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The Role of Bone Phosphorus, Calcium and Magnesium on Bone Reserves of Indigenous Mixed Breed Cattle Grazing Natural Pasture during Times of Mineral Stress Induced by Drought in Mogosane Village of North West Province, South Africa

B.G. Mokolopi

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ABSTRACT

Background: Bone consists of Ca and P deposited within an organic collagen matrix. The highly porous nature of this matrix provides bone with an extensive surface area, making bone a highly labile source of both Ca and P and serves as metabolic reservoir for Ca, P and other minerals. Phosphorus in the skeleton provides large reserve, which may be mobilized when the diet is deficient in phosphorus, the measurement of bone minerals is important to evaluate bone reserves such as bone volume, bone specific gravity and bone thickness.

Methods: Ten male mix breed cattle between the ages of 6 and 12 months therefore, were randomly selected from a herd feeding exclusively on communal grazing and receiving no supplements and were used to determine the role of bone phosphorus, calcium and magnesium on bone reserves in cattle grazing natural pasture. Bone samples were analysed for P through the FASPac II Version R2MI Auto- Analyzer and were analysed for Ca and Mg through an Atomic Absorption Spectrometer.

Results: Bone volume, bone specific gravity and bone thickness were reacting with same pattern almost throughout research. Grass phosphorus was positively correlated to bone Mg (r^2= 0.466) with the P value of 0.053. Concentration of Ca in the grass was positively correlated to bone Mg (r^2= 0.524) and bone specific gravity (r^2= 0.593) with the P values of 0.051 and 0.048 respectively. The rainfall measured during the research shown to have positive correlation on the concentration of P in the grass r^2= 0.690) and of Mg (r^2= 0.848) with the P values of 0.04 and 0.001 respectively.

Key words: Bone volume, Bone specific gravity, Bone thickness.

INTRODUCTION

Rib biopsy can be used in studies related to calcium (Ca), phosphorus (P) and magnesium (Mg) metabolisms (Beighle *et al*., 1993). According to Malafaia *et al*. (2017) the rib biopsy is a very useful technique to confirm the diagnosis of P deficiency in cattle raised on pasture, as well as in experiments which evaluate the dynamics of bone tissue; because it allows the assessment of bone mineral content in cattle, by obtaining a greater number of bone samples to be used for evaluations involving radiographic images and histopathological studies. Livestock plays an important role in national economy (Madke *et al*., 2006; Kathiravan and Selvan 2011; Bairwa *et al*., 2013). Kathiran and Selvan also explained the importance of livestock as producing food, providing security, boosting crop production, generating cash income, particularly for rural people and forming a major reserve of capital for farming households. According to Satyanarayan and Jagadeeswary (2010) the livestock sector is an important component of the agricultural economy of developing countries and acts as a means of capital accumulation and a barter commodity in societies where the currency does not circulate.

And the livestock industry in the Republic of South Africa is almost entirely dependent on the native pasture for satisfying their nutritional needs. Unfortunately, several nutritional deficiencies put a limit on animal production in most pastoral areas covered mainly by grass (Ateba and Beighle, 2011). Thus, livestock production is often badly influenced by mineral defiencies or imbalances (Bhat *et al*., 2011).

As the phosphorus in the skeleton provides a large reserve, which may be mobilized when the diet is deficient in phosphorus, the measurement of bone minerals is

Department of Agriculture and Animal Health, College of Agriculture and Environmental Sciences, University of South Africa, Science Campus, Private Bag X6, Florida 1710, Johannesburg, South Africa.

Corresponding Author: B.G. Mokolopi, Department of Agriculture and Animal Health, College of Agriculture and Environmental Sciences, University of South Africa, Science Campus, Private Bag X6, Florida 1710, Johannesburg, South Africa.
Email: kgobebg@unisa.ac.za

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important to evaluate P status (Dixon *et al*., 2019). According to them, bone mineral reserves of cattle are usually evaluated from rib bone biopsies and changes in those reserves are thus important for understanding and managing the P and Ca nutrition of ruminants.

