Blood, rib bone and rumen fluid as indicators of phosphorus status of grazing beef cows supplemented with different levels of phosphorus at Armoedsvlakte

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Received 10 November 1995; accepted 14 November 1997

Sixty-six grazing beef cows at Armoedsvlakte were supplemented *per fistulam* with different levels of phosphorus (P) from 1984 to 1989. Supplementation was provided according to a 3 × 2 factorial of three levels of P (Low, Medium and High), in the periods of either all year round (LP 12, MP 12 and HP 12) or six months only (LP 6, MP 6 and HP 6), i.e. September to February (late gestation to late lactation). Blood, rib bone and rumen fluid were evaluated as indicators of the P status of cattle. Animals which did not receive P supplement from March to August (LP 6, MP 6 and HP 6), had lower inorganic phosphorus (P_i) levels in blood plasma than the 12-month groups (LP 12, MP 12 and HP 12) by late pregnancy (August). By late lactation (March), after having received P supplement for six months, there was little difference between treatments. This pattern was repeated during each of the successive calving seasons, indicating that plasma P_i level reflected the P intake of animals, not necessarily their P status. There was a tendency for an increased plasma calcium (Ca) concentration to compensate for a low P_i level. At the end of the first year (August 1985), differences in bone P concentration were apparent, with the HP 6, MP 12 and HP 12 groups showing their superiority; by late lactation of the first calving season (March 1986), only the MP 12 and HP 12 cows were able to maintain bone P reserves. The rib bone P levels of the other treatments were considerably lower in March 1986, falling well short of the accepted 'normal' level of 140 mg P/cm^3 fresh bone, and even below that which is classified as deficient, i.e. 120 mg P/cm^3 fresh bone. In contrast to plasma P_i levels, bone P levels of the 6-month groups did not recover after six months of P supplementation. The pattern in bone Ca concentration was similar to that of bone P, as might be expected given the composition of the bone crystal. The Ca:P ratio in rib bone varied little from 2:1. In general, slightly wider ratios were apparent when cows were only supplemented for 6 months of the year. Bone density (SG, specific gravity) followed the same trends as observed for the individual bone minerals and may be used as a measure of bone mineralisation, although it is not as sensitive an indicator as a single mineral, e.g. P, nor does it provide information concerning the bone reserves of a specific mineral. In the 6-month groups, a sharp decline in rumen P_i level was observed during those months without any P supplement, followed by a rapid increase in rumen P_i once P supplementation recommenced in September. Rumen fluid P_i concentration was insufficiently sensitive to distinguish at all times between the different levels of supplementation (LP, MP and HP) but it readily distinguished between unsupplemented and supplemented (6- or 12-month) groups.

Ses en sestig weidende vleisbeeskoeie is vanaf 1984 tot 1989 op Armoedsvlakte *per fistulam* van verskillende peile van fosfor (P) voorsien. Aanvulling is voorsien in 'n 3 × 2 faktoriaal van drie P peile (Laag, Medium en Hoog), in periodes van 12 maande (LP 12, MP 12 en HP 12) of slegs ses maande van die jaar (LP 6, MP 6 en HP 6). Bloed, ribbebene en rumenvloeistof is geëvalueer as indikators van die P-status van beeste. Tydens laat dragtigheid (Augustus), het koeie wat vanaf Maart tot Augustus (LP 6, MP 6 en HP 6) geen P-aanvulling ontvang het nie, laer anorganiese fosfor- (P_i) vlakke in bloedplasma as die 12 maande groepe (LP 12, MP 12 en HP 12) gehad, maar tydens laat laktasie (Februarie/Maart), nadat hulle weer vir ses maande P-aanvulling ontvang het, was die verskille tussen behandelings gering. Hierdie patroon is herhaal gedurende die opeenvolgende kalfseisoene, wat daarop dui dat P_i -vlakke in plasma die P-inname van diere reflekteer, maar nie noodwendig hulle P-status nie. Daar was 'n neiging vir verhoogde vlakke van kalsium (Ca) in plasma, skynbaar as kompensasie vir lae P_i -vlakke. Aan die einde van die eerste jaar (Augustus 1985) was verskille in been P-vlakke duidelik aanwesig, met die hoogste vlakke in die HP 6, MP 12 en HP 12 groepe, maar teen laat laktasie van die eerste kalfseisoen (Maart 1986) was slegs die MP 12 en HP 12 groepe in staat om been-P-reserwes te handhaaf. Die P-vlakke in ribbebeen van die ander behandelings was aansienlik laer in Maart 1986, party selfs heelwat laer as die aanvaarde 'normale' vlak van 140 mg P/cm^3 vars been en sommige ook selfs laer as die aanvaarde norm vir 'n P-tekort, naamlik 120 mg P/cm^3 vars been. In teenstelling met plasma P_i vlakke, het die been-P-vlakke van die ses-maande-groepe nie weer na ses maande van P-aanvulling verhoog nie. Die patroon van been-Ca-konsentrasie was soortgelyk aan die vir P in been, soos verwag kon word gegewe die samestelling van beenkristal. Die verhouding van Ca:P in ribbebeen, het min vanaf 2:1 afgewyk. Oor die algemeen het die verhouding effens vergroot waar koeie slegs vir ses maande van die jaar met P aangevul is. Digtheid van been (SG, soortlike gewig) het dieselfde patroon gevolg as die van die individuele beenminerale en kan dus dien as maatstaf van mineralisasie van been, alhoewel dit nie so 'n sensitiewe indikator is as byvoorbeeld P nie en ook nie inligting verskaf oor die beenreserwes ten opsigte van 'n spesifieke mineraal nie. In die ses-maande-groepe het die rumen-P_i -vlakke skerp gedaal gedurende die maande sonder P-aanvulling, gevolg deur 'n skerp styging met die hervatting van P-aanvulling in September. Rumen-P_i-konsentrasie was nie sensitief genoeg om te alle tye tussen die verskillende peile van aanvulling (LP, MP en HP) te onderskei nie, maar kon geredelik 'n onderskeid tref tussen die groepe wat geen aanvulling of aanvulling (6- of 12-maande) ontvang het.

