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Bone mineral density in the tail-bones of cattle: effect of dietary phosphorus status, liveweight, age and physiological status

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Abstract. In three grazing experiments in the seasonally dry tropics of Australia, growing steers (Experiment 1), first-calf cows (Experiment 2) and mature breeder cows (Experiment 3), ingested diets for 12–17 months, which were either adequate or severely deficient in phosphorus (P) (P_{adeq} and P_{defic}, respectively). Bone mineral density (BMD) at the proximal end of the ninth coccygeal vertebra (Cy9) was measured at intervals using single photon absorptiometry (SPA). Liveweight (LW) and plasma inorganic phosphorus (PIP) concentrations were monitored at intervals and rib-bone cortical bone thickness (CBT) of biopsy samples was measured at the end of Experiments 1 and 3. Measurements of LW change, PIP concentrations and CBT confirmed that diet P intakes of cattle in the P_{adeq} treatments were adequate whereas there was severe and chronic P deficiency in the P_{defic} treatments. In Experiment 1 BMD in P_{adeq} steers increased with LW and age from ~0.25–0.27 g/cc (8 months, 200 kg LW) to ~0.34 g/cc (32 months, 490 kg LW), whereas in P_{defic} steers BMD decreased progressively to ~0.23–0.24 g/cc. Although BMD decreased in the P_{defic} steers bone volume of Cy9 (calculated from tail-bone thickness) increased, and some net bone deposition in the Cy9 continued. Rib-bone CBT and tail-bone BMD at the end of Experiment 1 were closely correlated ($r = 0.93$). In Experiment 2 BMD was initially 0.33 g/cc (~25 months, 400 kg LW) and did not change through pregnancy and lactation in P_{adeq} cows. However, in the P_{defic} cows there was a gradual decline in BMD to ~0.25 g/cc. There was no change in dimensions of the Cy9 so the decreases in BMD involved net demineralisation of bone. In Experiment 3 BMD was less responsive to P deficiency than in Experiments 1 and 2. Only after ~11 months was BMD reduced ($P < 0.05$) in the P_{defic} cows, and then only by 15%. In contrast, rib-bone CBT decreased by 30% due to P deficiency, and BMD was poorly correlated with CBT ($r = 0.4$). The effects of animal weight, age and maturity on tailbone BMD of P-adequate animals, and the different responses to P deficiency observed in young growing steers, first-calf cows and mature breeders are discussed in relation to the use of SPA measured tail-bone BMD to diagnose P deficiency in grazing cattle.

Additional keywords: bone mineral concentration, bone mineral mass, cortical bone thickness, phosphorus deficiency.

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Introduction

Phosphorus (P) deficiency commonly occurs in grazing cattle, particularly in many rangeland regions of northern Australia, Africa and South America, and may severely reduce growth and productivity (Winks 1990; McCosker and Winks 1994). The reliable diagnosis of dietary P deficiency and P status in free-ranging beef cattle is difficult despite efforts over many decades to identify and develop effective and practical diagnostic techniques (Wadsworth *et al*. 1990). The measurement of P concentration in soil or the pasture on offer is of limited benefit because of the over-riding influence, particularly in extensive rangeland systems, of selective grazing. As diet P concentration in cattle ingesting forage diets is correlated with faecal attributes including the P concentration in faeces, and with some diet attributes that can be measured with near-infrared reflectance spectroscopy of faeces, diet P concentration may

be estimated from faeces (Moir 1960; Holechek *et al*. 1985; Dixon and Coates 2011; Dixon 2016). However, such estimation of diet P concentration from faecal measurements may involve considerable error (Dixon 2016). In growing cattle the inorganic P concentration in plasma (PIP) or in whole blood appears to be the most reliable method for diagnosing current diet P deficiency provided due consideration is given to the effect of age, site of sampling (e.g. jugular versus caudal), sample preparation, expected growth rates and animal stress on the measured values (Wadsworth *et al*. 1990; McCosker and Winks 1994; Coates 1994). However, diagnosis from PIP has several limitations. First, PIP concentration primarily reflects current P intake with little consideration of any P mobilised from or deposited into the soft and skeletal tissues (AFRC 1991). Second, insufficient information is available to interpret PIP concentrations of breeder cows in pregnancy or lactation; limited data suggests that breeder productivity as measured by calf growth and cow liveweight and reconception can be quite satisfactory even when PIP concentrations are much lower than the accepted threshold values for growing cattle (Coates and Ternouth 1992; Miller *et al*. 1997; Ternouth and Coates 1997). Third, diagnosis of cattle P status from PIP has seldom been applied in extensive rangeland grazing systems such as in the north Australian beef cattle industry due to practical difficulties such as the availability of the skills for blood sampling and processing, access to laboratory analyses, and the interpretation of results. The cortical or compact bone thickness (CBT) of rib-bone obtained from biopsy samples (Little 1972, 1984; Read 1984) may be used to assess the status of bone P reserves in cattle. However, although this technique has often been used in research contexts it is not suitable for routine on-farm diagnosis due to the need for specialist veterinary and surgical skills, difficulties with obtaining repeated-measurements, and because of animal welfare considerations.

