^{a}	44.7^{a}	34.9^{b}	41.4^{a}	45.2^{a}	31.9^{b}	35.5^{b}	45.7^{a}	33.1^{b}	30.5^{b}	1.726
BUN (uMOL/L)	4.84^{cd}	6.28^{ab}	6.90^{a}	5.35^{bc}	3.88^{def}	3.02^{f}	6.31^{ab}	4.00^{de}	4.16^{de}	5.75^{bc}	4.20^{de}	3.44^{ef}	0.33
CREA (FLMOL/L)	139.7^{b}	154.7^{a}	148.5^{ab}	152^{ab}	81.1^{cd}	84.4^{cd}	153.3^{a}	80^{cd}	91.5^{c}	145.7^{ab}	72.8^{d}	77.3^{d}	4.266
GLU (MMOL/L)	2.46^{de}	2.04^{e}	2.43^{de}	1.69^{e}	3.61^{bc}	2.87^{d}	1.86^{e}	2.95^{cd}	4.26^{ab}	1.96^{e}	4.4^{a}	3.87^{ab}	0.246
TP (G/L)	74.7^{a}	75^{a}	68.3^{bc}	71.9^{ab}	64.1^{cd}	70.7^{ab}	74.2^{a}	63.4^{cd}	60.7^{de}	74.5^{a}	63.6^{cd}	57.7^{e}	1.847

^{abcdef}: Means along the same row with different superscripts are significantly different (P <0.05)

Table 11: Interaction effects of months x diets on faecal mineral excretions

	APRIL			MAY			JUNE			JULY			
Parameters	D1	D2	D3	D1	D2	D3	D1	D2	D3	D1	D2	D3	SEM
Faecal P	0.61^{f}	1.14^{def}	1.43^{def}	1.00^{ef}	2.51^{bc}	3.69^{a}	2.01^{bcd}	1.91^{cde}	2.89^{ab}	2.08^{bcd}	2.04^{bcd}	2.60^{bc}	0.30
Faecal Ca	8.11^{de}	10.86^{cd}	11.40^{cd}	8.93^{de}	16.04^{ab}	12.93^{bc}	10.25^{cde}	15.69^{ab}	13.69^{bc}	7.02^{e}	13.302^{bc}	17.78^{a}	1.14
Faecal Mg	1.50^{c}	2.22^{c}	2.01^{c}	1.71^{c}	2.65^{ab}	1.49^{c}	5.27^{b}	1.39^{c}	8.64^{a}	5.27^{b}	1.27^{c}	1.64^{c}	1.00

^{abcde}: Means along the same row with different superscripts are significantly different (P <0.05)

The decline in body weight (BW) during the winter season may be attributed to the poor nutritional composition in pastures and this is in consonance with reports by Mokolopi & Beighle, (2006) and Chipfupa (2012), as well as the need for more energy and nutrients for maintaining physiological and metabolic activities during winter (King 2000). Mokolopi & Beighle (2006) further stressed that the nutritional quality of pastures declined towards winter as a result of the poor quality of overgrown pastures as they approach maturity. These ideas are also reflected in the gradual decline in the body conformation scores (BCS) as the winter season progressed. The observations are in agreement with those reported by Mapiye *et al.* (2010) that body condition scores reflect the plane of nutrition the animals were exposed to over a reasonable length of time.

The unstable nature of blood phosphorus and calcium could be attributed to the gradual shift in the season. However, Mokolopi (2019) reported a reduced phosphorus and calcium level from summer to winter in grasses in communal grazing lands. The reduced blood phosphorus and magnesium concentrations might be attributed to the low quality of range pastures towards the end of summer or due to the fact that plants tend to be lignified as they age. This can also stand for reduced phosphorus as winter progresses. Calcium concentrations in blood, however, were within the critical range (Meyer *et al.,* 1992) for clinically healthy cattle. Although the blood phosphorus, calcium and magnesium levels were lower than the recommended ranges (Phosphorus- 1.38-2.55 mmol/L, Calcium- 1.95-5.62 mmol/L and Magnesium- 0.52-1.00 mmol/L) by Merck (2016) at the onset of the experiment, the steady increase brought the levels back within the optimal ranges suggested for clinically healthy cattle.

The decline in blood albumin and creatinine as the winter season progresses could be attributed to the low-quality nutritional profile of available pastures in winter, which is also reflected in the total protein values (Mapiye *et al.,* 2010). This further buttresses the fact that plant nutrition decreases as winter progresses. The increase in Glucose mean concentrations at the approach of winter can be hinged on the fact that the animals tend to consume more to meet metabolic needs to cater for the decline in environmental temperatures during the winter while continuing optimal physiological conditions. Although albumin values were higher at the beginning (April and May) of the experiment, all the values recorded through the experimental phase for all the other parameters were within optimal ranges prescribed for clinically healthy cattle (Meyer *et al.,* 1992). Suffice it to say, serum constituents and mineral levels in animals can be affected by various factors such as seasonal and physiological variations (Rathwa *et al.,* 2017).

Breves *et al*. (1985) opined that low phosphorus intakes lead to reduced plasma and salivary phosphorus, but irreducible faecal endogenous losses as a result of poor nutritional quality and as indicated by non-significant level of the grazed pasture, could probably result in the predominant source of faecal phosphorus. This is further supported by the report of Iqbal *et al*., 2005, that excessive endogenous P is excreted through faeces.