Keywords: Blood plasma, grazing cattle, native pasture, rumen fistula, phosphorus deficiency, phosphorus status, phosphorus supplementation, veld

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Introduction

Although phosphorus (P) has been identified by Theiler (1912) and more recently by Read *et al.* (1986b,c,d) and De Waal *et al.* (1996) as a major limiting nutrient for beef cattle at Armoedsvlakte in the Northern Cape (now part of the North West Province), the supplementary P requirements of grazing beef cattle remain uncertain. Therefore, recommendations on P supplementation via licks are to a large extent based on speculation. Specific clinical symptoms like aphosphorosis may be useful in diagnosing a mineral deficiency, but by the time such symptoms appear, the deficiency usually is quite advanced (Little, 1972). Blood samples are readily obtainable and have formed the basis of diagnostic tests (Cohen, 1975), despite their providing little information about the status of mineral reserves and being influenced by several factors, as summarized by Read *et al.* (1986d). Recently, studies at this Institute have shown that, in some instances and localities, implementation of a more sensitive and reliable indicator of P status, namely P levels in rib bone (Little, 1972), can identify a P deficiency in reproducing sheep (Read *et al.*, 1986a) and reproducing cattle (Read *et al.*, 1986b,c). Early detection of a P deficiency, the P requirement of grazing cattle and the correct level of P supplementation, have important economic consequences; many producers oversupply cattle with supplementary P, while others undersupply. Both scenarios can severely impact on animal production and financial returns, as illustrated by Read *et al.* (1986b) and De Waal *et al.* (1996), as part of this study.

The objective of this study was to quantify the supplementary P requirement of grazing beef cows at Armoedsvlakte by investigating the effects of 12-month supplementation with different levels of P, in contrast with supplementation at corresponding levels of P for six months only of the year, i.e. during late pregnancy to late lactation. De Waal *et al.* (1996) have reported on the effects on reproduction and body mass; this paper reports on blood, rib bone and rumen fluid as indicators of the P status of cattle.

Experimental procedure

The experimental site (Armoedsvlakte Research Station), animals used, treatments applied and general experimental procedures have been described elsewhere (De Waal *et al.*, 1996). In brief, four to five months prior to being mated for the first time, 66 Bonsmara type, crossbred, heifers were fitted with rumen cannulae (80 mm inner diameter) and allotted randomly to six treatments of 11 animals each. They were managed as a single grazing herd at a stocking rate of 7 ha/Large Stock Unit (LSU; Meissner *et al.*, 1983), since supplementary P was introduced *per fistulam* directly into the rumen. The six treatments were arranged as a factorial combination of 2 Periods and 3 Levels of P supplementation, as detailed in Table 1.

Supplementation of P *per fistulam* began in September 1984, with dicalcium phosphate (minimum 16% P) as the source of P. From March to August, cows in the 12-month groups (LP 12, MP 12 and HP 12) received their weekly allowance of P in two doses and from September to February, all the groups (LP 6, MP 6, HP 6, LP 12, MP 12 and HP 12) received their weekly allowance of P in three doses (Table 1). Besides the P supplementation *per fistulam*, they had free access to a salt (sodium chloride) lick only.

Table 1 The treatment design, with the different levels and periods of phosphorus (P) supplementation *per fistulam* to the breeding beef cows in the six treatments

	Method and interval of supplementation	
	March to August Weekly allowance[2], was given in 2 doses (Monday & Friday)	September to February Weekly allowance[3], was given in 3 doses (Monday, Wednesday & Friday)
Treatment Labels[1]	Level of supplementation (g P day^{-1})	
LP 6	–	5
MP 6	–	10
HP 6	–	16
LP 12	3	5
MP 12	6	10
HP 12	9	16

[1] LP 6, MP 6, HP 6: Low, medium and high levels of P for 6 months/year.

LP 12, MP 12, HP 12: Low, medium and high levels of P for 12 months/year.

[2] LP 6, MP 6, HP 6: None.

LP 12, MP 12, HP 12: Provided as 65, 130 or 197 g dicalcium phosphate, 2 times per week.

[3] Provided as 73, 146 or 233 g dicalcium phosphate, 3 times per week.

Blood and rib bone samples were collected twice annually from the same eight cows in each of the six treatments during late lactation (February or March) and late pregnancy (August). These dates were also chosen to coincide with the six-monthly changes in level of P supplementation (Table 1). Blood samples were taken by jugular puncture, gently mixed with three drops of heparin to prevent clotting, and spun down within 3 h of sampling; plasma was analysed for inorganic phosphorus (P_i), calcium (Ca) and magnesium (Mg) concentration according to De Waal (1979). Prior to and during sampling of blood, every endeavour was made to avoid those factors or situations known to influence blood analysis of minerals (De Waal, 1979; Read *et al.*, 1986d). Rib bone samples were taken according to the biopsy technique described by Little (1972), with a modification as described by Read (1984). In the modified technique, full core samples of rib bone were taken instead of a single layer of cortical bone as described in the original bone biopsy technique. This should be kept in mind, because it may have an important influence on the results reported here and in other trials where the modified technique was used (Read *et al.*, 1986c), in comparison to those trials where the unmodified technique of Little (1972) was used. Rib bone samples were analysed for P, Ca and Mg concentration and specific gravity (SG) was determined according to De Waal (1979).