A diagnostic approach that has received limited attention for estimating skeletal P reserves, and long-term P deficiency in beef cattle, is the measurement of bone mineral density (BMD) and bone mineral mass (BMM) of limb bones or tail-bones. Murray (1989) described an instrument developed for on-farm (crush-side) measurements of BMD of tail-bone using single photon absorptiometry (SPA). The tail was considered the most appropriate measurement site for several reasons. First, this is a convenient practical site to take *in vivo* measurements on untrained cattle held in a cattle crush. Second, mobilisation of bone minerals occurs primarily from the axial skeleton rather than from the appendicular skeleton (Benzie *et al*. 1955; Hill 1962; Murray *et al*. 1994). Coates and Murray (1994) reported the results of an experiment where BMD measurements in the ninth coccygeal vertebra (Cy9) using the Murray *et al*. (1994) densitometer were compared with PIP and rib CBT measurements in groups of P-deficient and P-adequate steers. These results indicated that PIP gave the highest level of discrimination between P-deficient and P-adequate steers, whereas CBT and tail-bone BMD gave lesser but useful degrees of discrimination. More recently, Coates *et al*. (2016) evaluated the accuracy of *in vivo* measurements of tail-bone BMD using the densitometer, by comparing SPA measurements with laboratory measurements of bone ash density made on resected tails. It was concluded that the accuracy of *in vivo* SPA measurements of BMD was sufficient to warrant continued research in the evaluation of tail-bone BMD for diagnosing P deficiency and as an indicator of body P status in cattle. The present study reports results from three experiments where the SPA densitometer was used to make sequential measurements of tail-bone BMD in growing steers, in first-calf cows, and in mature breeder cows consuming P-deficient and P-adequate tropical forage diets for extended intervals of 12–17 months.

Materials and methods

Three grazing experiments were conducted in the seasonally dry tropics of northern Australia. Experiments 1 and 2 were conducted at the CSIRO Lansdown Pasture Research Station (19°41′S, 146°51′E) near Townsville, whereas Experiment 3 was conducted at Springmount Station (17°13′S, 145°12′E) near Mareeba in north Queensland. Some aspects of Experiments 1 and 3 have been reported previously (Miller *et al*. 1997; Coates *et al*. 2016).

Experiment 1. Grazing steers at Lansdown Research Station

Two age groups (each n = 20) of *Bos indicus* × *Bos taurus* (Droughtmaster) steers were used. The older steers (Group 1) were born in November–December 1992. From September 1993 to mid-March 1994 they were managed as a single herd and grazed mixed grass-legume pasture containing stylo legumes (*Stylosanthes hamata* cv. Verano and *S. scabra* cv. Seca). Because drought conditions prevailed until the end of January the steers were supplemented with low quality grass hay and supplement feed blocks containing protein, energy and minerals including P. In mid-March the herd was allocated by stratified randomisation based on liveweight (LW) into low P (P_{defic}) and higher P (P_{adeq}) treatment groups and grazed unfertilised and P fertilised paddocks, respectively, until July 1994 when they moved to a trial area of twenty 3.8-ha paddocks. The pasture comprised native grasses with stylo legumes and all paddocks were acutely P deficient with soil bicarbonate extractable P (P_B, Colwell 1963) of <4 ppm in the top 10 cm of soil. Supplementary P (sodium orthophosphate, 20% P) was included in the drinking water in 10 of the paddocks to provide the P_{adeq} treatment. One steer was allocated to each of the paddocks, the P_{adeq} and P_{defic} treatment steers being allocated to paddocks with and without the P supplement, respectively. Salt (sodium chloride) blocks were provided in all paddocks. In addition a second group of steers of similar genotype but 12 months younger (Group 2) were also allocated by stratified randomisation based on LW to the P_{defic} and P_{adeq} treatments so that each paddock was stocked with one yearling and one weaner steer. Water intake in the P_{adeq} paddocks was monitored and the concentration of sodium orthophosphate in the drinking water was adjusted so that the P_{adeq} steers ingested, on average, 5–7 g supplementary P per head per day. The steers grazed in the 20 paddocks until the end of the experiment in July 1995. Measurements of LW (unfasted), PIP of jugular blood and tail-bone BMD were made at intervals of ~2 months. Biopsy samples of rib-bone were obtained from steers in half the paddocks (5 P_{adeq} and 5 P_{defic}) at the end of the experiment.