The elevated faecal calcium throughout the experimental period can be hinged on the adequate amount of Ca in the grass minerals which were the recommended range (6.45-15.22 mg/g) in grasses (NRC, 2001). This might be a function of the observed reduced blood P as a result of the imbalanced ratio of Ca: P ratio is detrimental to cattle (Hale & Olson, 2001). Variations in Mg concentration may be attributed to the body's inability to maintain and store Mg in the animals' bodies (Mokolopi *et al*., 2019).

The increased body weight, hair coat colour, blood phosphorus, calcium and magnesium across the dietary treatment groups can be attached to the added supplements. As attributed by De Waal (1996) and De Waal *et al.*, (1997) mineral supplementation had proven to have a significant effect on animal weight gains. This was attested by Horst *et al.,* (1994) that anionic diets influence metabolism, homeostasis, cell development and cell proliferation. In addition, mineral supplementation stimulates bone mineral deposition, skeleton development and anabolic body growth, which results in animal mass gains (Gunther & Tekin, 1987).

The results of this study are in agreement with those reported by De Brouwer *et al.,* (2000) that anionic diets influence metabolism, homeostasis and cell proliferation, which result in animal mass gain. Other independent researchers in South Africa affirm that dicalcium phosphate supplementation not only correct mineral deficiencies, but it also plays a very significant role in the mass gain of free ranging beef cattle during the winter dry seasons (De Waal *et al*., 1996; Bakunzi *et al*., 2012).

Consistent increases in phosphorus, calcium and magnesium levels observed in the study may also be attributed to metabolism, bone mineralization and homeostasis. These results are in agreement with those reported by Bakunzi *et al.* (2012), that during any dietary deficiency, serum mineral levels increase, thereby making circulating levels an unreliable index of mineral status.

The decreasing albumin, blood urea nitrogen, creatinine and total protein could be because the supplemented diets (D2 and D3) were used for growth rather than for immune system balancing. Despite this reduction, the serum metabolites of the supplemented cattle were still within the required level (Merck, 2011) for optimum production. This indicated that there is

enough Ca required for other enzymatic or physiological functions in the body (Suttle *et al.,* 2003); hence, supplemented diets were optimal for normal immune system balancing. Increased Ca metabolism as a result of the supplementation will require high energy to maintain optimal physiological conditions, thus aiding the increasing glucose levels.

The decreasing albumin, blood urea nitrogen, creatinine and total protein could be that the supplemented diets (D2 and D3) are used for growth rather than immune system balancing. Despite this reduction, the serum metabolites of the supplemented cattle were still within the required level (Merck, 2011) for optimum production. This indicated that there is enough Ca required for other enzymatic or physiological functions in the body (Suttle *et al.,* 2003) hence, supplemented diets were optimal for normal immune system balancing. Increased Ca metabolism as a result of the supplementation will require much energy to maintain optimal physiological conditions, thus aiding the increasing glucose levels.

The increasing faecal phosphorus and calcium values can most likely be attributed to the mineral supplementations offered across the treatment groups as excess minerals in the diets are shed through urine or faeces. The fluctuation in the faecal magnesium could be as a result of the use of magnesium for physiological purposes as the animals in D2 could have more need for the magnesium available in their diets compared to the other groups. These variations in Mg concentration are consistent with those reported by Mokolopi (2019) that the body is unable to retain and store Mg in the body.

The interaction between month and mineral supplementation shows that body weight was still reducing as the season progressed, which is attributed to the quality of feed consumed by the cattle. Nevertheless, the data recorded suggests that the supplementations made their means comparable as the season continued. HCC results suggest that hair coat colour improves as the season continues with or without supplementation as the means increased towards the culmination of the experiment. The highly comparable means for BCS shows that mineral supplementation, as the season progressed, had little effect on the body conformation of the experimental cattle.

The results obtained from this study indicate that albumin values tended to reduce with mineral supplementation as the winter season progressed; as were reflected in total protein and blood urea nitrogen with or without mineral supplementation. Glucose on the other hand, tended to increase with supplementation at the culmination of the experimentation. Creatinine values showed that mineral supplementation tended to reduce its concentration in contrast to no supplementation.

Faecal phosphorus values tended to increase as the experiment progressed, with or without supplementation. Faecal calcium values increased as the experiment continued towards termination with mineral supplementation, while the values reduced in animals without supplementation. This suggests that cattle offered mineral supplementation, as in the case of this study, may be unable to utilize a good portion of the supplements as it may be in excess of their requirements, which is then shed in their faeces. On the other hand, faecal magnesium tended to increase without supplementation as the experiment progressed, while mineral supplementation caused a relative decrease. This may suggest that the magnesium concentration increases through winter towards autumn and this is supported by (Mokolopi & Beighle, 2006) who reported increased plant and blood magnesium from winter to autumn in grasses from communal rangeland and cattle fed on the rangeland in the North West region of South Africa.