At the monthly weighing of animals (towards the end of each month), rumen fluid was sampled from the same eight cows per treatment that were used for blood and rib bone sampling. Rumen fluid was sampled in the morning according to De Waal *et al.* (1989), prior to dosing of the dicalcium phosphate *per fistulam*. Rumen fluid was analysed for soluble P_i concentration according to the method described by Witt & Owens (1983), which involves centrifugation of the samples followed by the analysis of the supernatant for P, using the

colorimetric phosphomolybdate reaction of Fiske & Subbarow (1925, as cited by Witt & Owens, 1983).

The treatment design was a 3 × 2 factorial, three Levels of phosphorus by Period (supplementation in only six months of the year *vs.* all year round). Most analyses were done using the General Linear Model procedure (PROC GLM) of the Statistical Analysis System (SAS, 1989), the exceptions arising in the case of binary data, where PROC LOGISTIC was used. In these cases, the error was assumed to be binomial and a logit link function was used.

Results and Discussion

Mineral levels in blood plasma

Mineral concentrations were determined in blood plasma according to the recommendation of Little *et al.* (1971) and like analysis in previous studies at this Institute (De Waal, 1979; Read *et al.*, 1986a,d). Values presented here may differ from other published values, which are based on analysis of blood serum (Read *et al.*, 1986d).

In a trial run of techniques and procedures, blood was sampled from the heifers during the early part of their first pregnancy (February 1985). At this point, after having received supplementary P for the six months since September 1984, and being pregnant but not having lactated yet, little difference in average plasma P_i levels of the heifers in each of the 6- or 12-month groups was detected (mg P_i/100 ml plasma: LP 6 – 5.38; MP 6 – 5.50; HP 6 – 5.98; LP 12 – 4.70; MP 12 – 4.89 and HP 12 – 6.23), but there was a clear trend for P_i to increase with the level of P supplementation.

The average mineral concentrations in blood plasma, sampled twice annually over five years during late pregnancy (August) and late lactation (February or March) from cows in the different treatments, are presented in Table 2. The results are also displayed graphically in Figures 1, 2 and 3, respectively, for blood plasma P_i, Ca and Mg.

Table 2 Mineral concentrations in blood plasma, sampled twice a year from cows in the different treatments over five years of the trial[1]

		6			12			max.
Time	Model	LP	MP	HP	LP	MP	HP	SE[2]
Phosphorus (P_i) concentrations (mg/100 ml)								
Feb/Mar	—	4.63	4.47	4.57	4.09	4.64	4.64	0.243
August	P × L	2.92	2.50	2.72	4.00	5.49	6.14	0.220
Difference	P × L	–1.71	–1.97	–1.86	–0.09	0.85	1.50	0.273
Calcium (Ca) concentrations (mg/100 ml)								
Feb/Mar	P	7.83	7.58	7.78	7.59	7.23	7.36	0.153
August	P × L	8.29	8.47	8.41	8.18	7.91	7.61	0.143
Difference	—	0.46	0.83	0.62	0.60	0.68	0.26	0.193
Magnesium (Mg) concentrations (mg/100 ml)								
Feb/Mar	P × L	2.37	2.45	2.51	2.54	2.39	2.33	0.0698
August	—	2.16	2.21	2.22	2.21	2.19	2.22	0.0578
Difference	P × L	–0.21	–0.25	–0.29	–0.34	–0.21	–0.10	0.0566

[1] LP 6, MP 6, HP 6: Low, medium and high levels of P for 6 months/year. LP 12, MP 12, HP 12: Low, medium and high levels of P for 12 months/year.

[2] The standard error (SE) quoted is the largest SE of the six treatment means shown; the variation in SEs is small and is due to varying numbers in the six treatment groups.

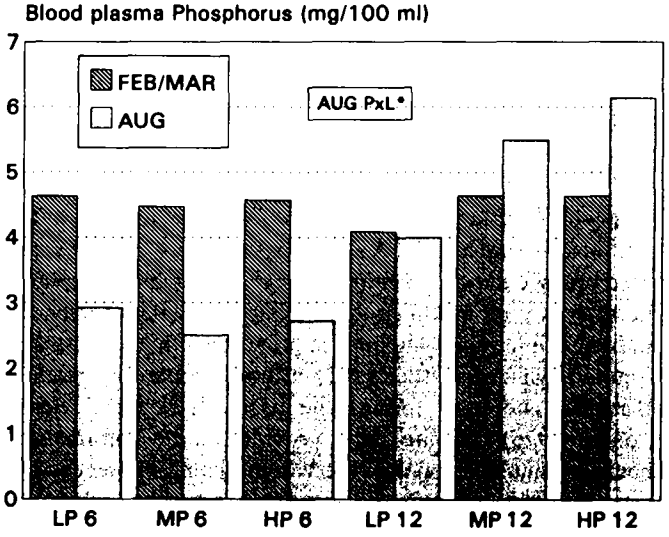

Figure 1 Average blood plasma P_i, sampled twice a year (February/March and August) from cows in the different treatments over five years of the trial.

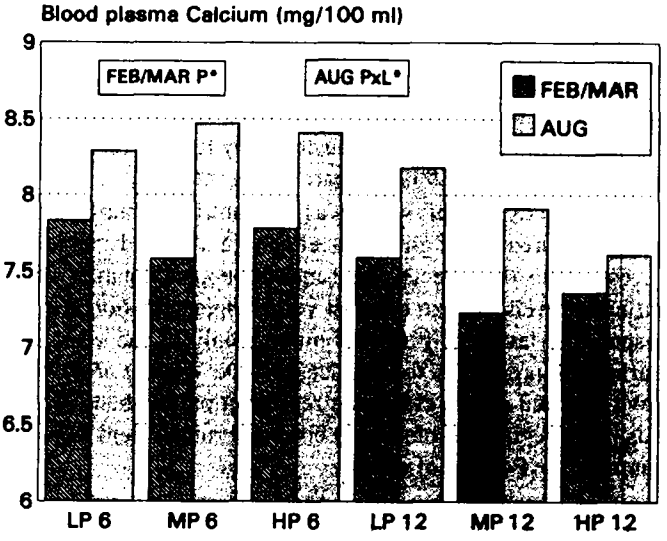

Figure 2 Average blood plasma Ca, sampled twice a year (February/March and August) from cows in the different treatments over five years of the trial.