Experiment 2. Grazing first-calf cows at Lansdown Research Station

Pregnant Droughtmaster females ~30 months old were allocated in April 1994 by stratified randomisation based on LW to low P (P_{defic}) and adequate P (P_{adeq}) treatment groups (each $n = 10$). The P_{defic} animals grazed as a single herd an area (~20 ha) of unfertilised pasture comprised predominantly of native grasses, sabi grass (*Urochloa mosambicensis* cv. Nixon) and stylo legumes growing on a soil low in available P (P_B of ~4 ppm). The P_{adeq} animals grazed as a single herd an area (~16 ha) of sabi grass/stylo pasture, which had been fertilised annually for >10 years with superphosphate at 10 kg P/ha. There was no paddock replication of the two treatments. The females calved in November–December 1994, calves were weaned on 11 May 1995, and the final measurements were made in late June 1995. Unfasted LW, PIP and tail-bone BMD measurements were made seven times through the experiment at intervals of 2–3 months. Calf growth rates were also measured. No rib-bone biopsy samples were taken.

Experiment 3. Mature breeder cows grazing as paddock groups at Springmount Station

Pastures and paddocks have been described briefly by Miller *et al*. (1996) and more comprehensively by Miller *et al*. (1997). There were six paddocks encompassing a range of soil P concentrations, pasture species and supplement regimes as indicated in Table 1. A maintenance application of superphosphate was applied to the moderate soil P (MP) paddock in September 1994 to maintain a moderate level of available P in the soil. Each paddock was grazed by eight pregnant, then lactating, *Bos indicus* × *Bos taurus cows*, 4–5 years of age when the experiment commenced in June 1994. The cows calved during October–November in the late dry season, the seasonal break occurred in late January, and calves were weaned on 19 April in the mid to late wet season. Loose mix supplements of calcium phosphate (Kynophos) and/or urea were fed year-round and offered twice weekly to provide 10 g P/cow.day and 28 g N/cow.day as appropriate, together with 50 g sodium chloride/cow.day as indicated in Table 1. The salt was provided in all paddocks. Results from a previous draft of breeders in the same experiment (Coates *et al*. 1996; Miller *et al*. 1996; Dixon *et al*. 2016) indicated that cows in the three P_{defic} paddocks (VLP, LP and LP + N) were acutely P deficient whereas cows grazing the three P_{adeq} paddocks (LP + P, LP + P + N and MP) had adequate P intakes.

Unfasted LW was recorded at frequent but irregular intervals throughout the experiment from June 1994 to July 1995. Jugular PIP and tail-bone BMD measurements of cows were made in late pregnancy (September), in mid-lactation (early March) and 5 weeks post-weaning (May). Rib-bone biopsy samples were taken from four of the eight cows in each paddock in June 1995.

Measurements

Measurements of jugular PIP were made as described by Coates (1994). Rib-bone biopsy samples from the 12th rib were obtained as described by Little (1972, 1984); the biopsy sample included external cortical bone, trabecular bone and internal cortical bone of the rib although only the measurements of external cortical bone are presented in this paper. Trabecular bone was scraped from the cortical bone, and CBT was measured using calipers along the longitudinal line of maximum thickness.

Tail-bone BMD at the proximal end of tail-bone Cy9 was measured by SPA as described by Murray *et al*. (1994) and Coates *et al*. (2016) while animals were restrained in a short section of crush. Calculation of BMD included a correction for displacement of soft tissue by bone mineral (Coates *et al*. 2016). BMM was calculated as BMD (g/cc) × bone volume (cc), and bone volume was calculated as tail-bone thickness (TBT, cm) × cross-sectional area (cm^2) of the core of bone being measured. To determine changes in BMM as a guide to net mobilisation or net deposition of bone mineral during a specified interval, the initial cross-sectional area was taken as the area of a 5-mm-diameter circle (0.1964 cm^2), which approximated the cross-sectional area of the core being scanned during BMD measurements and the bone volume was calculated as TBT × 0.1964 cc. For subsequent determinations of BMM (identified as BMM_2 in this paper), for comparison with the initial determination of BMM (identified as BMM_1),

Table 1. Experiment 3. Paddock nomenclature, grouping of the herds in the various paddocks into P_{adeq} and P_{defic} treatments, the available P in top 10 cm of soil, pastures and supplement offered to mature reproducing breeder cows

Paddock	VLP[A]	LP[B]	LP + N[C]	LP + P[D]	LP + P + N[E]	MP[F]
Treatment	P_{defic}	P_{defic}	P_{defic}	P_{adeq}	P_{adeq}	P_{adeq}
Soil P_B ppm[G]	2	3–4	3–4	3–4	3–4	6–10
Pasture[H]	NP	NP + stylo	NP + stylo	NP + stylo	NP + stylo	NP + stylo
Supplement[I]	Nil	Nil	NPN	P	P + NPN	Nil

[A]Very low soil P.
[B]Low soil P.
[C]Low soil P, supplemented with urea N.
[D]Low soil P, supplemented with P.
[E]Low soil P supplemented with urea N and with P.
[F]Moderate soil P.
[G]Bicarbonate extractable P in top 10 cm soil (Colwell 1963).
[H]NP = native grass pasture, NP + stylo = native grass pasture with *Stylosanthes* legumes.
[I]NPN = non-protein nitrogen as urea.

the bone volume was adjusted to take account of any increase in bone dimensions.