The interactive effect between the month and the Calcium phosphate /Vitamin D_3 supplementation further supported the fact that the effect of season trumps the effect of supplementation of BW and BCS reduced as the winter months progressed. This is further reflected in the serum parameters such as albumin, blood urea nitrogen, creatinine and total protein whose values drop with the advancement of winter despite the supplementations. This corroborates the idea that mineral and vitamin supplementation is not enough to meet the physiological needs of the animals in the winter season. However, a more balanced ration for energy and protein should be provided for the animals for optimum growth and performance, which will require little or no supplementations, as most of the vitamins and minerals will be present in the upgraded rations fed to the animals during the winter season.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Supplementation with (DCP) mineral lick only (D2) and lick + AD3injection (D3) improved the blood P and Ca concentrations of calves. However, over- supplementation caused the animals to shed more minerals through their faeces, as stated in table 2 above. The blood chemistry parameters also show that animals that were treated with licks and additional AD3E injectable (D2 and 3) had better values than those that were not treated (D1). An increased level of GLU and a decrease in TP concentrations clearly suggests that animals in the treated groups (D2 and D 3) did not have to travel long distances in search of feed as compared to decreasing GLU and increasing TP levels in the D1 group of calves which were not supplemented. A more accurate assessment of the nutritional status of cattle can be made using blood metabolites in combination with other physiological parameters like body weights, body condition scores and hair coat conditions. The study indicates that minerals play a vital role in forage digestion and they can be successfully utilized in the wintering of replacement heifers on communal grazing systems. Cattle farming communities, agricultural advisers and all stakeholders need to be educated on the determination and application of blood parameters and physiological parameters (BCS, BW and HCC) as management tool in beef production. On the basis of the overall mean correlation between blood P, blood Ca, and blood metabolites D2 calves were the most superior of all the groups tested. The results of this study revealed that calves treated with dicalcium phosphate lick performed best in most parameters. The use of dicalcium phosphate lick is recommended for wintering of communal free ranging beef cattle. Further research is needed to determine the correlation between blood mineral concentration, blood metabolites and the visual parameters like hair coat scores and body condition scores in beef cattle raised on poor winter pastures.

5.2 Study limitations

This study only concentrated on the mineral homeostasis of calves grazing normal pastures as compared to those supplemented with mineral lick only (D2) and those (D3) given additional injectable AD3E. It is clear that this study did not address the cost benefit of giving supplements and the addition of injectable supplements. The addition of injectable supplements improved the Ca and P status of animals, but on the other hand they lost more of the minerals through their faeces, which might indicate that farmers lose more by adding additional supplements by means

of injectable supplements. There are limited research studies reporting on hair coat condition and its relation to mineral supplements. Even though correlations between body weights and body condition scores were not done, the general observations in this study show a certain level of influence does exist in these parameters.

5.3 Study recommendations

The use of dicalcium phosphate lick is recommended for wintering of communal free ranging beef cattle. Further research is needed to determine the correlation between blood mineral concentration, blood metabolites and the visual parameters like hair coat scores and body condition scores in beef cattle raised on poor winter pastures. The government and other stakeholders must be involved in educating farmers in using mineral analyses as a management tool. Nutritional diseases have been proven in research to affect productivity in ruminants, hence accurate determination of grass minerals and biochemical profiles of blood will provide valuable information to assist in the diagnosis and treatment of those diseases of veterinary importance.

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Addendum A

Effects of dicalcium phosphate and complex vitamin ADE +minerals on minerals, metabolites and physiological growth parameters of calves

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Abstract

The effects of dicalcium phosphate (DCP) lick alone or in combination with complex vitamin ADE plus minerals injection on calves raised on poor quality roughages have not been studied thoroughly. This study investigated the effects of DCP and complex vitamin ADE plus minerals on blood and feacal minerals, blood metabolites, body weights, body condition score and hair coat condition. A total of thirty homogenous Bonsmara calves 15 to 18 months of age were randomly assigned to three groups with the aim of comparing treatment groups. The experimental design consisted of three groups of heifers D1 was allowed maintenance ration (control), D2 was given dicalcium phosphate lick and maintenance ration and D3 was fed dicalcium phosphate lick and were also inoculated with a shot of complex vitamin ADE plus minerals on top of maintenance ration on monthly intervals after sample collection. Grass mineral concentrations were found to be lower than those recommended to meet animal mineral requirements (NRC, 1996), with P concentrations ranging between 1.12mg g^{-1} to 1.52mg g^{-1}, Ca from 6.18mg g^{-1} to 13.03mg g^{-1} and Mg from 1.50mg g^{-1} to 2.48mg g^{-1}. Dicalcium phosphate alone or in combination with complex vitamin ADE plus minerals had a significant increase on physiological parameters that included body weights, body condition score, hair coat condition, blood minerals, and blood metabolites levels of both D2 and D3 groups, however, calves in D3 group lost minerals in feaces more than their counterparts. The outcomes of this study indicate that DCP lick is an effective supplementation method for calves raised under communal pastures during the winter season.

Keywords: Body condition score, body weights, blood and hair coat score
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Introduction

Native pastures and agricultural by-products are the main sources for cattle feeds in many communal farms of South Africa. The potential of any feed to support animal production depends on the quantity consumed and the extent to which it meets the animal's mineral requirements. In dry winter seasons the feeding of communal cattle mostly depends on native grasses and crop residues, however under these conditions calves may be exposed to some

mineral nutrition deficiencies (Bakunzi *et al*., 2012). Therefore for these reseason feed mineral analysis is necessary to accurately determine the nutritional status of our pastures (Mokolopi, 2019). Mineral deficiencies are a problem in our soil and subsequently in grasses and animals feeding on it (Chipfupa, 2012), and to mitigate through these problem calves were supplemented with dicalcium phosphate (DCP) lick and complex vitamin ADE plus minerals injections. Physiological parameters like body condition score, body weights, and hair coat conditions are increasingly been used in several studies as indicators of growth and productivity in beef cattle raised on communal areas (Mokolopi, 2019).