At the end of the period when the two groups (6-month and 12-month) received the same supplements (February), there were no significant differences in P_i concentration between the six treatment groups (Table 2) (i.e. not even according to the level of P supplementation; the entry '—' in the column headed 'Model' in Table 2 indicates that neither the interaction, P × L, nor the main effects, P or L, were significant). The mean concentration of the six treatments in February was 4.50 ± 0.0896 mg P_i/100 ml plasma. The analysis for the means in the next row of Table 2, August, displays Period by Level interaction. This is understandable because the animals in the LP, MP and HP groups were treated identically during the foregoing six months (Table 1). Hence, one would not expect these three means to differ significantly. However, in the 12-month group, there is clear evidence that level of P_i in

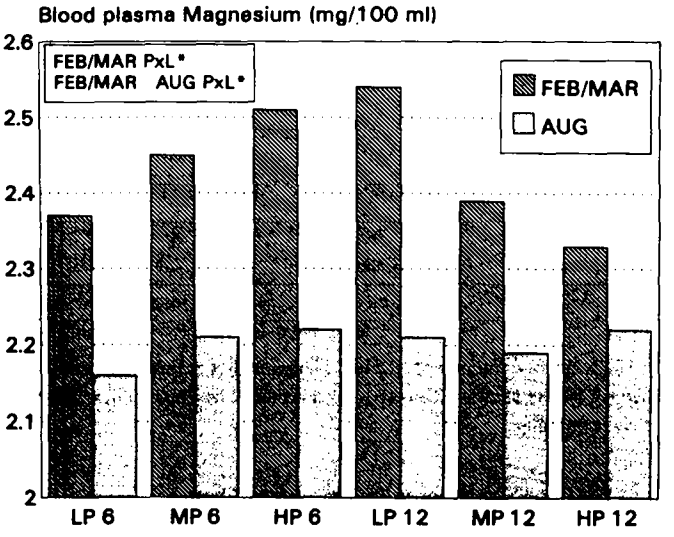

Figure 3 Average blood plasma Mg, sampled twice a year (February/March and August) from cows in the different treatments over five years of the trial.

plasma of the animals increased as the level of P supplement increased. The last row of Table 2 shows the difference between the measurements at the two times in the year. The significant interaction is due to a greater difference among the three groups receiving P supplementation all year round (LP 12, MP 12 and HP 12).

Reflecting on individual years, by late pregnancy of 1985 the three groups that did not receive any P supplement from March to August, clearly had lower P_i levels than the 12-month groups. However, by late lactation in March 1986, after having received P supplement for six months, there was little difference between treatments, except for a higher P_i level in the HP 12 group and a lower P_i level in the LP 12 group. This same general pattern was repeated during each of the successive calving seasons. Some values of cows in the 6-month groups in August were indicative of P deficiency (< 2 mg P_i/100 ml) according to Gartner *et al.* (1980). In the trial by Read *et al.* (1986d), unsupplemented cows (–P) had even lower plasma P_i levels, e.g. 1.45 and 1.25 mg P_i/100 ml plasma during lactation in 1982 and 1983, respectively, clearly demonstrating the severity of the P deficiency in the pasture at Armoedsvlakte. However, after having received supplementary P for six months in this study, the average plasma P_i levels of the LP 6, MP 6 and HP 6 groups compared favourably with those of the respective 12-month groups. This effect is clearly illustrated in Figure 1. In agreement with Moir (1966) and Read *et al.* (1986d), this general pattern indicates that plasma P_i reflects the dietary P intake of animals, not necessarily the P status. The analysis does not differentiate between physiological differences of individuals within sampling periods. However, examination of the original data clearly showed that, within each of the six treatments at a given sampling time, the dry or non-pregnant cows always had higher average plasma P_i levels than the lactating or pregnant cows.

Blood plasma Ca concentrations (Table 2) suggest that the difference observed in the six-month period ending in August carried over into the six-month period ending in February; there was a significant difference depending on Period in the first row of Table 2. This is supported further by the fact that the differences between the two periods seem to be constant; no effect is significant in the last row of Table 2. The difference between the two periods averaged 0.57 ± 0.0714. It is difficult to interpret and explain the Period by Level interaction in the row for August. One would expect the three means for the 6-month groups to be constant, but it is not clear why the means should be less when level of P supplementation increased. We are not aware of evidence showing that a higher level of plasma P_i reduces the level of Ca; in fact, the opposite seems to be possible (ARC, 1980; Read *et al.*, 1986d). If a higher level of P reduced the level of Ca, the three responses for the 12-month groups in February/March (Table 2) which are apparently constant could be explained by the fact that the P levels also are constant.

Blood plasma Ca concentrations of all groups rose sharply from August 1985 to March 1986 and remained fairly stable until they once again declined in March 1988. The lower levels prior to August 1985 may be indicative of Ca accretion (i.e. bone formation) and therefore lower labile Ca reserves in the young heifers; during the lactation that followed, the increase suggests a greater mobilisation of the labile Ca reserves, resulting in a higher plasma Ca level. The decrease in plasma Ca concentration observed during late lactation of 1988, may reflect a decrease in the available labile Ca reserves in the more mature cows, combined with the effects of lactation. There appears to be a tendency for an increased plasma Ca level in compensation for a low P_i level, a tendency also observed by Read *et al.* (1986d). However, Underwood (1966) suggested that this tendency may be a result from both Ca and P being resorbed from bone mineral reserves during dietary inadequacies. While P_i is immediately utilised, the excess Ca remains in circulation. Hence, plasma Ca levels increase. In general, the cows in this trial had lower plasma Ca levels than those reported by Read et al. (1986d) for Armoedsvlakte, but similar to those reported by them for Glen.