Approaches to estimate bone P reserves and net mobilisation or replenishment of P from the analysis of rib and hip (*tuber coxae*) biopsies are discussed. In at least some circumstances, breeder cows grazing P-deficient pastures mobilise bone P to alleviate the effects of diet P deficiency (Dixon *et al*., 2017). Thus, the mechanism involved in the role of bone phosphorus, calcium and magnesium on bone reserves in native cattle grazing natural pasture during times of mineral stress induced by drought remains to be elucidated.

Hale and Olson (2001) stated that beef cattle require a number of dietary mineral elements for normal body maintenance, growth and reproduction and according to them P, calcium (Ca) and magnesium (Mg) are amongst the major minerals that are required in relatively large amounts for body maintenance, growth and reproduction.

Determining the phosphorus (P) status of cattle grazing P-deficient rangelands is important for improving animal production in these areas. Markers of bone turnover offer potential as markers of P status in cattle, as they reflect bone mobilisation or bone formation. The subsequent low P content of the forage may result in subclinical or clinical P deficiencies in cattle (Malafaia *et al*., 2014). According to them subclinical deficiency of P most likely affects the majority of cattle raised exclusively on pastures in Brazil and causes considerable economic losses every year. Phosphorus (P), which is essential for animal health, has a number of important biological functions, such as providing structure and strength to bones, cell walls and phosphate buffer systems (Zhang *et al*., 2017)

In cattle, hypophosphatemia occurring at the onset of lactation is widely believed to be associated with periparturient recumbency and the downer cow syndrome (Geisert *et al*., 2010) After calcium (Ca), P is the second major component of bone mineral; P participates in the process of glycolysis and oxidative phosphorylation and maintains the integrity of cellular structures (Grünberg *et al*., 2005). Furthermore, studies in different regions of the world demonstrated that the concentrations of trace elements in pasture and rangelands can vary considerably with season and often drop to levels of concern for deficiencies in animal nutrition (Blanco-Penedo *et al*., 2009; Khan *et al*., 2008). Phosphorus's essentiality for all life on Earth is beyond doubt. As one of the three major macronutrients, it is a crucial building block of modern-day mineral fertilizers and, thus, an essential production factor for global food security (Geissler *et al*., 2019)

More than 45 years ago Cohen (1973a), explained that prolonged low levels of dietary P which cause P deficiency may be better detected from the measurement of bone P since bone P content significantly reflects a variation in P content of pasture and it can provide a better estimate of the P status of grazing cattle than blood or hair P. Furthermore, they explained that bone P is unlikely to be influenced by exercise or excitement of cattle when samples are collected.

Bone is a dynamic tissue that performs mechanical, biological and chemical functions. Bone development and chemical and physical properties are affected among others, by nutrition (Loveridge, 1999). According to him, the skeletal system forms the external structure and appearance of mammalian vertebrate species and has the obvious functions of locomotion, structural support of the body and protection of soft tissue (brain, heart, spinal cord and lungs). Bone also serves as a metabolic reservoir for Ca, P and other minerals and it houses cells responsible for bone formation and resorption.

Bone consists of Ca and P deposited within an organic collagen matrix. The highly porous nature of this matrix provides bone with an extensive surface area, making bone a highly labile source of both Ca and P (Knowlton *et al*., 2004). According to them bone mineral content is the result of the balance between rates of bone accretion and bone resorption. According to Keene *et al*. (2004), bone serves as a metabolic reservoir for Ca, P and other minerals and it houses cells responsible for bone formation and resorption.

Ca and P play a very important role in animals' growth, development and bodily functions (Grzegorczyk *et al*., 2019). Ca has been known to be important for healthy muscle and nerve activities for more than 30 years, among other minerals, hence Mg was known to have a positive effect on muscle relaxation and could improve muscle function (Clausen and Everts 1989). Ca is a fundamental element contained in almost all the structured tissues (Choucair *et al*., 2006). It has been involved in nearly every metabolic process and plays a major role in bone development, blood coagulation processes and the activation of certain enzymes, such as adenyl cyclase (Simpson and Dale 1972).