In South Africa, blood biochemical parameters (metabolites) are becoming important in determining the energy status of beef cattle (Mapiye *et al*., 2010). On the same note, blood serum mineral concentrations of micro and macro minerals are used to monitor their status in beef cattle (Ndlovu *et al*., 2010). The alternations of blood mineral concentration and blood serum biochemical profile have a considerable influence on the production parameters responsible for growth and productivity. Physiological parameters like body condition scores (BCS), body weights (BW) and hair coat conditions (HCC) also widely accepted to assess the changes in blood metabolites and the energy status of calves (Aktas *et al*., 2011). Determination of grass, feacal, blood mineral concentration and blood metabolites will provide data or information for the diagnosis, treatment, and prognosis of diseases that could affect calves kept communal free-range rearing systems in the commonage of the South African municipalities (Mapiye *et al*., 2010). The objective of the current study was to evaluate effects of dicalcium phosphate in comparison with injectable complex vitamin AD3E plus minerals as supplements during the winter season. The study also aimed at determining the variations in nutritionally related blood metabolites concentrations and the relations with blood P, Ca and Mg levels.

Material and Methods

Animals were balanced for age and size, condition and randomly allocated into three experimental groups of 10 each. The groups were allowed to graze on similar available pastures as maintenance ration throughout the trial. The first group (D1) control was grazed on natural pastures as maintenance ration throughout the experiment. The second group (D2) was allowed access to grazing on natural pastures as maintenance ration and dicalcium phosphate (DCP) lick, whilst the third group D3 was also allowed to graze on natural pastures as maintenance and dicalcium phosphate but was further injected with complex vitamin ADE plus minerals during monthly interval throughout the experiment from April to July. The animal monitoring sheets were filled on a daily basis to monitor the animal health status during the experiment.

Grass samples were collected from the veld where the animals were grazing once a month for the duration of the experiment. Sites 5m × 5m homogenous vegetation unit (HVU) were marked in different directions to be used as the replicates. In a HVU, three $1m^2$ quadrats were randomly put to sample grass species. All the species were harvested, dried, grinded and bulked together and placed in a tight container pending analysis. Feacal samples were collected directly from the rectum of calves using sterile arm length gloves and harvested feacal sample were dried,

grinded and bulked together in a tight container pending analysis. Samples were collected during the months of April, May, June and July.

Triplicate blood samples were collected in the morning from all animals available for research. The animals were calmly driven into a crush pen to minimize stress where they were bled from the jugular vein into a 10ml EDTA vacutainer tubes (K_2 EDTA, 10.8mg) to harvest plasma and also into the red stoppered tubes (Vacuette with Z serum Clot Activator) to harvest serum, placed on ice and transported to the laboratory for analysis. Samples were collected during the months of April, May, June and July.

Body weights (BW) and body condition score (BCS) measurements were collected during monthly intervals whilst hair coat condition (HCC) were recorded monthly with those reported by Foster *et al.* (2016). The ethical clearance for experimentation of the animals was approved by the interfaculty animal ethics committee at University of the Free State with a project number: UFS-AED2017/0049.

Grass and feacal samples were analysed using the dry ashing macro and trace minerals methods for feed and plants (AgriLASA, 1998) and digested using a microwave digestion system conditions stated in the methods for the Anton- Paar Multiwave 3000 reaction system and subjected to the Nexion ICP-MS machine (Perkin-Elmer Inc., Pretoria, South Africa) for analysis.

Blood from serum harvesting was collected and stored at -20ºC and transported to the laboratory. Blood samples were centrifuged at 1500× g for 15min. Samples were analysed for minerals (Magnesium, Phosphorus and Calcium) using Nexion ICP-MS machine (Perkin-Elmer Inc., Pretoria, South Africa) Protein and energy metabolites [albumin (ALB), total protein (TP), blood urea nitrogen (BUN), glucose (GLU) and a hepatic and enzyme marker creatinine (CREA) were determined using the idexx vet test chemistry Analyzer (IDEXX Laboratories, Inc., Pretoria, South Africa).

Data collected during the trial was subjected to a general two-factor analysis of variances using the procedure of SAS (2018) to determine whether or not the inclusion of dicalcium phosphate cum complex vitamin ADE plus minerals supplement and month of collection had an effect on blood mineral and metabolites, faecal mineral levels, hair coat condition, and body condition scoring. The probability of variance was considered significant at 5% or less.

Results and Discussion

Grass P concentrations ranged between 1.12mg g-1 to 1.52mg g-1, Ca from 6.19mg g-1 to 13.03 mg g-1 and Mg from 1.50 mg g-1 to 2.48mg g-1. No significant ($P>0.05$) differences between grass P and Mg were recorded between months except for grass Ca, where a significant decrease was seen from April to May and then an increase in June and July with means ranging from 6.19mg g-1 to 13.03 mg g-1.In general, the grass minerals levels were too low to meet the animal maintenance requirements for minerals, as stated in the NRC, (2001) which then led to the study. Most farmers also give mineral licks with vitamin and minerals

injectable, not taking into consideration the loss of excess minerals thought the faeces. Grass mineral concentrations reported here are in agreement with findings reported Bakunzi et al. (2012) and more recently Mokolopi (2019).

All the parameters considered in this study were significantly (P <0.05) different throughout the experimental period. Faecal P values (2.40mg g-1, 2.27mg g-1 and 2.24mg g-1) and Faecal Ca values (12.63mg g-1, 13,21mg g-1 and 12.70mg g-1) were highest in May, June and July, respectively, while the lowest Faecal P value (1.06mg g-1) and Faecal Ca (10.13mg g-1) were recorded at the beginning of the experiment. Higher (5.10mg g-1) Faecal Mg was documented in June with comparable mean values (1.91mg g-1, 1.95mg g-1 and 2.73mg g-1) recorded for April, May and July, respectively.