Blood plasma Mg concentration decreased with increasing level of P supplementation in the 12-month group; the differences between the means for the three levels in the 6-month group are not significant. In the second row of Table 2, no effect is significant, while the last row reflects the interaction. As was the case with Ca, one must ask whether a higher level of P reduces the level of Mg. The Mg levels in blood plasma for all groups were at a given sampling time, similar to those reported by Read *et al.* (1986d) for Armoedsvlakte and Glen.

Examination of Figures 2 and 3 reveals an interesting phenomenon. Higher values for plasma Mg (Figure 3) were observed at the end of the period of lower levels of P supplementation, i.e. August, while plasma Ca values (Figure 2) were higher at the end of the period of higher levels of P supplementation, i.e. February/March. The reasons for this phenomenon cannot be explained.

Mineral levels and SG of rib bone

In agreement with Little (1972), Little & McMeniman (1973), De Waal (1979) and Read *et al.* (1986a,c), who concluded that mineral concentrations are more sensitive when expressed per unit volume (mg/cm^3) of fresh bone than when

expressed as percentage (mg/mg) of dry bone, bone mineral concentrations are expressed as mg/cm³ fresh bone. Biopsies were performed on the same rib of the same side of the animals at a specific sampling occasion (Read *et al.*, 1986c). Therefore, comparisons between treatments are valid for a specific sampling occasion, but not necessarily between different occasions because the degree of mineralization of one rib may differ from that of another (Little & Minson, 1977).

In a trial run of techniques and procedures, mentioned previously, rib bone was sampled from the heifers during the early part of their first pregnancy (February 1985). At this point, after having received supplementary P for six months since September 1984, and being pregnant but not having lactated yet, the differences in rib bone P levels of the heifers in the 12-month groups and one of the 6-month groups (mg P/cm³ fresh bone: HP 6 – 124.3; LP 12 – 122.7; MP 12 – 125.0 and HP 12 – 126.6) were small; those of the other two 6-month groups (mg P/cm³ fresh bone: LP 6 – 112.5 and MP 6 – 114.7) were lower. In the present trial, the rib bone P levels for heifers were much lower than the 183.4 (–P) and 171.5 (+P) mg P/cm³ fresh bone reported by Read *et al.* (1986c) for the heifers at Armoedsvlakte and also the 181.4 (–P), 185.5 (+P) and 184.6 (PR) mg P/cm³ fresh bone for the heifers at Glen, when these heifers were at a comparable stage of development.

The average chemical composition and SG of rib bone, sampled twice annually over five years during late pregnancy (August) and late lactation (February or March) from cows in the different treatments, are presented in Tables 3 and 4. The results are also displayed graphically in Figures 4, 5, 6 and 7, respectively, for rib bone P, Ca, Mg and SG.

In Table 3 the notation 'P+L' indicates that although the interaction between Period and Level for P concentration of rib bone was not significant for the period ending in February,

Table 3 Mineral concentrations of rib bone, sample twice a year from cows in the different treatments ove five years of the trial[1]

		6			12			max.
Time	Model	LP	MP	HP	LP	MP	HP	SE[2]
Phosphorus (P) concentrations (mg/cm³ fresh bone)								
Feb/Mar	P + L	116.1	122.1	128.9	124.8	130.9	138.7	3.00
August	P × L	116.4	118.4	126.3	127.3	142.4	155.2	3.51
Difference	P × L	0.3	–3.7	–2.6	2.4	11.5	16.4	3.35
Calcium (Ca) concentrations (mg/cm³ fresh bone)								
Feb/Mar	L	240	253	266	243	248	261	6.62
August	P × L	247	245	252	259	279	298	6.93
Difference	P × L	7	–9	–14	16	31	37	6.22
Magnesium (Mg) concentrations (mg/cm³ fresh bone)								
Feb/Mar	P × L	4.54	4.76	5.62	4.47	4.89	4.98	0.214
August	—	5.20	4.70	5.30	5.49	5.98	6.90	0.293
Difference	P × L	0.66	–0.07	–0.32	1.02	1.09	1.91	0.235

[1] LP 6, MP 6, HP 6: Low, medium and high levels of P for 6 months/year
LP 12, MP 12, HP 12: Low, medium and high levels of P for 12 months/year

[2] The standard error (SE) quoted is the largest SE of the six treatment mean shown; the variation in SE's is small and is due to varying numbers in the si treatment groups.

Table 4 Specific gravity (SG) of rib bone, sampled twice a year from cows in the different treatments over five years of the trial[1]

		6			12			max.
Time	Model	LP	MP	HP	LP	MP	HP	SE[2]
Feb/Mar	P + L	1.467	1.498	1.514	1.494	1.513	1.567	0.0181
August	P + L	1.433	1.432	1.460	1.455	1.522	1.556	0.0193
Difference	P × L	–0.034	–0.066	–0.054	–0.039	0.009	–0.011	0.0140

[1] LP6, MP6, HP6: Low, medium and high levels of P for 6 months/year.
LP12, MP12, HP12: Low, medium and high levels of P for 12 months/year.

[2] The standard error (SE) quoted is the largest SE of the six treatment means shown; the variation in SEs is small and is due to varying numbers in the six treatment groups.