Tail-bone thickness at the measurement site was calculated from total tail thickness using a ratio of 0.602 for the steers in Experiment 1 (Coates *et al*. 2016) and a ratio of 0.643 for the first-calf cows and mature cows in Experiments 2 and 3. The latter ratio had been previously determined on 60 resected tails from a comparable group of 4-year-old cows (D. B. Coates, unpubl. data). In this paper BMD and BMM always refer to the SPA measurements made at the proximal end of Cy9 unless otherwise stated.

Animal welfare and the use of radioactive substances

Surgical and other experimental procedures were carried out according to the code of practice for the care and use of animals for scientific purposes and with the approval of the relevant Animal Ethics Committees operating at the time the experiments were conducted. Regulations regarding the use and storage of radioactive substances incorporated into the instrument used to measure BMD were followed.

Statistical analyses

Statistical analysis was conducted using GENSTAT release 16.1 (VSN International Ltd, Hemel Hemstead, UK). In Experiment 1 where the P_{adeq} and P_{defic} paddocks were replicated and when each paddock was stocked with one steer from each age group (Groups 1 and 2), a 2-strata ANOVA was used to determine the effects of P status and steer age on each of the measurements (LW, BMD, BMM, PIP and rib-bone CBT). In Experiment 2 where there was no paddock replication, differences between the P_{adeq} and P_{defic} treatment herds were again determined using ANOVA with individual animals as the experimental units. In Experiment 3, paddocks VLP, LP and LP + N were considered as replicates of the P_{defic} treatment whereas paddocks LP + P, LP + P + N and MP were considered as replicates of the P_{adeq} treatment, and the paddocks were considered as the experimental units. In each experiment, separate analyses were conducted for each sampling occasion and for each constituent. Paired *t*-tests were used to determine differences in a constituent between sampling occasions with respect to individual paddock groups. Statistical significance was deemed to have occurred when $P < 0.05$, whereas situations where $P > 0.05$ but <0.10 are described as non-significant trends or tendencies. In most cases the actual *P*-values have been provided to allow readers a greater level of discernment regarding the outcome of statistical analysis.

Results

Experiment 1. Growing steers

From September 1993 to mid-March 1994, when Group 1 steers grazed as a single herd, the average daily gain was only 0.2 kg/day due to drought for much of that period (Fig. 1*a*). Nevertheless, BMD increased from 0.240 to 0.278 g/cc during that interval (Fig. 1*b*). When the two treatments grazed as separate herds from March to July 1994, average daily gains increased to 0.45 and 0.37 kg/day for P_{adeq} and P_{defic} herds, respectively. However, BMD measured in late July 1994 did not differ ($P = 0.91$) between the P_{adeq} (0.289 g/cc) and P_{defic} (0.283 g/cc) groups. After Group 1 steers were transferred to the 20 small paddocks, P_{adeq} steers continued to make good LW gains averaging 187 kg/head (0.55 kg/day) for the 49 weeks July 1994 to July 1995. Gains for the Group 1 P_{defic} steers for the same period averaged only 77 kg/head (0.23 kg/day). During the same 49-week interval BMD of the P_{adeq} steers increased from