Table 1 Means square analysis of grass and faecal minerals

Parameters	April	May	June	July	SEM
Grass P mg g-1	1.31±0.02^{a}	1.12±0.05^{a}	1.19±0.03^{a}	1.52±0.08^{a}	0.03
Grass Ca mg g-1	8.23±1.03^{ab}	6.19±0.54^{b}	9.47±1.52^{ab}	13.03±1.85^{c}	1.46
Grass Mg mg g-1	2.02±0.22^{a}	2.48±0.40^{a}	1.50±0.03^{a}	1.71±0.06^{a}	0.07
Faecal P	1.06^{b}	2.40^{a}	2.27^{a}	2.24^{a}	0.18
Faecal Ca	10.13^{b}	12.63^{a}	13.21^{a}	12.70^{a}	0.66
Faecal Mg	1.91^{b}	1.95^{b}	5.10^{a}	2.73^{b}	0.56

ab: Means along the same row with different superscripts are significantly different (P <0.05)

Highest mean (P <0.05) values (245.13kg and 239.00kg) for BW were recorded in April and May, respectively, while the lowest mean value (204.20kg) was recorded in July. BLP and BLCA had highest (P <0.05) mean values (1.41 and 3.55) in May while the lowest mean values (1.05 and 2.27) were recorded in April and July, respectively. Blood Mg was highest (P <0.05) in July with comparable mean values (0.47mmol/L and 0.61mmol/L) in May and June, respectively, while the lowest value (0.35mmol/L) was observed in April. HCC had highest (P <0.05) mean values (3.60 and 3.87) in June and July, respectively, while the lowest value (2.50) was recorded in April. BCS recorded highest (P <0.05) mean value (5.87) in April with comparable mean values (5.70 and 5.67) in May and June, respectively, while the lowest mean value (5.50) was observed in July (Table 2) below.

Table 2 Growth parameters, blood minerals and blood metabolites

Parameters	APRIL	MAY	JUNE	JULY	SEM
BW	245.13^{a}	239.00^{a}	223.90^{b}	204.20^{c}	5.08
HCC	2.50^{c}	2.87^{b}	3.60^{a}	3.87^{a}	0.11
BCS	5.87^{a}	5.70^{ab}	5.67^{ab}	5.50^{b}	0.10
Blood P	1.05^{c}	2.03^{a}	1.41^{b}	1.33^{b}	0.66
Blood Ca	1.88^{d}	3.55^{a}	3.22^{b}	2.47^{c}	0.11
Blood Mg	0.35^{b}	0.47^{ab}	0.61^{ab}	0.95^{a}	0.06
ALB (G/L)	43.67^{a}	40.33^{b}	37.53^{c}	36.43^{c}	1.00

BUN (uMOL/L)	6.01[a]	4.08[c]	4.83[b]	4.46[ab]	0.19
CREA (FLMOL/L)	147.63[a]	105.83[b]	108.27[b]	98.60[c]	2.46
GLU (MMOL/L)	2.31[c]	2.73[b]	3.02[ab]	3.41[a]	0.14
TP (G/L)	72.67[a]	68.90[b]	66.10[ab]	65.27[c]	1.07

abc: Means along the same row with different superscripts are significantly different (P <0.05)

Albumin was highest (P <0.05) at the onset of the experiment while the lowest values (37.53G/L and 36.43G/L) were recorded in June and July, respectively. Blood Urea Nitrogen had the highest (P <0.05) mean value (6.01) in April with comparable mean (4.46uMOL/L) in July, while the lowest value (4.08 uMOL/L) was documented in May. Creatinine had the highest (P <0.05) mean value (147.63FLMOL/L) in April while the lowest mean value (98.60 FLMOL/L) was recorded at the culmination of the experiment. Glucose values increased (P <0.05) from the onset to the end of the experiment. Total Protein was highest (P <0.05) in April with the comparable mean value (66.10G/L) in June, while the lowest mean value (65.27G/L) was recorded in July.

All the parameters considered were significantly (P <0.05) influenced by the dietary treatments except for BCS. BW and Blood Ca increased across the treatments with highest values (246.23kg and 3.70mmol/L, respectively) in animals receiving D3, while the lowest means (212.43kg and 1.57mmol/L, respectively) were documented in animals fed with D1. HCC was highest (P <0.05) in animals offered D3 with comparable mean value (3.2) in animals treated with D2, while the lowest value (3.08) was recorded in animals receiving the D1 diet. Blood P and Blood Mg were higher (1.74mmol/L, 0.60mmol/L and 1.85mmol/L, 0.75mmol/L) in animals receiving D2 and D3, respectively, while the least values (0.78 mmol/L and 0.44mmol/L) were documented in animals receiving the D1 diet (Table 3).

Table 3 Influence of supplemented diets on growth parameters, blood minerals and metabolites

Parameters	D1	D2	D3	SEM
BW	212.43[c]	225.53[b]	246.23[a]	4.402
HCC	3.08[b]	3.2[ab]	3.35[a]	0.091
BCS	5.6	5.7	5.75	0.085
Blood P	0.78[b]	1.74[a]	1.85[a]	0.057
Blood Ca	1.57[c]	3.08[b]	3.70[a]	0.097
Blood Mg	0.44[b]	0.60[a]	0.75[a]	0.054
ALB (G/L)	45.33[a]	35.65[b]	37.50[b]	0.86
BUN (uMOL/L)	5.56[a]	4.59[b]	4.39[b]	0.17
CREA (FLMOL/L)	147.68[a]	97.15[b]	100.43[b]	2.13
GLU (MMOL/L)	1.99[b]	3.25[a]	3.36[a]	0.12
TP (G/L)	73.83[a]	66.53[b]	64.35[b]	0.92

abc: Means along the same row with different superscripts are significantly different (P <0.05)

The dietary treatments significantly (P <0.05) influenced all the parameters considered. Albumin, Blood Urea Nitrogen, Creatinine and Total Protein followed the same trend with highest (P <0.05) mean values (45.33G/L, 5.56uMOL/L. 147.68FLMOL/L and 73.83G/L) were recorded in animals receiving D1, while lowest values were observed in animals receiving diets D2 and D3, respectively. Higher glucose mean values (3.25G/L and 3.36G/L MMOL/L) were recorded in D2 and D3 respectively, with lowest value (1.99MMOL/L) recorded in D1 (Table 3).