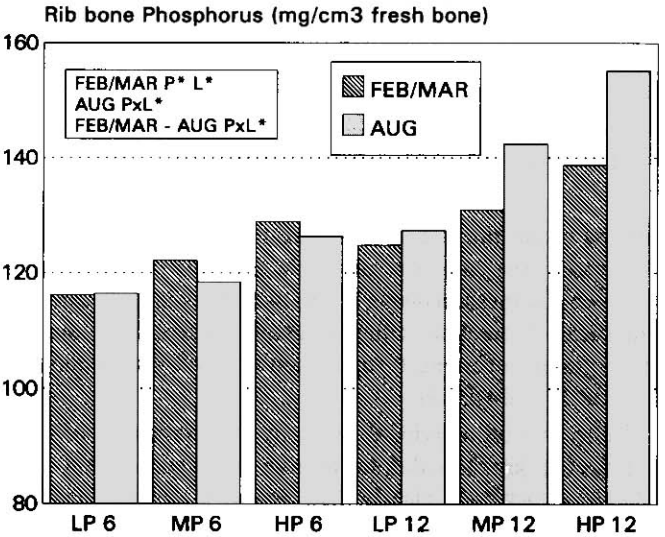

Figure 4 Average rib bone P, sampled twice a year (February/March and August) from cows in the different treatments over five years of the trial.

both main effects were. Rib bone P displays the same general pattern of response as plasma P_i (Table 2). Thus, the difference between February/March and August seems to be constant (possibly zero) in the 6-month groups but increasing in the 12-month groups, with the difference at the low level of P supplementation (LP 12) not significantly different from zero. It is noticeable in Figure 4, that except for the MP 12 and HP 12 treatments, the differences in rib bone P between the 6-month groups and the 12-month groups were relatively small. In general, the corresponding differences were larger for rib bone Ca (Figure 5) and Mg (Figure 6).

At the end of the first year (August 1985) differences in bone P concentration were apparent, with the HP 6, MP 12 and HP 12 groups showing their superiority. However, by late lactation of the first calving season (March 1986) only the MP 12 and HP 12 groups were able to maintain bone P reserves. The mean values of all the other groups were considerably lower in March 1986, falling well short of the accepted 'normal' P concentration of 140 mg/cm³, as proposed by Little & Ratcliff (1979), and even below that which may be classified as P deficient, i.e. 120 mg/cm³ (Little & Shaw, 1979). In contrast to plasma P_i concentration, the rib bone P concentration of the 6-month groups did not recover

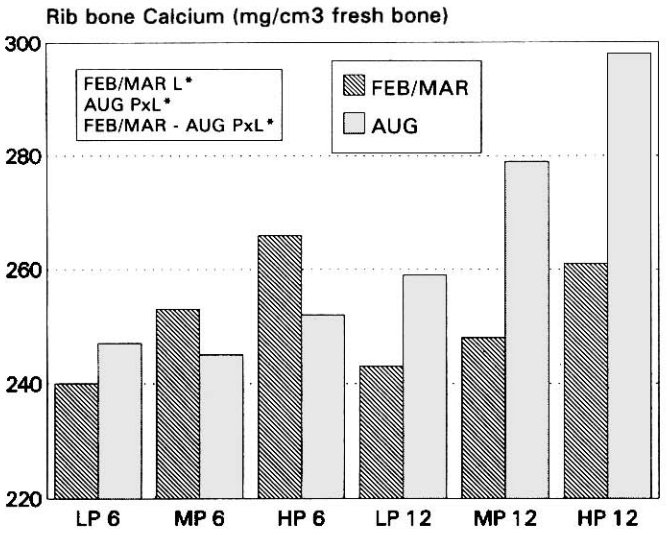

Figure 5 Average rib bone Ca, sampled twice a year (February March and August) from cows in the different treatments over fiv years of the trial.

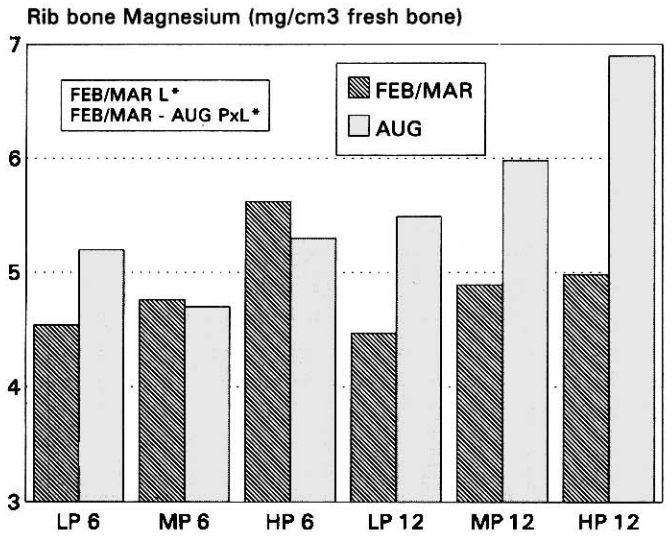

Figure 6 Average rib bone Mg, sampled twice a year (February March and August) from cows in the different treatments over fiv years of the trial.

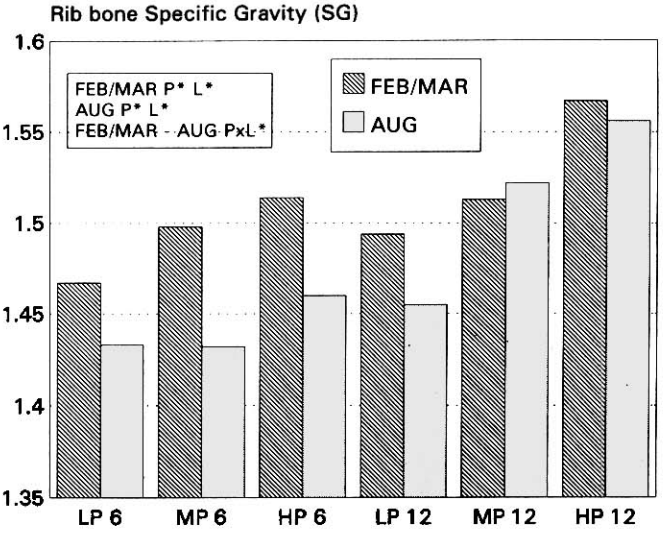

Figure 7 Average Specific Gravity (SG) of rib bone, sampled twice a year (February/March and August) from cows in the different treatments over five years of the trial.

