Phosphorus and the grazing ruminant. 3. Rib bone samples as an indicator of the P status of cattle

Marion V.P. Read and E.A.N. Engels

Agricultural Research Institute, Glen

W.A. Smith

Department of Animal Science, University of Stellenbosch, Stellenbosch

Rib bone samples were taken from free-grazing, crossbred, beef-type cows at different stages of their reproductive cycles to monitor the P status of supplemented and unsupplemented groups. Two experimental sites, Glen and Armoedsvlakte were used, the latter notorious for its P-deficient pastures. P content of bone samples (expressed as mg P/cm^3 fresh bone) proved a reliable and sensitive indicator of the P status of grazing cattle. Concentrations suggesting adequacy (140 – 150 mg P/cm^3) agree with those published elsewhere. During lactation, concentrations below 100 mg/cm^3 were observed in the severely P-deficient (– P) group at Armoedsvlakte. There was no suggestion of even a sub-clinical deficiency at Glen; the only advantage of supplementation was maintenance of higher bone mineral reserves during late lactation, especially in the young heifer.

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Ribbebeenmonsters is van vryweidende, kruisgeteelde vleisbeeskoeie in verskillende stadia van hul reproduktiewe siklus geneem om die P-status van groepe met of sonder P-aanvulling te monitor. Twee proefpersele naamlik Glen en Armoedsvlakte (laasgenoemde bekend vir P-gebrekkige weidings) is vir die ondersoek gebruik. P-inhoud van beenmonsters (uitgedruk in mg P/cm^3 varsbeen) blyk 'n betroubare en sensitiewe maatstaf van die P-status van weidende beeste te wees. Konsentrasies wat genoegsame P aandui (140 – 150 mg/cm^3) stem goed ooreen met gepubliseerde gegewens. Konsentrasies minder as 100 mg/cm^3 is tydens laktasie in die ernstig-P-gebrekkige (– P) groep by Armoedsvlakte waargeneem. Daar is geen aanduidings van selfs 'n subkliniese tekort by Glen gevind nie. Die enigste voordeel van aanvulling hier is 'n verbeterde instandhouding van die mineraalreserwes tydens laktasie in veral die jong verse.

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Marion V.P. Read* and E.A.N. Engels
Agricultural Research Institute, Glen, 9360
Republic of South Africa

W.A. Smith
Department of Animal Science, University of Stellenbosch, Stellenbosch, 7600 Republic of South Africa

*To whom correspondence should be addressed

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Introduction

Specific clinical symptoms like aphosphorosis may be useful in diagnosing a mineral deficiency, although by the time these are apparent, the deficiency is usually in an advanced stage (Little, 1982). Most mineral imbalances and particularly the borderline or sub-clinical cases may not produce symptoms specific to a single mineral and therefore chemical analyses and biological assays are essential (McDowell, Conrad & Ellis, 1984).

Although feed is the only sample suitable for diagnosing a P deficiency (Committee on Mineral Nutrition, 1973, cited by Reid & Horvath, 1980) determining the P status of grazing ruminants is complicated by their selective grazing behaviour. Hand-plucked pasture samples are clearly not representative of the diet selected by grazing animals (e.g. Langlands, 1974; Engels, De Waal, Biel & Malan, 1981; Read, 1984), and although sampling the pasture with oesophageally fistulated animals is the most reliable approach (Van Dyne & Torell, 1964), salivary contamination makes these samples unsuitable for estimating dietary P levels (Langlands, 1966) unless the salivary P is labelled with ^{32}P (Little, McLean & Winter, 1977).

Various other criteria have therefore been used as diagnostic aids of the P status of grazing ruminants: soil analyses (McDowell, Bauer, Galdo, Koger, Loosli & Conrad, 1982); pica or depraved appetite (Bisschop, 1964); various aspects of bone composition (Priboth & Koni, 1980; Schroter & Seidel, 1980; Little, 1982); bone growth (Wise, Smith & Barnes, 1958) and breaking strength (Shirley, Kirk, Davies & Hodges, 1971, cited by Cohen, 1973b); blood analyses (McDowell, *et al.*, 1982); rumen contents (Marshall, Torell & Bredon, 1967) and rumen fluid (Witt & Owens, 1983); faecal P concentration (Cohen, 1974); P levels in milk (Theiler, Green & Du Toit, 1927); P balance studies (Field, Coop, Dingwall & Munro, 1982); analysis of muscle tissue (Belonje, 1978) and body organs (Kiatoko, McDowell, Bertrand, Chapman, Pate, Martin & Conrad, 1982); hair (Tischer, 1977); and the performance of animals in terms of bodymass (growth) and reproductive efficiency (Holmes, 1981).