The diets significantly (P <0.05) influenced the selected faecal minerals. FCP was highest (P <0.05) in animals offered D3 and lowest mean value (1.43mg g-1) in those receiving D1 diet. FCCA increased in those fed D2 and D3 diets, respectively, while lowest value (8.58mg g-1) was recorded in those on the D1 diet. Higher FCMG were documented in groups offered D1 and D3 diets (3.44mg g-1 and 3.45mg g-1, respectively) while the lowest value (1.89 mg g-1) was documented in animals receiving the D2 diet.BW had highest (P <0.05) means (261.9kg and 258.4kg) documented for cattle fed D3 in April and D3 in May, which is comparable to those on D1 and D2 in April, D2 in May and D3 in June, while the lowest means (196.5kg and 196.5kg) was recorded for cattle in groups D1 and D2 in July. Highest (P <0.05) HCC mean values (4 and 4) were observed in animals assigned to D1 and D2 in the month of July, while the lowest mean values were recorded in animals receiving D1 in May, D1 in June. BCS was highest (P <0.05) in animals treated in D3 for the month of May while the lowest values (5.0 and 5.0) were recorded in animals assigned to D1 and D2 in July. Blood P had the highest mean value (3.08mmol/L) in cattle fed D3 in May with the lowest values (0.59mmol/L and 0.65mmol/L) recorded in animals receiving D1 in June and D1 in July respectively.

Blood Ca recorded the highest mean value (5.06mmol/L and 5.13mmol/L) in animals receiving D3 in May and June, respectively, while the lowest values (1.22mmol/L) were recorded in those receiving the control diet in July. Blood Mg had the highest (P <0.05) mean value (1.60mmol/L) in animals treated with D3 in July, while the lowest value (0.31mmol/L) was recorded in animals receiving D2 in April; which is comparable with animals receiving D1 and D3 in April; D1, D2 and D3 in May; D1 and D3 in June and D1 in July.

Table 4: shows the interaction effect of months and diets on blood metabolites. Albumin recorded comparable highest (P <0.05) mean values (45.72, 42.7, 42.6; 44.7, 41.4; 45.2 and 45.7) in cattle receiving D1, D2, D3 in April; D1, D3 in May, D1 in June and D1 in July, respectively, while comparable lowest values (34.9; 31.9; 33.1 and 30.5) were recorded in animals offered D2 in May; D2 in June; D2 and D3 in July, respectively. BUN was highest (P <0.05) in those assigned the D3 diet in April, while the lowest mean value (3.02) was recorded in animals receiving D3 in May. Creatinine recorded highest values (154.7 and 153.3) in animals receiving D2 in April and D1 in June while the lowest values (72.8 and 77.3) were in animals receiving D2 and D3 in July. Glucose mean value (4.4) was highest in animals receiving D2 in July but lowest (2.87) in animals receiving D3 in May. Total protein highest mean values (74.7,

75; 74.2 and 74.5) were recorded in animals offered D1, D2 in April; D1 in June and D1 in July.

As shown in Table 4 the interactive effect of months and diets on faecal mineral excretion. All the parameters considered were significantly (P <0.05) influenced. Faecal P was recorded in animals offered D3 in May which is comparable to animals receiving D3 in June, while the lowest value (0.61) was recorded in animals receiving D1 in April. Faecal Ca had the highest value (17.78) in animals receiving D3 in July, while the lowest value (7.02) was recorded in animals on the D1 diet in July. Faecal Mg had the highest mean value (8.64) in animals treated with D3 in July, while the lowest mean values (1.50, 2.22, 2.01; 1.71, 1.49; 1.39; 1.27 and 1.64) were recorded in animals receiving D1, D2, D3 in April; D1, D3 in May; D2 in June; D2 and D3 in July.

Table 4 Interaction effect of the month × diet for physiological parameters, metabolites and faecal mineral excretions