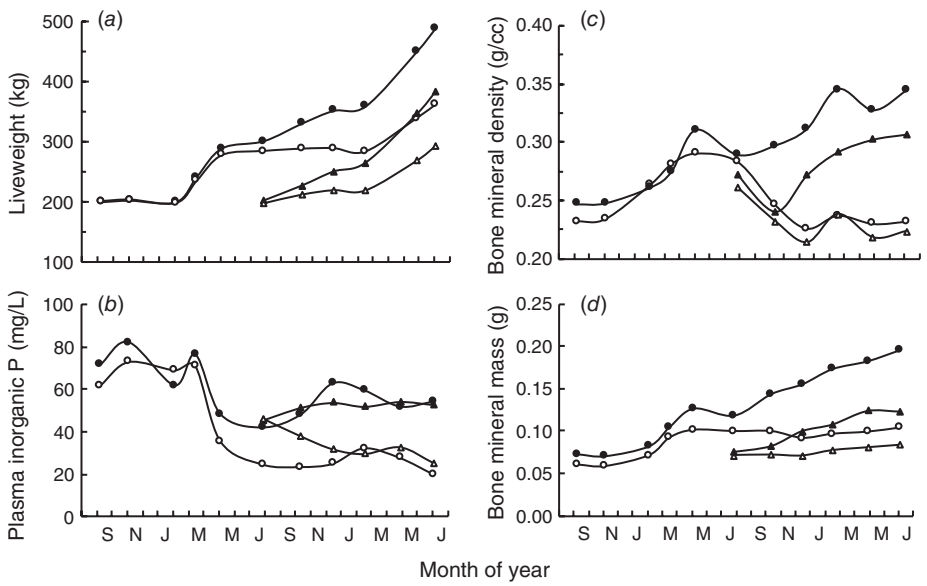

Fig. 1. Grazing steers in Experiment 1. Profiles of (*a*) liveweight, (*b*) plasma inorganic P concentration (PIP), (*c*) bone mineral density (BMD) at the proximal end of the ninth coccygeal vertebra (Cy9), and (*d*) bone mineral mass (BMM) at the proximal end of Cy9 from September 1993 to July 1995. Group 1 steers (●, ○) were 1 year older than Group 2 steers (▲, △), and the diets were either adequate in P (filled symbols ●, ▲, P_{adeq}) or deficient in P (○, △, P_{defic}).

0.289 to 0.344 g/cc whereas BMD of Group 1 P_{defic} steers declined rapidly from 0.283 to 0.226 g/cc for the period July to mid-December 1994, and then remained at ~0.23 g/cc till the end of the experiment. Differences between Group 1 P_{adeq} and P_{defic} steers were significant for the final 9 months of the experiment ($P = 0.012$ in October 1994 and $P < 0.001$ thereafter).

Liveweight changes in Group 2 steers followed a similar pattern to those of the Group 1 steers with LW gains over 49 weeks of 182 and 98 kg/head in P_{adeq} and P_{defic} steers, respectively (Fig. 1*a*). Except for an initial decline in BMD of the P_{adeq} steers from July to October, a decline for which we have no explanation, changes in BMD followed similar patterns to those observed for Group 1 steers (Fig. 1*c*). The decline in BMD of the P_{defic} steers from 0.261 g/cc in July to 0.214 g/cc in mid-December, and the relative stability of BMD at ~0.22 g/cc for the remainder of the experiment, paralleled that of the Group 1 P_{defic} steers. Mean BMD of Group 2 P_{adeq} steers was higher than that of the P_{defic} steers for the four sampling occasions December 1994 to July 1995 ($P = 0.02$ in December and $P < 0.01$ thereafter). There was an effect of steer age on BMD of P_{adeq} steers where mean BMD values of Group 1 steers were higher than those of Group 2 steers throughout the period July 1994 to July 1995 (Fig. 1*c*) and the differences were significant in October ($P = 0.008$) and December ($P = 0.02$) of 1994 and in February of 1995 ($P = 0.001$). Conversely, mean BMD values for Group 1 and Group 2 P_{defic} steers did not differ on any sampling occasion (Fig. 1*c*) with *P*-values ranging between 0.17 and 0.94. Previously, unpubl. data obtained from resected tails of the steers in Experiment 1 (see Coates *et al.* 2016) were used to determine the relationship between the volume of entire Cy9 vertebrae and TBT. There was a linear relationship between volume and TBT ($P < 0.001$, $r = 0.74$) where: Volume of Cy9 (cc) = 5.3499 TBT (cm) – 4.2023. This regression was used to estimate the increase in the volume of Cy9 as TBT increased. On the assumption that the proportional increase in volume is uniform across various sections of Cy9 it was possible to calculate the BMM of the core, initially 5 mm in diameter, at the beginning and end of an interval (BMM_1 and BMM_2, respectively), accounting for changes in both BMD and bone volume. Thus BMM was adjusted for changes in bone dimensions between sequential SPA measurements. Profiles of core BMM during the experimental period were calculated for the growing steers in Experiment 1 (Fig. 1*d*). Despite the large decreases in BMD of Group 1 and Group 2 P_{defic} steers during the July 1994 to July 1995 interval (Fig. 1*c*), there was no decline in volume adjusted BMM. Calculations indicated that there were increases in BMM of 4% and 18% for Groups 1 and 2 P_{defic} steers, respectively, despite reductions of 18% and 14% in BMD. The BMM of P_{adeq} steers over the same interval increased by 65% for steers in both age groups.