after six months of P supplementation. The results of the current study support the thesis by Moir (1966) and the results of Read *et al.* (1986c,d) namely that plasma P_i level is indicative of the P intake of animals rather than the P status, while bone P concentration more accurately reflects the mineral reserves. As was the case with blood, the analysis does not differentiate between physiological differences of individuals within sampling periods. However, in a comparison of the original data of the dry or non-pregnant cows with either lactating or pregnant cows within a treatment, at a given sampling time, the extent of withdrawal of reserve minerals for reproduction is apparent. Considering that the HP 12 group received enough supplementary P *per fistulam* to provide in their total daily requirement (ARC, 1980), which was further augmented by P intake from the pasture itself, the extent of withdrawal of reserve minerals for reproduction and lactation becomes even more apparent.

The results in Table 3 indicate a significant response of Ca concentration in rib bone to Level of P supplementation during the period ending in February, as well as a significant interaction between Period and Level of supplementation during the period ending in August. However, the result suggests little response in concentration of bone Ca with increasing P supplementation in the 6-month groups but an increasing concentration of bone Ca in the 12-month groups.

The pattern in bone Ca concentration was similar to that of bone P, as might be expected given the composition of the bone crystal: $Ca_{10}(PO_4)_6OH_2$ (Ganong, 1977, as cited by Read *et al.*, 1986c). The Ca:P ratio in bone in this study varied little from 2:1, except for late pregnancy in 1986 when it appeared narrower and for late lactation in 1988 when it appeared wider. In general, slightly wider Ca:P ratios were apparent when cows were only supplemented for six months of the year. Considering the lower blood Ca concentration during late lactation in 1988, the wider Ca:P ratio during this time (late lactation in 1988) probably indicates less labile Ca, but a greater proportion incorporated into immobilisable bone min eral.

The results in Table 3 indicate a significant response of Mg concentration in rib bone to a Period by Level interaction to P supplementation during the period ending in February. Means show a relatively stable concentration of bone Mg with increasing P supplementation in the 6-month groups but an increasing concentration of bone Mg in the 12-month groups. This is reminiscent of the results for rib bone Ca levels. Similar to the observations made by Read *et al.* (1986c), the rib bone Mg reserves seem to have been depleted and repleted together with bone P and Ca, probably because of the interrelationships among these minerals (Jacobson *et al.*, 1972). In general, it is apparent that the response in rib bone Ca (Figure 5) and Mg (Figure 6) to supplementation, i.e. for Period and Level of P supplementation, differed from that observed in blood plasma for the same two minerals (Figures 2 and 3).

In Table 4, the notation 'P+L' indicates that although the

interaction between Period and Level for SG of rib bone was not significant, both main effects were. This interaction effect was close to significance in August (p = 0.0652). As expected, bone density followed the same trends as observed for the individual bone minerals; loss of minerals increases the porosity of bone which is reflected in a reduced SG. Therefore, specific gravity may be used as a measure of bone mineralisation, although it is not as sensitive an indicator as a single mineral, e.g. P, nor does it provide information concerning the bone reserves of a specific mineral. However, Read *et al.* (1986c) showed that in severe cases of a P deficiency, e.g. at Armoedsvlakte where the control group of cows (–P) received no P at all, both the SG and bone P content proved equally conclusive in identifying a P deficiency. In general, the SG of rib bone of cows in this trial was similar to those reported by Read *et al.* (1986c) for Armoedsvlakte, but lower than those reported for Glen, where no clear P deficiency could be diagnosed. The graphic presentation in Figure 7 shows that the differences in SG of rib bone between the 6-month and 12-month groups were the smallest for the MP 12 and HP 12 treatments.

P_i levels in rumen fluid

The average P_i concentrations in rumen fluid, sampled monthly over five years from cows in the different treatments, are presented in Table 5. The results are also displayed graphically in Figure 8 for ruminal P_i.

The means for rumen P_i in Table 5 correspond to the model which is significant in the particular instance. For example, the means for March were the same for the three levels in the 6-month and 12-month groups because the marginal means (one for each Period) were entered into Table 5. In March, the first month in which the 6-month and 12-month groups were treated differently, the significant model contains Period

Table 5 Phosphorus (P_i) concentrations (mg/100 ml) of rumen fluid, sampled monthly over five years from cows in the different treatments[1]

		6			12			max.
Month	Model	LP	MP	HP	LP	MP	HP	SE[2]
Mar	P	19.8	19.8	19.8	22.9	22.9	22.9	0.627
Apr	P × L	13.7	13.7	13.7	15.8	19.2	20.4	0.868
May	P × L	16.1	15.7	15.6	18.9	25.3	26.2	1.13
Jun	P × L	14.0	14.1	14.4	17.8	22.9	26.0	0.990
Jul	P × L	16.3	15.3	16.3	21.0	26.3	26.7	1.22
Aug	P × L	11.9	11.7	12.3	18.5	22.0	25.0	1.10
Sep	L	19.1	25.0	25.2	19.1	25.0	25.0	1.75
Oct	L	23.6	29.0	28.9	23.6	29.0	28.9	1.12
Nov	L	21.7	25.4	26.7	21.7	25.4	26.7	1.06
Dec	L	18.6	23.1	24.7	18.6	23.1	24.7	0.861
Jan	L	18.2	22.1	22.4	18.2	22.1	22.4	1.15
Feb	L	25.9	29.0	30.0	25.9	29.0	30.0	1.11

[1] LP6, MP6, HP6: Low, medium and high levels of P for 6 months/year. LP12, MP12, HP12: Low, medium and high levels of P for 12 months/year.