	APRIL			MAY			JUNE			JULY			
Parameters	**D1**	**D2**	**D3**	**D1**	**D2**	**D3**	**D1**	**D2**	**D3**	**D1**	**D2**	**D3**	**SEM**
BW	234,7^{abc}	238,8^{abc}	261,9^{a}	216,4	242,2^{abc}	258,4^{a}	202,1^{de}	224,6^{bc}	245^{ab}	196,5^{e}	196,5^{e}	219,6^{bcde}	8,05
HCC	2,4^{d}	2,7^{d}	2,4^{d}	2,4^{d}	3,3^{bc}	2,9^{cd}	4^{a}	3,4^{bc}	3,4^{bc}	2^{d}	2^{d}	3,6^{ab}	0,181
BCS	5,8^{ab}	5,8^{ab}	5,9^{ab}	5,5^{bcd}	6,0^{c}	6,8^{a}	5,2^{bc}	5,7^{ab}	6,4^{b}	5,0^{cd}	5.0^{cd}	5,8^{bcd}	0,17
Blood P	1,03^{fg}	0,89^{gh}	1,23^{ef}	0,85^{gh}	2,16^{b}	3,08^{a}	0,59	2,04^{b}	1,61^{cd}	0,65^{h}	1,87^{bc}	1,46^{de}	0,11
Blood Ca	1,93^{e}	1,82^{e}	1,89^{e}	1,66^{ef}	3,94^{b}	5,06^{a}	1,46^{ef}	3,08^{cd}	5,13^{a}	1,22^{f}	3,48^{bc}	2,70^{d}	0,19
Blood Mg	0,38^{cd}	0,31^{d}	0,37^{cd}	0,37^{cd}	0,5^{ecd}	0,52^{cd}	0,44^{cd}	0,88^{b}	0,50^{cd}	0,57^{bcd}	0,68^{bc}	1,60^{a}	0,11
ALB (G/L)	45.72^{a}	42.7^{a}	42.6^{a}	44.7^{a}	34.9^{b}	41.4^{a}	45.2^{a}	31.9^{b}	35.5^{b}	45.7^{a}	33.1^{b}	30.5^{b}	1.726
BUN (uMOL/L)	4.84^{cd}	6.28^{ab}	6.90^{a}	5.35^{bc}	3.88^{def}	3.02^{f}	6.31^{ab}	4.00^{de}	4.16^{de}	5.75^{bc}	4.20^{de}	3.44^{ef}	0.33
CREA (FLMOL/L)	139.7^{b}	154.7^{a}	148.5^{ab}	152^{ab}	81.1^{cd}	84.4^{cd}	153.3^{a}	80^{cd}	91.5^{c}	145.7^{ab}	72.8^{d}	77.3^{d}	4.266
GLU (MMOL/L)	2.46^{de}	2.04^{e}	2.43^{de}	1.69^{e}	3.61^{bc}	2.87^{d}	1.86^{e}	2.95^{cd}	4.26^{ab}	1.96^{e}	4.4^{a}	3.87^{ab}	0.246
TP (G/L)	74.7^{a}	75^{a}	68.3^{bc}	71.9^{ab}	64.1^{cd}	70.7^{ab}	74.2^{a}	63.4^{cd}	60.7^{de}	74.5^{a}	63.6^{cd}	57.7^{e}	1.847
Faecal P	0.61^{f}	1.14^{def}	1.43^{def}	1.00^{ef}	2.51^{bc}	3.69^{a}	2.01^{bcd}	1.91^{cde}	2.89^{ab}	2.08^{bcd}	2.04^{bcd}	2.60^{bc}	0.30
Faecal Ca	8.11^{de}	10.86^{cd}	11.40^{cd}	8.93^{de}	16.04^{ab}	12.93^{bc}	10.25^{cde}	15.69^{ab}	13.69^{bc}	7.02^{e}	13.302^{bc}	17.78^{a}	1.14
Faecal Mg	1.50^{c}	2.22^{c}	2.01^{c}	1.71^{c}	2.65^{ab}	1.49^{c}	5.27^{b}	1.39^{c}	8.64^{a}	5.27^{b}	1.27^{c}	1.64^{c}	1.00

abcde: Means along the same row with different superscripts are significantly different (P <0.05)

DISCUSSION

The decline in body weight (BW) during the winter season may be attributed to the poor nutritional composition in pastures and this is in consonance with those reported by Chipfupa (2012), as well as the need for more energy and nutrients for maintaining physiological and metabolic activities during winter Gibbens, 2012 . Mokolopi, 2019 further stressed that the nutritional quality of pastures declined towards winter as a result of the poor quality of overgrown pastures as they approach maturity. These is reflected in the gradual decline in the body conformation scores (BCS) as the winter season progressed. The observations are in agreement with those reported by (Mapiye et al., 2010) that body condition scores reflect the plane of nutrition the animals were exposed to over a reasonable length of time.

The unstable nature of blood phosphorus and calcium could be attributed to the gradual shift in the season. However, Mokolopi (2019) reported a reduced phosphorus and calcium level from summer to winter in grasses in communal grazing lands. The reduced blood phosphorus and magnesium concentrations might be attributed to the low quality of range pastures towards the end of summer or due to the fact that plants tend to be lignified as they age. This can also stand for reduced phosphorus as winter progresses. Calcium concentrations in blood, however, were within the critical range (Hadzimusic & Krnic, 2012) for clinically healthy cattle. Although the blood phosphorus, calcium and magnesium levels were lower than the recommended ranges (Phosphorus- 1.38-2.55 mmol/L, Calcium- 1.95-5.62 mmol/L and Magnesium- 0.52-1.00 mmol/L) by Merck (2016) at the onset of the experiment, the steady increase brought the levels back within the optimal ranges suggested for clinically healthy cattle.

The decline in blood albumin and creatinine as the winter season progresses could be attributed to the low quality nutritional profile of available pastures in winter, which is also reflected in the total protein values (Mapiye et al., 2010). This further buttresses the fact that plant nutrition decreases as winter progresses. The increase in Glucose mean concentrations at the approach of winter can be hinged on the fact that the animals tend to consume more to meet metabolic needs to cater for the decline in environmental temperatures during the winter while continuing optimal physiological conditions. Although albumin values were higher at the beginning (April and May) of the experiment, all the values recorded through the experimental phase for all the other parameters were within optimal ranges prescribed for clinically healthy cattle. Suffice it to say, serum constituents and mineral levels in animals can be affected by various factors such as seasonal and physiological variations (Rathwa et al., 2017).