Mg serves as a major cofactor in activating many enzyme systems (Sarma and Gambhir 1995) and it is important in biological systems (Hasanat *et al*., 2017). It has a direct effect on blood vessel tone, contractility and reactivity and thus plays an important role in the physiological regulation of blood pressure (Touyz 2003).

The aim of this study was therefore, to obtain data for the subsequent establishment of the relationship among the levels of P, Ca and Mg in the bone of indigenous mixed breed cattle depending on natural grazing, on bone thickness (BT), bone volume (BV) and bone specific gravity (BSG). The assessed data included the P, Ca and Mg contents of the pastures the cattle were grazing.

Forage has been a key component of the diet of grazer and indirectly of human diet (Bashir *et al*., 2019). However, little is known about how bone P, Ca and Mg contents are affected by pasture and how they affect bone BT, BV and BSG of indigenous mixed breed cattle grazing natural pasture during times of mineral stress therefore hypothesised that:

- High levels of grass P, Ca and Mg will increase levels of bone P, Ca and Mg
- Low levels of grass P, Ca and Mg will decrease levels of bone P, Ca and Mg
- Bone P, Ca and Mg contents will have significant impact on BV, BT and BSG measured.

MATERIALS AND METHODS

The work was done in 2006 in Mogosane Village of North West Province, South Africa and it was conducted from March 2006 to March 2007, with the annual rainfall of 384.38 mm maximum. Ten male mix breed cattle between the ages of 6 and 12 months therefore, were randomly selected from a herd feeding exclusively on communal grazing. Animals were depending on natural pasture, with no supplements given. Grass samples where the research animals were grazing were also collected from the field once a month for 12 months and were analysed for P, Ca and Mg. Grass samples were collected as prescribed by Mokolopi and Beighle (2006) and were cut using a stainless- steel knife and placed in clean cloth bags at the site (Khan *et al*., 2006) Rainfall data was also recorded.

Experimental procedures

Rib biopsy was conducted for bone sampling. A trephine was used to remove a 12 mm circular core of bone that consisted of cortical and some trabecular bone. Two percent lignocaine was used for local anesthesia. The hair was shaved and the area over the rib was scrubbed for surgery. The operation was done aseptically to prevent infection in the biopsy site. A scalpel blade was used to make a 3 cm incision in the skin over the bone to be sampled, carried down through the muscle to the periosteum over the rib. The trephine was introduced into the bone and with proper movement of the trephine the core was collected from the rib. The muscle and skin were closed using absorbable suture for the muscle and non-absorbable suture for the skin.

Sandpaper was used to completely remove trabecular bone that might have been taken out with the bone sample, leaving only cortical bone for use in analysis. Bone thickness was measured in mm using calipers, as described by (Beighle, 2000). Trabecular bone was removed to eliminate inconsistent mineral values because it contained a variable number of red blood cells that contain minerals (Beighle, 2000).

A thread was used to hold the bone sample and was allowed to hang still in an analytic scale and the weight was recorded as the weight of the bone in air. While the bone sample was hanging in the scale a glass beaker containing water was introduced in such a way that the sample was lowered into the glass beaker with the water so that the bone sample was submerged in the water and the weight was recorded as the weight of the bone in water. The volume of the bone samples was calculated from the difference of the air weight and the water weight (air weight-water weight) and air weight divided by the volume was recorded as the specific gravity of the bone sample. Samples were then divided into half to provide duplicate samples.

Laboratory procedures

All laboratory equipment used in the digestion and analysis of grass and bone samples were soaked in 36% Hydrochloric acid (HCl) overnight. They were then rinsed with distilled water 3 times and were dried in a hot air oven for 16 hours at 106°C. After drying, crucibles were allowed to cool in a desiccator for 6 hours when they were then weighed to determine the empty weight. Samples were then digested as described by Beighle *et al.* (1990) and were analysed for P through the FASPac II Version R2MI Auto-Analyzer: (Astoria Pacific International 1992-2005) and were analysed according to the method of Fiske and Subarrow (1925). Pasture samples were analysed for Ca and Mg through an Atomic Absorption Spectrometer (The Analyst 700 model, 110 Bridgeport Avenue Shelton, CT 06484- 4794, USA) and were analysed as described by Tradeau and Freier (1967).