Considering that in cattle the most common mineral deficiency in the world is that of P (McDowell, *et al.*, 1984) and that South African natural pastures, especially the grassveld areas, are thought to be low in P for at least part of the year (Du Toit, Louw & Malan, 1940), it is imperative to identify the most precise yet practical indicator of the grazing ruminants' P status.

The skeleton is probably the most reliable guide to P nutrition because it contains a large proportion (80 – 85%, Simesen, 1970) of the total body P, which can be resorbed during times of scarcity (Hill, 1962). Most studies of the skeleton were conducted on animals slaughtered specifically for that purpose, until the development of a rib bone biopsy technique (Little, 1972) which enabled the serial sampling of the same individual. This technique appears to have been used successfully in various studies using both cattle (Cohen, 1973a, b; Little & Shaw, 1979) and sheep (Little & McMeniman, 1973; Little, Siemon & Moodie, 1978). However, there have also been some doubts of its reliability with sheep (Belonje, 1978; Belonje & Van den Berg, 1983).

One of the main objectives of this study was to evaluate bone, blood and faeces as indicators of the P status of grazing ruminants. The results of bone and blood analyses of sheep have already been reported (Read, Engels & Smith, 1984a). This article and the following one will therefore be confined mainly to the results obtained when using these indicators to monitor the P status of cattle grazing at two experimental sites, Glen and Armoedsvlakte.

Experimental procedure

The animals used, treatments applied, experimental sites, forage P levels, and general experimental procedure have already been described (Read, Engels & Smith, 1984b). Rib bone samples were usually taken from eight cattle in each group at Glen and six from each group at Armoedsvlakte, at different stages of the animals' reproductive cycle. A slightly modified version of Little's (1972) biopsy technique, as described by Read (1984) was used. Bone samples were analysed for P, Ca, Mg, percentage ash, and specific gravity (SG) according to De Waal (1979). At weaning, rib bone samples were also taken from the calves of the dams sampled at Glen.

Results and Discussion

In agreement with the findings of Little (1972) and Little & McMeniman (1973) mineral concentrations expressed per unit volume of fresh bone (i.e. mg/cm^3) were found to be more sensitive than those expressed as percentage of dry bone. All bone mineral concentrations are therefore expressed as mg/cm^3 fresh bone.

Because biopsies were performed on the same rib of the same side of the animals at a specific sampling period, comparisons between treatments are valid for a specific sampling period, but not necessarily between different periods because the degree of mineralization of one rib may differ from that of another (Little & Minson, 1977).

Changes in bone parameters of cattle at Glen

During early lactation of the first calving season (1978/79) the P reserves of the supplemented groups were slightly more favourable (Table 1) but by weaning the −P dams had mobilized considerably more reserves than the +P and PR groups. Both the −P and +P groups seem to have restored these reserves by late pregnancy (1979), whereas the P status

Table 1 Chemical composition of rib bone samples taken from the cattle at Glen[a]