Concentrations of PIP were high in all Group 1 steers (mean 71 mg/L) before the P_{adeq} and P_{defic} treatments were imposed in mid-March 1994 (Fig. 1*b*). There was a sharp decrease in PIP in both P_{adeq} and P_{defic} steers from mid-March 1994 when these steers were grazed as two herds in the fertilised and unfertilised paddocks, respectively, but the decrease in the P_{defic} steers was greater. In July 1994 the mean PIP concentrations in the P_{adeq} and P_{defic} steers were 42 and 25 mg/L, respectively. Following the relocation of the steers to the individual paddocks and with the P supplementation of the P_{adeq} steers, PIP in the P_{adeq} steers increased to 63 mg/L in December 1994 and averaged 55 mg/L from October 1994 to July 1995. From July 1994 to July 1995 PIP in Group 1 P_{defic} steers averaged only 25 mg P/L, and was thus indicative of acute P deficiency. From May 1994 to July 1995, PIP in P_{defic} steers averaged only half that of the P_{adeq} steers ($P < 0.001$ on all seven sampling occasions). Similarly, in the younger Group 2 steers, PIP in P_{defic} steers was lower than in P_{adeq} steers from October 1994 to July 1995 ($P < 0.05$ in October and $P < 0.01$ thereafter).

At the end of the experiment rib CBT of Group 1 steers was greater in P_{adeq} than in P_{defic} steers with means of 3.59 and 2.22 mm, respectively ($P < 0.001$). Similarly, CBT of Group 2 steers was greater in the P_{adeq} than in the P_{defic} steers with means of 3.19 and 1.80 mm, respectively ($P < 0.001$). Rib CBT was greater in the older steers than in the younger steers for both P_{adeq} and P_{defic} treatments ($P = 0.015$). There was a strong linear correlation between the BMD measurements made at the end of the experiment and the rib CBT measurements (Fig. 2). At the end of the experiment both BMD and CBT of P_{defic} steers were ~0.6 that of P_{adeq} steers.

Experiment 2. First-calf cows

The P_{adeq} and P_{defic} females did not differ in mean LW from April to mid-December 1994 (*P* ranging from 0.38 to 0.92, Fig. 3*a*). The April to mid-October interval coincided with mid- to late pregnancy, whereas the large LW losses from mid-October to mid-December were associated with calving. From mid-December 1994 to June 1995 the mean gain of the P_{adeq} females was 92 kg (0.49 kg/day), whereas the P_{defic} females underwent an average weight loss of 11 kg. The P_{adeq} females were heavier than the P_{defic} females from mid-February 1995 to the end of the experiment ($P = 0.007$ in February and <0.001 thereafter) when the P_{adeq} females were, on average, 114 kg heavier than the P_{defic} females. Despite the large differences between the P_{adeq} and P_{defic} females in post-calving LW

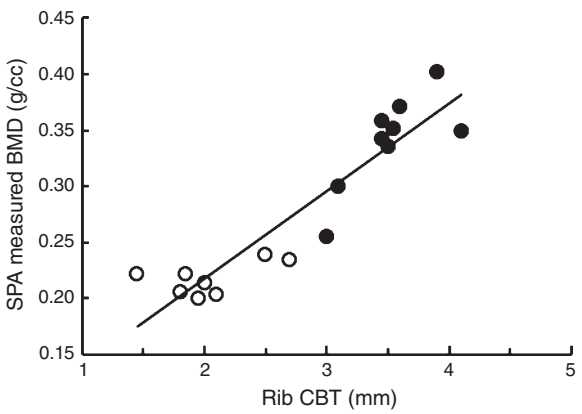

Fig. 2. Grazing steers in Experiment 1. End of experiment relationship between bone mineral density (BMD) measured using single photon absorptiometry at the proximal end of the ninth coccygeal vertebra and rib compact bone thickness (CBT) in two age groups of steers ingesting P adequate (P_{adeq}, ●) or P deficient (P_{defic}, ○) diets for 17 months (Group 1 steers) or for 12 months (Group 2 steers). Two outlier points were excluded. The equation was: BMD = 0.0777 Rib CBT + 0.0624 ($n = 18$, $r = 0.93$).

change and body condition, calf growth rates did not differ between the treatment groups ($P > 0.05$), with average daily gains for the period 13 December 1994 to weaning on 11 May 1995 of 0.91 and 0.87 kg/head for the P_{adeq} and P_{defic} treatments, respectively.

In P_{adeq} females there was little change over time in mean BMD despite the large fluctuations in LW; the overall mean BMD was 0.330 g/cc ranging from 0.319 to 0.347 g/cc on different sampling occasions (Fig. 3*c*). In contrast, there was a progressive and linear decline in BMD of P_{defic} females over the 12 months from April 1994 to April 1995, from 0.344 to 0.240 g/cc ($P < 0.001$, $R^2 = 0.97$). The mean measured increase in the BMD of P_{defic} females during the interval April to July 1995 following weaning (see Fig. 3*c*) was not significantly different from zero ($P = 0.45$). Mean BMD did not differ between the P_{defic} and P_{adeq} females on the first two sampling occasions ($P = 0.38$ in April and $P = 0.55$ in July). However, during that interval the mean measured BMD of P_{defic} females decreased by 0.012 g/cc whereas that of the P_{adeq} females increased by 0.024 g/cc and there was a strong trend for these changes to differ ($P = 0.067$). Treatment mean BMD measurements were higher for P_{adeq} than for P_{defic} females for the remainder of the experiment ($P < 0.05$ to $P < 0.001$). At the end of the experiment the mean BMD of P_{defic} females was 30% lower than that of P_{adeq} females.