Statistical analysis

The role of P, Ca and Mg contents of BV, BSG and BTH of indigenous mixed breed cattle grazing natural pasture were analysed using General Linear Model (GLM) procedures of the statistical analyses system (SAS, 2010). The statistical model used was:

$$Y_{ijk} = \mu + T_{1} + “_{ijk}$$

Where,

Y_{ijk} = the overall observation (BV, BSG and BTH)

μ = population means

T_{1} = Effect of bone minerals (P, Ca and Mg)

$“_{ijk}$ = Residual effect

Where there was a significant T-test ($P<0.05$), Duncan Multiple Range was used to test the significance of differences between means.

The correlation between rainfall and grass P, Ca and Mg bone micro- minerals were measured with SPSS version 20.0, using Quadratic model.

RESULTS AND DISCUSSION

Although bone P and Ca concentrations did not show significant difference between March' 06 and April'06, bone Mg concentration increased significantly ($P<0.05$) from March'06 until June'06. (Table 1). Bone P and Ca concentration reacted oppositely from May'06 until November'06 and the concentration increased from November' 06 to January'07 and reacted oppositely from January' 07 to March'07. Bone Mg concentration fluctuated from June '06 until the end of the trial. Animals in March'07 had the highest value of bone Ca concentration (598.8 mg/g) and had the lowest values of bone P concentration (120.1 mg/g) and bone Mg concentration (1.8 mg/g). This indicates that the animals were trying very hard to maintain mineral homeostatic mechanism in the body and this is the reason why most researchers used dietary supplement in order to balance Ca and P level in the body of the animals or to supply

Table 1: Mean bone P, Ca and Mg concentration (mg/g, dry weight) by months.

Months	March'06	April'06	May'06	June'06	July'06	Aug'06	Sept'06	Oct'06	Nov'06	Jan'07	March'07
Bone P (mg/g)SEM	164.9 ^{a}	148.3^{a}	134.2^{a}	144.9^{a}	146.3^{a}	146.6^{a}	153.5^{a}	137.50^{a}	129.00^{a}	193.9^{a}	120.7^{a}
	± 14.17	±14.15	±13.58	±14.15	±14.52	±14.51	±14.58	±13.62	±13.06	±26.50	±13.00
Bone Ca (mg/g)SEM	300.6^{a}	224.9^{a}	269.1^{a}	245.3^{a}	265.7a	270.7^{a}	259.6^{a}	270.1^{a}	269.9^{a}	398.7^{a}	598.8^{a}
	±42.20	±4013	±44.17	±41.30	±44.17	±66.20	±64.34	±43.07	±42.00	±69.17	±8108
Bone Mg (mg/g)SEM	1.17^{a}	2.28^{ab}	3.67^{bc}	3.76^{bc}	3.00^{abc}	3.85^{bc}	3.30^{bc}	3.53^{bc}	5.22^{c}	5.15^{c}	1.83^{ab}
	±0.54	±0.53	±0.75	±0.76	±0.48	±0.78	±0.47	±0.50	±0.90	±0.90	±0.49

^{abcd}Means with different letters in the same row are significantly (*P< 0.05*) different.

Table 2: Mean bone volume, bone specific gravity and bone thickness (mm) by months.

Months	March'06	April'06	May'06	June'06	July'06	Aug'06	Sept'06	Oct'06	Nov'06	Jan'07	March'07
Bone volume SEM	0.05±0.01	0.07±0.01	0.07±0.01	0.08±0.01	0.07±0.01	0.09±0.02	0.11±0.02	0.10±0.02	0.10±0.02	0.09±0.02	0.05±0.01
Bone specific gravity SEM	2.17±0.17	2.21±0.17	1.94±0.17	2.25±0.16	1.95±0.26	1.94±0.17	1.70±0.25	1.95±0.26	2.08±0.32	2.20±0.32	1.81±0.25
Bone thickness SEM	1.19±0.19	1.45±0.19	1.48±0.19	1.93±0.18	1.41±0.18	1.91±0.19	2.21±0.28	2.26±0.30	1.77±0.35	1.92±0.35	1.70±0.33

minerals that are missing or not consumed in sufficient quantity in the animal's diet.