Year	Physiological status	Treatment	Mineral content (mg/cm^3) P	Ca	Mg	Ca:P ratio	Ash (%)	SG
1978	Late pregnancy	−P	181,4^a	378,8^a	7,4^a	2,09	64,6^a	1,82^a
		+P	185,5^a	378,2^a	6,7^a	2,03	63,9^a	1,81^a
		PR	184,6^a	390,2^a	7,4^a	2,11	64,6^a	1,81^a
1979	Early lactation	−P	164,1^a	348,0^a	5,5^a	2,12	65,8^a	1,72^a
		+P	179,8^b	376,7^a	5,7^a	2,10	67,2^a	1,78^a
		PR	171,6^{ab}	368,6^a	6,0^a	2,15	66,4^a	1,73^a
	Late lactation/weaning	−P	112,4^a	211,3^a	3,4^a	1,88	53,3^a	1,49^a
		+P	136,1^b	258,7^b	4,1^{ab}	1,90	55,8^b	1,58^b
		PR	149,1^b	278,4^b	4,5^b	1,87	57,5^b	1,62^b
	Late pregnancy	−P	134,5^a	258,6^a	4,4^a	1,92	58,6^a	1,55^a
		+P	155,4^a	307,7^b	5,7^a	1,98	60,1^a	1,66^a
		PR	151,1^a	309,9^b	4,7^a	2,05	62,2^a	1,62^a
1980	Early lactation	−P	129,2^a	281,9^a	4,4^a	2,18	58,4^a	1,53^a
		+P	131,8^a	290,7^{ab}	5,6^a	2,21	59,0^a	1,52^a
		PR	144,4^a	325,2^b	7,0^b	2,25	60,2^a	1,65^a
	Late lactation/weaning	−P	128,5^a	266,1^a	7,1^a	2,07	60,0^a	1,57^a
		+P	136,4^{ab}	275,1^a	7,1^a	2,01	60,4^a	1,62^a
		PR	150,0^b	314,1^b	8,2^b	2,09	62,9^a	1,64^a
1981	Late lactation/weaning	−P	146,5^a	324,5^a	4,4^a	2,21	60,6^a	1,65^a
		+P	155,8^{ab}	336,7^{ab}	4,6^a	2,16	62,7^{ab}	1,71^{ab}
		PR	166,6^b	366,0^b	5,1^a	2,19	64,6^b	1,77^b
1982	Late lactation/weaning	−P	149,1^a	325,6^a	7,8^a	2,18	61,5^a	1,64^a
		+P	143,2^a	307,3^a	8,6^a	2,15	58,9^a	1,65^a
		PR	157,5^a	340,9^a	8,6^a	2,16	61,7^a	1,69^a
	Late pregnancy	−P	145,4^a	290,8^a	11,8^a	2,00	58,2^a	1,61^a
		+P	165,2^b	326,7^b	15,5^a	1,98	61,8^b	1,64^a
		PR	159,9^a	319,6^a	15,9^a	2,00	61,6^a	1,64^a

[a]Differences between treatments tested within individual periods and years; treatments with same superscripts do not differ significantly ($P < 0,05$).

of the PR group remained essentially the same. During lactation of 1980, the P reserves were again depleted to rank the groups according to their level of P supplementation at weaning (i.e. $-P < +P < PR$). The seemingly higher concentrations of bone minerals during 1981 and 1982 (in comparison to those of 1979 and 1980) may suggest a P intake inadequate for both the growth and development of the young dam and the products of conception, whereas greater reserves probably became available as the cattle matured.

Despite the observed differences in bone mineralization at weaning, chemical analyses of bone samples taken at the same time from calves of these dams failed to show any differences over the experimental period (Table 2). In times of inadequacy the dam therefore attempts to ensure the survival of her offspring by mobilizing P reserves to the detriment of her own.

Data collated by the ARC (1965) indicated that the Ca:P ratio in bone differs very little from the theoretically accepted ratio of 2:1, as is also apparent from Table 1. Except during late pregnancy (1979) and lactation (1983) not even the severely P-deficient cattle at Armoedsvlakte exhibited a disturbed Ca:P ratio (Table 3). This agrees with the earlier conclusion of Theiler, Du Toit & Malan (1937) that this ratio fails to explain the production of osteodystrophic diseases when Ca and/or P intake is deficient. However, this may not be totally unexpected because of the composition of the bone crystal: $Ca_{10}(PO_4)_6OH_2$ (Ganong, 1977) and considering that there is no hormone (known to the authors) whose function is to bring about the differential resorption of P alone but rather, because of their interrelationship, the homeostasis of Ca and P is regulated by calcitonin and parathormone in response to circulating levels of ionized serum Ca^{++} (White, Handler & Smith, 1973). This probably justifies the conclusion of Belonje & Van den Berg (1983) that Ca is 'dominant' and '. . . has a more profound effect on bone phosphorus than does dietary phosphorus . . .' and that '. . . the use of bone phosphorus analyses to assess phoshorus intake should therefore be viewed with caution'. Admittedly if the diet was deficient in Ca (which is highly unlikely at Armoedsvlakte) and P, a positive response in bone mineral concentration could hardly be expected from supplementary P, although probably not because of the 'dominant role' of Ca but rather the chemical composition of bone.