Hadzimusic & Krnic, (2012) opined that low phosphorus intakes lead to reduced plasma and salivary phosphorus, but irreducible faecal endogenous losses as a result of poor nutritional quality and as indicated by non-significant level of the grazed pasture, could probably result in the predominant source of faecal phosphorus. This is further supported by the report of Rahman *et al.*, 2014, that excessive endogenous P is excreted through faeces.The elevated faecal calcium throughout the

experimental period can be hinged on the adequate amount of Ca in the grass minerals which were the recommended range (6.45-15.22 mg/g) in grasses (NRC, 2001). Variations in Mg concentration may be attributed to the body's inability to maintain and store Mg in the animals' bodies (Mokolopi , 2019).

The increased body weight, hair coat colour, blood phosphorus, calcium and magnesium across the dietary treatment groups can be attached to the added supplements. As attributed by Viljoen, 2011 mineral supplementation had proven to have a significant effect on animal weight gains. This was attested by Bakunzi et al. (2012) that anionic diets influence metabolism, homeostasis, cell development and cell proliferation.

Other independent researchers in South Africa affirm that dicalcium phosphate supplementation not only correct mineral deficiencies, but it also plays a very significant role in the mass gain of free ranging beef cattle during the winter dry seasons (Bakunzi et al., 2012).Consistent increases in phosphorus, calcium and magnesium levels observed in the study may also be attributed to metabolism, bone mineralization and homeostasis. These results are in agreement with those reported by Bakunzi et al. (2012), that during any dietary deficiency, serum mineral levels increase, thereby making circulating levels an unreliable index of mineral status.

The decreasing albumin, blood urea nitrogen, creatinine and total protein could be because the supplemented diets (D2 and D3) were used for growth rather than for immune system balancing. Despite this reduction, the serum metabolites of the supplemented cattle were still within the required level (Merck, 2011) for optimum production.

The decreasing albumin, blood urea nitrogen, creatinine and total protein could be that the supplemented diets (D2 and D3) are used for growth rather than immune system balancing. Despite this reduction, the serum metabolites of the supplemented cattle were still within the required level (Merck, 2011) for optimum production. Increased Ca metabolism as a result of the supplementation will require much energy to maintain optimal physiological conditions, thus aiding the increasing glucose levels. The increasing faecal phosphorus and calcium values can most likely be attributed to the mineral supplementations offered across the treatment groups as excess minerals in the diets are shed through urine or faeces. The fluctuation in the faecal magnesium could be as a result of the use of magnesium for physiological purposes as the animals in D2 could have more need for the magnesium available in their diets compared to the other groups. These variations in Mg concentration are consistent with those reported by Mokolopi (2019) that the body is unable to retain and store Mg in the body.

The interaction between month and mineral supplementation shows that body weight was still reducing as the season progressed, which is attributed to the quality of feed consumed by the cattle. Nevertheless the data recorded suggests that the supplementations made their means comparable as the season continued. HCC results suggest that hair coat colour improves as the season

continues with or without supplementation as the means increased towards the culmination of the experiment. The highly comparable means for BCS shows that mineral supplementation, as the season progressed, had little effect on the body conformation of the experimental cattle.

The results obtained from this study indicate that albumin values tended to reduce with mineral supplementation as the winter season progressed; as were reflected in total protein and blood urea nitrogen with or without mineral supplementation. Glucose on the other hand, tended to increase with supplementation at the culmination of the experimentation. Creatinine values showed that mineral supplementation tended to reduce its concentration in contrast to no supplementation.

Faecal phosphorus values tended to increase as the experiment progressed, with or without supplementation. Faecal calcium values increased as the experiment continued towards termination with mineral supplementation, while the values reduced in animals without supplementation. This suggests that cattle offered mineral supplementation, as in the case of this study, may be unable to utilize a good portion of the supplements as it may be in excess of their requirements, which is then shed in their faeces. On the other hand, faecal magnesium tended to increase without supplementation as the experiment progressed, while mineral supplementation caused a relative decrease. This may suggest that the magnesium concentration increases through winter towards autumn and this is supported by (Mokolopi, 2019) who reported increased plant and blood magnesium from winter to autumn in grasses from communal rangeland and cattle fed on the rangeland in the North West region of South Africa.

The interactive effect between the month and the Calcium phosphate /Vitamin D3 supplementation further supported the fact that the effect of season trumps the effect of supplementation of BW and BCS reduced as the winter months progressed. This is further reflected in the serum parameters such as albumin, blood urea nitrogen, creatinine and total protein whose values drop with the advancement of winter despite the supplementations. This corroborates the idea that mineral and vitamin supplementation is not enough to meet the physiological needs of the animals in the winter season. However, a more balanced ration for energy and protein should be provided for the animals for optimum growth and performance, which will require little or no supplementations, as most of the vitamins and minerals will be present in the upgraded rations fed to the animals during the winter season.

Conclusions

The results of this study exhibited calves treated with dicalcium phosphate lick as the best performed in the most parameters. The use of dicalcium phosphate lick is recommended for overwintering of communal free-ranging beef cattle. Further research is needed to determine the correlation between blood mineral concentration, blood metabolites and the visual parameters like hair coat scores body condition scores in beef cattle raised on poor winter pastures.

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Compliance with ethics

Animal ethics committee of the University of the Free State approved the student project number UFS-AED 2017/0049 under strict recommendation on animal welfare and methodology.

Conflict of interest: The authors declare that they have no conflict of interest.

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