All the parameters were reacting with the same pattern almost throughout the research except that bone thickness and bone volume increased from July'06 to September'06 while bone specific gravity declined during that period (Table 2). This result also indicates the possible tendency for the animals to use their homeostatic mechanisms to increase bone thickness when bone specific gravity was declining and to increase bone specific gravity when bone thickness was declining. From there bone volume and bone specific gravity reacted differently until January'07, while bone thickness reacted with the same pattern as bone specific gravity during that period except in November'06. Although there were no significant differences among the stated parameters, all the parameters were low in March'06.

High values of bone volume, bone specific gravity and bone thickness were found in September'06 (0.11), in June'06 (2.25) and in October'06 (2.26 mm) respectively while low values were found in March'06 and March'07 (0.05), in September'06 (1.70) and in March'06 (1.19 mm) respectively. The content of P, Ca and Mg in the grass measured on a dry weight basis (Table 3) reacted similar to pattern of the rainfall in most of the trial except in September'06, October'06 and November'06 and in June'06, August'06 and November'06, respectively.

Furthermore, the content of Ca in the grass reacted differently with the pattern of the rainfall especially from March'06 to April'06 and from September'06 to November'06 and also in February'07. March'06 had the highest value of rainfall of 111.2 mm and during that period there were high values of P and Mg concentrations. All the minerals concentrations in the grass increased with rainfall in January'07 and March'07. The decreased in grass P, Ca and Mg concentrations with the rainfall was seen in May'06 and July'06.

The content of phosphorus in the cortical bone was following the same pattern as the one followed by the content of phosphorus in the grass almost throughout the trial except in June'06, July'06 and March'07 where bone P concentration increased while grass P concentration decreased during that period. Grass phosphorus (P) concentration (mg/g) found in pasture samples collected during this research, had a significant ($P<0.05$) influence in magnesium (Mg) concentration found in the bone. It was also found out that grass calcium (Ca) concentration (mg/g) had a significant ($P<0.05$) effect in the Mg concentration (mg/g) found in the bone, whereas the content of Mg in grass was significantly ($P<0.05$) related to Mg content found in bone.

It was unexpected to find an increase in the bone P concentration during the months of June'06, July'06, August'06 and September'06 indicating that animals were actively incorporating P into the bone compared to previous months, because the grass P concentrations were down in April'06, May'06 and June '06 and the rainfall was decreasing in

Table 3: Mean grass P, Ca and Mg concentration (mg/g, dry weight) and rainfall (mm) by months.

Months	March'06	April'06	May'06	June'06	July'06	Aug'06	Sept'06	Oct'06	Nov'06	Dec'06	Jan'07	Feb'07	Marc'07
Grass P (mg/g, dry weight) SEM	2.40^{d}	1.74^{bc}	1.17^{b}	0.34^{a}	0.33^{a}	0.35^{a}	1.35^{bc}	1.01^{b}	0.74^{b}		1.10^{b}	1.09^{b}	1.10^{b}
	±0.28	±0.19	±0.24	±0.09	±0.09	±0.09	±0.10	±0.09	±0.10		±0.10	±0.10	±0.09
Grass Ca (mg/g, dry weight) SEM	7.53^{c}	7.90^{c}	8.44^{c}	7.29^{b}	7.01^{b}	8.80^{c}	9.01^{d}	7.19^{b}	6.31^{a}		6.45^{b}	6.78^{b}	8.99^{d}
	±0.22	±0.20	±0.23	±0.23	±0.30	±0.23	±0.28	±0.23	±0.33		±0.30	±0.30	±0.29
Grass Mg (mg/g, dry weight) SEM	0.47^{d}	0.37^{c}	0.32^{b}	0.34^{c}	0.30^{a}	0.29^{a}	0.29^{a}	0.31^{b}	0.30^{b}		0.35^{c}	0.28^{a}	0.33^{c}
	±0.01	±0.01	±0.03	±0.01	±0.03	±0.01	±0.02	±0.01	±0.02		±0.01	±0.01	±0.01
Rainfall (mm)= x1	111.2	93.6	9.7	4.2	0	19.3	1	14.1	33.8	55	60.8	9.7	21.7

[abcd] Means with different letters in the same row are significantly *(P< 0.05)* different

Table 4: Correlation results among measured parameters.