In contrast to these reports concerning the relative stability of the Ca:P ratio, Cohen's (1973b) results suggested that P was being resorbed from bone without Ca. The only evidence in support of this was the observed decrease in the calcium phosphate:calcium carbonate ratio in bone of mature cows from 6,67:1 to 5,3:1 in P-deficient cows (Neal, Palmer, Eckles & Gullickson, 1931) and that in rats carbonate could be removed and deposited without phosphate (Rockenmaker & Kramer, 1945, cited by Cohen, 1973b).

Bone Mg concentration of all groups remained relatively stable except for the increasing tendency observed towards the end of the trial. Ash content and SG varied little between the groups although these parameters did tend to rank the groups according to their level of P supplementation.

Table 2 Chemical composition of rib bone samples taken from the calves at Glen, at weaning

Year	Treatment of dam	Mineral content (mg/cm^3) P	Ca	Mg	Ash (%)	SG
1979	$-P$	123,8	228,9	4,4	57,0	1,55
	$+P$	125,0	232,2	4,4	58,3	1,54
	PR	122,3	219,4	4,5	56,6	1,51
1980	$-P$	111,3	214,3	7,6	59,7	1,48
	$+P$	115,7	225,0	7,6	61,1	1,51
	PR	112,9	218,9	6,7	61,5	1,48
1981	$-P$	123,0	254,6	5,4	59,8	1,55
	$+P$	119,4	238,7	5,4	60,1	1,52
	PR	120,4	243,3	5,3	59,8	1,50
1982	$-P$	122,6	247,7	8,1	58,2	1,56
	$+P$	112,7	231,5	8,0	58,3	1,53
	PR	109,8	221,2	7,5	56,3	1,48

Changes in bone parameters of cattle at Armoedsvlakte

All bone parameters were very similar for both treatments throughout the first calving season (1978/79) and even during the following pregnancy. It was only during lactation in 1980 that the mobilization of bone mineral by the $-P$ group was not matched by the $+P$ group. For the remainder of the experimental period the mineral reserves of the unsupplemented group lagged seriously behind those of the $+P$ group (Table 3).

Because bone P concentration is a 'relatively new' indicator of the P status, its normal physiological range and level indicative of deficiency are not yet well established. Little & Shaw (1979) stated that levels over 150 mg P/cm^3 in samples from the 12th rib would indicate adequacy and levels 'around' 120 mg P/cm^3, deficiency, although the nutritional history of the animals must also be considered before pronouncing them as being P deficient because it is possible (according to these same authors) for concentrations higher than 150 mg P/cm^3 to be observed in bone samples from P-deficient animals. These levels may, however, not be totally comparable with those of the present study because full core samples of rib bone were taken in the modified technique instead of a single layer of cortical bone as described in the original biopsy technique by Little (1972).

Assuming that concentrations of between 140 and 150 mg P/cm^3 indicate adequacy, even the $+P$ group might be considered deficient at times (Table 3). This suggests that either the lick intake was insufficient or, more probably, that the samples were taken at those stages of the reproductive cycle when mineral reserves were at their lowest. In contrast, the $-P$ cattle could safely be described as suffering from an uncomplicated P deficiency from the first half of 1980, approximately 2 years after the trial commenced, whereas using a concentrate diet Theiler, *et al.* (1927) induced this condition in heifers after only 9 months.

Although the differences were not always significant at the 5% level, the bone Mg reserves of the $-P$ group seem to have been depleted considerably together with those of P and Ca, probably because of the interrelationship of these minerals (Jacobson, Hemken, Button & Hatton, 1972).

Following the mobilization of reserve minerals in bones, a concomitant decrease in bone ash percentage might well be expected, and this was observed in the $-P$ group of the present study from 1980 onwards. However, because the mechanism by which mineral matter is lost from the skeleton is that of resorption (i.e. the removal of both the bone matrix and mineral matter) and not demineralization, the mass of bones and ash would be reduced but not the ash percentage (Duckworth & Hill, 1953). The decrease observed in the $-P$ group may, according to these same authors, be explained by the '. . . persistence or accumulation of non-osseous organic matter . . .' or to growth of new osteoid during or after resorption.