There was very little change in predicted TBT in either P_{adeq} or P_{defic} females during the course of the experiment and changes in treatment mean TBT between sampling occasions were not significant ($P > 0.05$). Because there was no detectable change in bone dimensions, relative changes in BMM were the same as the relative changes in BMD so that an increase in BMD indicated net bone deposition whereas a decrease in BMD indicated net bone mineral mobilisation at the measurement site. The 30% decrease in measured BMD of P_{defic} females during the duration of the experiment was therefore indicative of substantial net bone mobilisation at the Cy9 measurement site. There was no change ($P = 0.44$) in measured BMD of the P_{adeq} females during the experiment.

The first measurement of PIP was made in late July 1994, 3 months after the experiment commenced, and the concentrations were lower in P_{defic} than in P_{adeq} females on all sampling occasions ($P < 0.001$, except in October where $P < 0.01$) (Fig. 3*b*). On average, PIP of P_{defic} females was ~one-third that of the P_{adeq} females (18 vs 53 mg/L) and indicated severe P deficiency in the P_{defic} females.

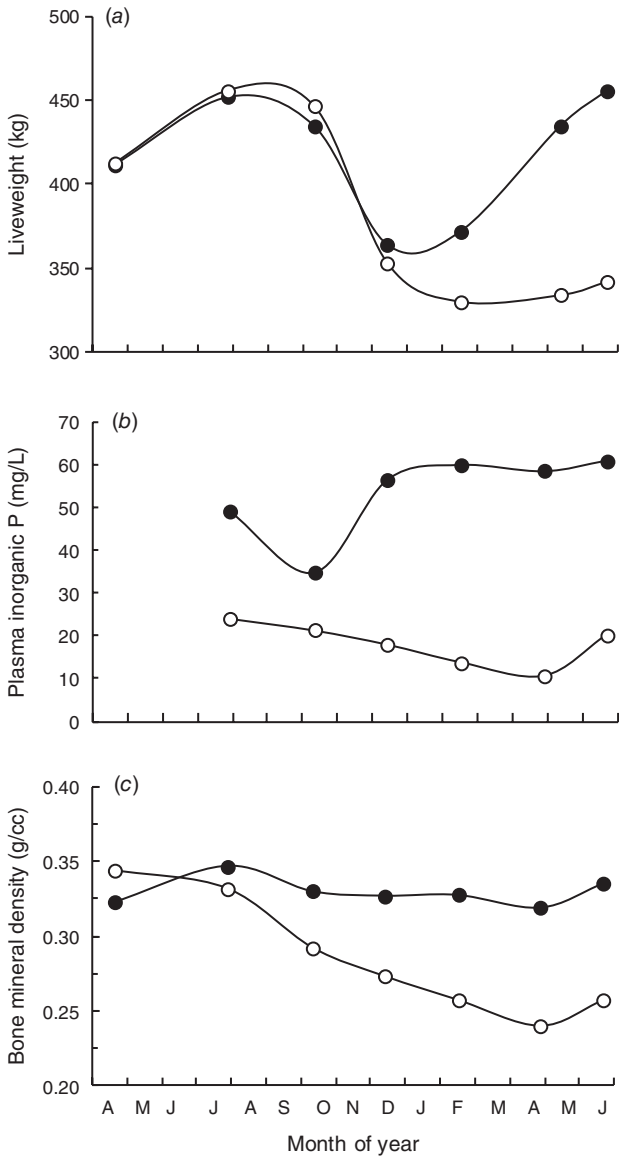

Fig. 3. Young breeder females in Experiment 2. Profiles of (*a*) liveweight, (*b*) plasma inorganic P concentration (PIP) and (*c*) bone mineral density (BMD) at the proximal end of the ninth coccygeal vertebra, for the period April 1994 to June 1995. Diets were either adequate in P (P_{adeq}, ●) or deficient in P (P_{defic}, ○).