[2] The standard error (SE) quoted is the largest SE of the six treatment means shown; the variation in SEs is small and is due to varying numbers in the six treatment groups.

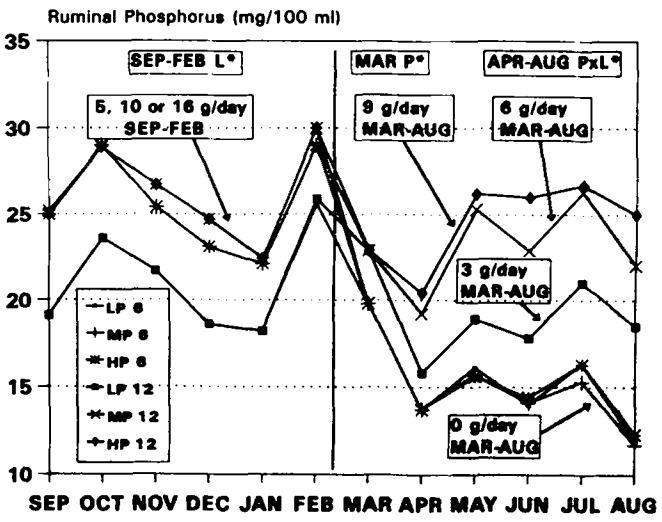

Figure 8 Average ruminal P_i, sampled monthly from cows in the different treatments over five years of the trial.

alone; in the remaining five months there was significant interaction between Period and Level. This is because the responses were constant at the three levels in the 6-month groups, but increased for the three levels in the 12-month groups — with March a transitional month. In the six-month period during which the 6-month and the 12-month groups were treated identically, only Level was significant.

Within each of the 6-month and 12-month groups, there was considerable variation in the monthly rumen P_i levels, probably related to differences in the sampling site (stratum) within the rumens; or the time since the animal had last drunk water, i.e. a dilution effect; or time elapsed since the last P supplementation, i.e. two to four days previously, depending on the time of the year (Table 1); increased P circulation of plasma P_i via the saliva to the rumen; or the extent of gut fill and the composition of the digesta, i.e. the ability of an individual to select parts of plants with higher P concentrations. There would also appear to be some seasonal variation in herbage P concentration, probably related to rainfall and the emergence of new plant growth. In several years during the early (December) or especially the late (February) growing season of the veld, all treatments showed a marked, but transitory, increase in rumen P_i concentration. A clear pattern was observed in the 6-month groups, related to supplementary P. A sharp decline in rumen P_i concentration was observed during the months without any P supplement (March to August), followed by a rapid recovery or increase in rumen P_i concentration once P supplementation was recommenced in September. This pattern resulted from either the addition of the supplement directly into the rumen, or recirculation of salivary P, or both. Although rumen fluid P_i concentration was insufficiently sensitive to distinguish at all times between the three different levels of supplementation (LP, MP and HP), it could be used to distinguish between the unsupplemented and supplemented (6-month or 12-month) groups. This conclusion is probably better illustrated by the graphic display of results in Figure 8.

Unlike for blood and rib bone, there is a paucity of information on the P_i concentration in rumen fluid, especially for grazing ruminants, as well as with regard to 'critical' values,

i.e. those values at which digestion by the rumen micro-organisms are depressed. According to Read *et al.* (1986b), the anorexia of their –P cattle may have been related to decreased microbial digestion caused by poor availability of P for the rumen micro-organisms. However, Witt & Owens (1983) failed to demonstrate such an effect and concluded that tissue stores of P may be mobilized before the digestibility of nutrients is reduced and that this permits survival through short periods of P deficiency. Besides these possible intraruminal effects, Read *et al.* (1986b) concluded that the stiff-legged gait, which is a characteristic symptom of aphosphorosis, severely impaired locomotion, grazing behaviour and probably feed intake. The intake of digestible organic matter (DOM) from the pasture at Armoedsvlakte by the –P cows (Read *et al.*, 1986b), averaged 2 915 g DOM day^{-1}, compared to 4 746 g DOM day^{-1} for the +P cows (averages of eight intake determinations each, between 1978 and 1982). In sharp contrast to those at Armoedsvlakte, the intake of cows in a similar trial at Glen (Read *et al.*, 1986b), averaged 6 064, 6 118 and 6 124 g DOM day^{-1}, respectively, for the –P (salt lick *ad lib.*), +P (salt/P lick *ad lib.*) and PR (salt lick *ad lib.* and P *per fistulam*) groups (averages of seven intake determinations each, between 1979 and 1982). It seems unlikely that at any time, even in August 1985 when all six treatments had very low P_i levels in rumen fluid or August 1987 when only the LP 6, MP 6 and HP 6 groups had low ruminal P_i levels, low P_i levels could have reduced digestion by the rumen micro-organisms and therefore affected the animals.

Conclusions

Blood plasma P_i concentration was indicative of the P intake of animals, but not necessarily their P status. Rib bone P concentration more accurately reflected the mineral reserves. Rumen fluid P_i concentration was insufficiently sensitive to distinguish at all times between the different levels of supplementation (LP, MP and HP), but it readily distinguished between the unsupplemented and supplemented (6-month or 12- month) groups.

De Waal *et al.* (1996) concluded that grazing animals (breeding beef cows) did not make up for the lack of P supplement in one half of the year during the other half of the year, when they received the same P supplements. Blood, rib bone and rumen fluid may be used, in conjunction with data on body mass and reproduction, as indicators of the P status of grazing beef cows.

Acknowledgements

The authors acknowledge the contribution of all those involved in this research programme, both in the past and present, especially Dr Elias Engels, Dr Marion Read, Dr Johan Grobbelaar, Mr Hannes Vermeulen, Mrs Heila Terblanché and the late Mr Alfonso Malan and Dr Daan Els.

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