Table 3 Chemical composition of rib bone samples taken from the cattle at Armoedsvlakte[a]

Year	Physiological status	Treatment	Mineral content (mg/cm³) P	Ca	Mg	Ca:P ratio	Ash (%)	SG
1978	Late pregnancy	−P	183,4[a]	388,4[a]	6,6[a]	2,12	66,6[a]	1,80[a]
		+P	171,5[a]	375,6[a]	6,7[a]	2,19	65,8[a]	1,78[a]
1979	Lactation	−P	159,7[a]	320,9[a]	4,0[a]	2,00	63,3[a]	1,65[a]
		+P	157,2[a]	313,8[a]	4,2[a]	2,00	62,2[a]	1,66[a]
	Late pregnancy	−P	133,9[a]	226,8[a]	4,7[a]	1,69	59,6[a]	1,44[a]
		+P	135,7[a]	245,6[a]	4,9[a]	1,81	56,6[a]	1,46[a]
1980	Lactation	−P	109,5[a]	231,6[a]	3,2[a]	2,11	55,7[a]	1,48[a]
		+P	136,1[b]	288,8[b]	4,2[a]	2,12	60,1[b]	1,60[b]
	Late pregnancy	−P	107,6[a]	223,2[a]	3,6[a]	2,07	55,5[a]	1,46[a]
		+P	146,7[b]	293,1[b]	6,2[b]	2,00	61,2[b]	1,63[b]
1981	Lactation	−P	98,2[a]	214,4[a]	2,7[a]	2,18	54,6[a]	1,38[a]
		+P	142,7[b]	304,3[b]	5,6[b]	2,13	59,5[b]	1,59[b]
	Late pregnancy	−P	120,7[a]	283,5[a]	2,1[a]	2,35	59,6[a]	1,51[a]
		+P	142,0[a]	308,1[a]	4,0[a]	2,17	59,4[a]	1,59[a]
1982	Late pregnancy	−P	101,1[a]	223,8[a]	5,7[a]	2,21	50,3[a]	1,45[a]
		+P	132,7[b]	275,4[b]	6,8[a]	2,08	57,8[b]	1,59[b]
1983	Lactation	−P	98,6[a]	151,7[a]	3,0[a]	1,54	53,1[a]	1,41[a]
		+P	134,6[b]	225,8[b]	5,4[b]	1,90	57,4[a]	1,56[b]

[a,b]Differences between treatments tested within individual periods and years; treatments with same superscripts do not differ significantly ($P < 0,05$).

A loss of mineral matter increases the bone's porosity and is reflected in a reduced SG (Table 3). The determination of SG of cancellous tissue from the tuber coxae of cattle has been considered a high-speed test with sufficient accuracy for identifying bone disorders which accompany a reduced ash mass in both single cases or for herd diagnosis (Priboth & Koni, 1980). Unfortunately bone SG may prove less sensitive than the P content expressed as mg P/cm³ fresh bone (Little & McMeniman, 1973; Little & Minson, 1977; Little & Ratcliff, 1979) when investigating a possible sub-clinical aphosphorosis (Table 1). However, in more severe cases (e.g. at Armoedsvlakte) both the SG and bone P content may be equally conclusive in identifying a P deficiency.

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References

AGRICULTURAL RESEARCH COUNCIL, 1965. The nutrient requirements of farm livestock No. 2. Ruminants. Technical reviews and summaries. HMSO, London.

BELONJE, P.C., 1978. An investigation into possible methods of assessing the intake of calcium and phosphorus by grazing sheep. *Onderstepoort J. vet. Res.* 45, 7.

BELONJE, P.C. & VAN DEN BERG, A., 1983. Failure of bone phosphorus to indicate dietary intake of phosphorus by sheep. *Onderstepoort J. vet. Res.* 50, 1.

BISSCHOP, J.H.R., 1964. Feeding phosphates to cattle. S.A. Dept. A.T.S., Science Bulletin 365.

COHEN, R.D.H., 1973a. Phosphorus nutrition of beef cattle. 2. Relation of pasture phosphorus to phosphorus content of blood, hair and bone of grazing steers. *Aust. J. exp. Agric. Anim. Husb.* 13, 5.

COHEN, R.D.H., 1973b. Phosphorus nutrition of beef cattle. 3. Effect of supplementation on the phosphorus contents of hair and bone of grazing steers. *Aust. J. exp. Agric. Anim. Husb.* 13, 625.

COHEN, R.D.H., 1974. Phosphorus nutrition of beef cattle. 4. The use of faecal and blood phosphorus for the estimation of phosphorus intake. *Aust. J. exp. Agric. Anim. Husb.* 14, 709.