Dependent Variables	Dependent Variables													
	Grass P		Grass Ca		Grass Mg		Bone P		Bone Ca		Bone Mg		Rainfall	
	r^2	P value	r^2	P value	r^2	P value	r^2	P value	r^2	P value	r^2	P value	r^2	P value
Grass P													0.690	0.04
Grass Ca													0.111	0.396
Grass Mg													0.848	0.001
Bone P	0.078	0.822	0.117	0.983	0.165	0.805								
Bone Ca	0.093	0.676	0.250	0.196	0.067	0.477								
Bone Mg	0.466	0.053	0.524	0.051	0.363	0.698								
Bone Volume	0.285	0.407	0.223	0.193	0.428	0.039	0.027	0.899	0.239	0.591	0.616	0.054		
Bone Specific Gravity	0.171	0.326	0.593	0.048	0.506	0.051	0.187	0.850	0.127	0.557	0.199	0.411		
Bone Thickness	0.396	0.242	0.170	0.245	0.374	0.154	0.013	0.751	0.004	0.875	0.403	0.130		

April'06, May'06, June'06 and July'06. During these months the grazing was extremely limited and animals were not receiving a good level of nutrition. Content of Mg in the cortical bone was reacting differently from the Mg content found in the grass almost throughout the research except in July'06, September'06 and January'07 where they were following the similar pattern.

High values of bone volume, bone specific gravity and bone thickness were found in September'06 (0.11), in June'06 (2.25) and in October'06 (2.26 mm) respectively while low values were found in March'06 and March'07 (0.05), in September'06 (1.70) and in March'06 (1.19 mm) respectively. This result indicates the possible tendency for the animals to use their homeostatic mechanisms to increase bone thickness when bone specific gravity was declining and to increase bone specific gravity when bone thickness was declining.

When bone minerals were observed during this period, it was found out that bone P content decreased in October '06 while bone Ca and Mg concentrations increased. In January'07 both bone P and Ca concentrations increased while bone Mg content declined during that month. These results also suggest ability of the animals to maintain the mineral homeostatic mechanisms in the body by maintaining a constant level of minerals in their body. The rainfall recorded in October (14.1 mm) and the one recorded in January'07 (60.8 mm) could have influence in the increase in bone thickness and bone P and Ca concentrations in January'07.

When correlation was done (Table 4), it was found out that the concentration of P in the grass was positively correlated to bone Mg (r^2= 0.466) with the P value of 0.053. Concentration of Ca in the grass was positively correlated to bone Mg (r^2= 0.524) and bone specific gravity (r^2= 0.593) with the P values of 0.051 and 0.048 respectively. The concentration of Mg in the grass showed a positive correlation to bone volume (r^2 = 0.428) and to bone specific (r^2= 0.506) with the P values of 0.039 and 0.051 respectively. The concentration of Mg in the bone was positively correlated to bone volume (r^2 = 0.616) with the value of 0.054. The rainfall measured during the research shown to have positive correlation on the concentration of P in the grass r^2= 0.690) and of Mg (r^2= 0.848) with the P values of 0.04 and 0.001 respectively.

CONCLUSION

For animals to maintain an adequate concentration of P in the bone during this time of nutritional stress was much unexpected. In this research the animals were able to maintain or improve bone P during the winter season when P in the diet was limiting. Furthermore, this study, indicates possible tendency for the animals to use their homeostatic mechanisms to increase bone thickness when bone specific gravity was declining and to increase bone specific gravity when bone thickness was declining. Further research is therefore needed to verify these results and further investigate the ability of the bovine to store P in bone during times of drought and P deficient pastures.

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Ethics statement

The research animals were handled under the supervision of the University Veterinarian from the Animal Health Department. This survey was part of a PhD study which was approved by a research ethics committee at the Institutional level. Therefore, the welfare and handling of the research animals were properly followed.

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