DE WAAL, H.O., 1979. Die voedingswaarde van veldweiding van die sentrale Oranje-Vrystaat vir skape met spesiale verwysing na die rol van proteïen- en fosforaanvullings. M.Sc.-tesis, Universiteit, Stellenbosch.

DUCKWORTH, J. & HILL, R., 1953. The storage elements in the skeleton. *Nutr. Abstr. Rev.* 23, 1.

DU TOIT, P.J., LOUW, J.A. & MALAN, A.I., 1940. A study of the mineral content and feeding value of natural pastures in the Union of South Africa. *Onderstepoort J. vet. Sci.* 14, 123.

ENGELS, E.A.N., DE WAAL, H.O., BIEL, L.C. & MALAN, A., 1981. Practical implications of the effect of drying and treatment on nitrogen content and *in vitro* digestibility of samples collected by oesophageally fistulated animals. *S. Afr. J. Anim. Sci.* 11, 247.

FIELD, A.C., COOP, R.L., DINGWALL, R.A. & MUNRO, C.S., 1982. The phosphorus requirements for growth and maintenance of sheep. *J. agric. Sci., Camb.* 99, 311.

GANONG, W.F., 1977. In: Review of Medical Physiology (8th Ed.). Lange Medical Publications, California, p. 294.

HILL, R., 1962. The provision and metabolism of calcium and phosphorus in ruminants. *Wld. Rev. Nutr. Diet.* 3, 129.

HOLMES, J.H.G., 1981. Phosphate deficiency in cattle on the Sepik plains of Papua New Guinea. *Trop. Anim. Hlth. Prod.* 13, 169.

JACOBSON, D.R., HEMKEN, R.W., BUTTON, F.S. & HATTON, R.H., 1972. Mineral nutrition, calcium, phosphorus, magnesium and potassium interrelationships. *J. Dairy Sci.* 55, 935.

KIATOKO, M., McDOWELL, L.R., BERTRAND, J.E., CHAPMAN, H.L., PATE, F.M., MARTIN, F.G. & CONRAD, J.H., 1982. Evaluating the nutritional status of beef cattle herds from four soil order regions of Florida. 1. Macroelements, protein, carotene, vitamins A and E, hemoglobin and hematocrit. *J. Anim. Sci.* 55, 28.

LANGLANDS, J.P., 1966. Studies on the nutritive value of the diet selected by grazing sheep. I. Differences in composition between herbage consumed and material collected from oesophageal fistulae. *Anim. Prod.* 8, 253.

LANGLANDS, J.P., 1974. Studies on the nutritive value of the diet selected by grazing sheep. VII. A note on hand-plucking as a technique for estimating dietary composition. *Anim. Prod.* 19, 249.

LITTLE, D.A., 1972. Bone biopsy in cattle and sheep for studies of phosphorus status. *Aust. vet. J.* 48,. 668.

LITTLE, D.A., 1982. Utilization of minerals. In: Nutritional Limits to Animal Production from Pastures. Ed. Hacker, J.B., Commonwealth Agricultural Bureaux, Farnham Royal, U.K. p. 259.

LITTLE, D.A., McLEAN, R.W. & WINTER, W.H., 1977. Prediction of the phosphorus content of herbage consumed by grazing cattle. *J. agric. Sci., Camb.* 88, 533.

LITTLE, D.A. & McMENIMAN, N.P., 1973. Variation in bone composition of grazing sheep in South-Western Queensland, related to lactation and type of country. *Aust. J. exp. Agric. Anim. Husb.* 13, 229.

LITTLE, D.A. & MINSON, D.J., 1977. Variation in the phoshorus content of bone samples obtained from the last three ribs of cattle. *Res. Vet. Sci.* 23, 393.

LITTLE, D.A. & RATCLIFF, D., 1979. Phosphorus content of bovine rib: influence of earlier biopsy of the same rib. *Res. Vet. Sci.* 27, 239.

LITTLE, D.A. & SHAW, N.H., 1979. Superphosphate and stocking rate effects on a native pasture oversown with *Stylosanthes humilis* in central coastal Queensland. 5. Bone phosphorus levels in grazing cattle. *Aust. J. exp. Agric. Anim. Husb.* 19, 645.

LITTLE, D.A., SIEMON, N.J. & MOODIE, E.W., 1978. Effects of varying phosphorus intake and requirements on measures of skeletal mineralization in the ewe. *Aust. J. exp. Agric. Anim. Husb.* 18, 514.

MARSHALL, D.T., TORELL, D.T. & BREDON, R.M., 1967. Comparison of tropical forage of known composition with samples of these forages collected by oesophageal fistulated animals. *J. Range Manage.* 20, 310.

McDOWELL, L.R., BAUER, B., GALDO, E., KOGER, M., LOOSLI, J.K. & CONRAD, J.H., 1982. Mineral supplementation of beef cattle in the Bolivian tropics. *J. Anim. Sci.* 55, 964.

McDOWELL, L.R., CONRAD, J.H. & ELLIS, G.L., 1984. Mineral deficiencies and imbalances, and their diagnosis. In: Herbivore nutrition in the subtropics and tropics. Eds. Gilchrist, F.M.C. & Mackie, R.I. The Science Press, Graighall, South Africa. p. 67.

NEAL, W.M., PALMER, L.S., ECKLES, C.H. & GULLICKSON, T.W., 1931. Effect of age and nutrition on the calcium phosphate:calcium carbonate ratio in the bones of cattle. *J. Agric. Res.* 42, 115.

PRIBOTH, W. & KONI, A., 1980. Bedeutung einfacher bioptisch — physikalischer Methoden für Osteopathiediagnostik beim Rind. *Mh. Vet. Med.* 35, 380.

READ, MARION V.P., 1984. Animal performance from natural pastures and the effects of phosphorus supplementation. M.Sc. thesis, University of Stellenbosch.

READ, MARION V.P., ENGELS, E.A.N. & SMITH, W.A., 1984a. Phosphorus and the grazing ruminant. 1. The effect of supplementary P on sheep at Armoedsvlakte. *S. Afr. J. Anim. Sci.* 16, 1.

READ, MARION V.P., ENGELS, E.A.N. & SMITH, W.A., 1984b. Phosphorus and the grazing ruminant. 2. The effect of supplementary P on cattle at Glen and Armoedsvlakte. *S. Afr. J. Anim. Sci.* 16, 7.

REID, R.L. & HORVATH, D.J., 1980. Soil chemistry and mineral problems in farm livestock. A review. *Anim. Feed. Sci. Techn.* 5, 95.

SCHROTER, J. & SEIDEL, H., 1980. Verlaufsuntersuchungen sum verhalten eniger Parometer des Mineralstoffwechsels unter besonderer Berucksichtigung des Mineralisationsgrades des Skeletts der Milchkuh im Zeitbereich 4 Wochen ante bis 16 wochen post partum. *Arch. exp. Vet. Med.* 34, 291.

SIMESEN, M.G., 1970. Calcium, inorganic phosphorus and magnesium. In: Clinical Biochemistry in Domestic Animals. Eds. Kaneko, J.J. & Cornelius, C.E. Academic Press, New York. p. 313.

THEILER, A., DU TOIT, P.J. & MALAN, A.I., 1937. Studies in mineral metabolism. XXXVII. The influence of variations in the dietary phosphorus and in the Ca:P ratio on the production of rickets in cattle. *Onderstepoort J. Vet. Sci. Anim. Ind.* 8, 375.

THEILER, A., GREEN, H.H. & DU TOIT, P.J., 1927. Minimum mineral requirements in cattle. *J. Agric. Sci.* 17, 291.

TISCHER, I., 1977. Untersuchungen zum latenten Phosphormangel in der Ernährung wachsender Wiederkauer. 14. Physiologische kennzahlen im Haar im Hinblick auf ihre Eignung zum rechtzeitigen Erkennen latenten Phosphormangels. *Nutr. Abstr. Rev.* 51, 439.

VAN DYNE, G.M. & TORELL, D.T., 1964. Development and use of the esophageal fistula: A review. *J. Range Manage.* 17, 7.

WHITE, A., HANDLER, P. & SMITH, E.L., 1973. In: Principles of Biochemistry (5th Ed.). McGraw-Hill: Tokyo. p. 1004.

WISE, M.B., SMITH, S.E. & BARNES, L.L., 1958. The phosphorus requirement of calves. *J. Anim. Sci.* 17, 89.

WITT, K.E. & OWENS, F.N., 1983. Phosphorus: ruminal availability and effects on digestion. *J. Anim. Sci.* 56, 930.