Experiment 3. Mature breeder cows

The direction and magnitude of cow LW changes during the annual cycle (Fig. 4) were made up of three distinct periods. First, the late pregnancy period (17 June to 10 October) corresponded with the early- and mid-dry season. Although there was a moderate LW increase of 20 kg in the P_{adeq}

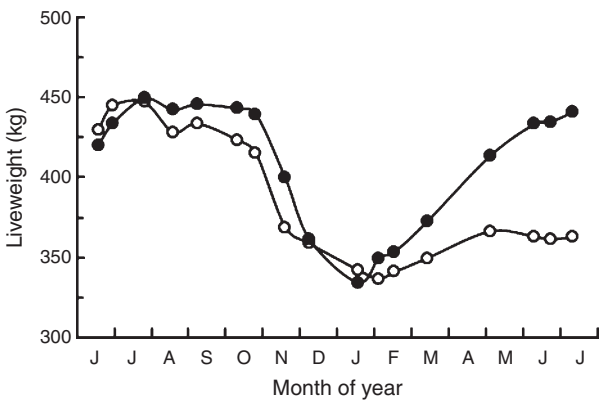

Fig. 4. Mature breeder cows in Experiment 3. Liveweight profiles from June 1994 to July 1995 as the cows progressed from mid-pregnancy through to 6 weeks post-weaning. Diets were either adequate in P (P_{adeq}, ●) or deficient in P (P_{defic}, ○).

treatment compared with a 6-kg loss in the P_{defic} treatment, the difference was not significant ($P > 0.05$) and nor did mean LW differ between the treatments pre-calving (early October). The second period encompassed calving and early lactation (10 October to 17 January) and corresponded primarily with the late dry season. Cows in both P_{adeq} and P_{defic} treatments underwent large LW losses (mean losses of 109 and 81 kg, respectively), but the mean cow LW did not differ between treatments during that interval (P ranged between 0.19 and 0.93). The third period (17 January to 10 July), encompassed mid- and late-lactation as well as a short post-weaning interval and coincided with the wet season and early dry season. Cows in both treatments gained LW but the gains were much greater ($P < 0.001$) in the P_{adeq} treatment (106 kg) than in the P_{defic} treatment (20 kg). Mean liveweight of P_{adeq} cows was higher than that of P_{defic} cows from mid-March to the end of the experiment with the probability of a significant treatment difference increasing through that interval ($P = 0.089$ in March and 0.003 in July). At the end of the experiment the mean LW of P_{adeq} cows was 441 kg, 21 kg heavier than at the beginning of the experiment. This compared with a final LW of 362 kg for P_{defic} cows, and a 67 kg LW loss during the experiment. Calf growth rate was higher in the P_{adeq} treatment ($P = 0.007$) with mean LW gains of 103 and 92 kg/head for P_{adeq} and P_{defic} treatment calves, respectively, during the interval 17 January to weaning in May.

Mean PIP concentrations in September 1994 were 59 and 42 mg/L in the P_{adeq} and P_{defic} treatments, respectively (Table 2) with a trend towards a significant difference ($P = 0.089$). Mean PIP of P_{adeq} cows in paddock MP at that time (43 mg/L) was similar to that in the three P_{defic} paddocks (mean 42 mg/L), and much lower than that of cows in the other two P_{adeq} paddocks that received P supplement (mean 67 mg/L). After the seasonal break to the wet season in late January the mean PIP concentrations of cows in paddock MP were similar to concentrations measured in the other two P_{adeq} paddocks. In the P_{defic} paddocks PIP declined markedly so that, in March and May, mean PIP of cows in the P_{defic} treatment (18 and 20 mg P/L, respectively) were less than half those in the P_{adeq} treatment ($P = 0.008$ in March and <0.001 in May, Table 2), and provided a clear indication of the severity of diet P deficiency in the P_{defic} cows.

In September 1994, 3 months after the experiment commenced, BMD did not differ between treatments ($P = 0.234$; Table 2). In March 1995, when the cows were in mid-lactation, there was a close to significant trend for BMD to be higher in the P_{adeq} treatment ($P = 0.053$), whereas in May the treatment difference was significant ($P = 0.024$). However, the proportional difference in BMD between treatments was quite small such that BMD of the P_{defic} treatment was only 13.5% lower in March and 14.8% lower in May than the corresponding measurements in the P_{adeq} treatment. Moreover the decline in BMD within the P_{defic} treatment was much less than in Experiments 1 and 2, with only an 8% reduction in BMD for the September–March interval, and an even lesser reduction of 6.5% for the September–May interval. The lesser reduction for this latter interval may have been the result of some bone deposition after the calves were weaned in April. Mean BMD in the P_{adeq} treatment did not differ ($P = 0.20$) between initial and final measurements in September and May. As in Experiment 2 there was little change in predicted TBT in either P_{adeq} or P_{defic} cows during the course of Experiment 3 and measured changes in treatment mean TBT between sampling occasions were not significant ($P > 0.05$). Therefore, relative changes in BMM were similar to those in BMD.

Rib-bone CBT at the end of the experiment was lower in P_{defic} than in P_{adeq} cows ($P = 0.021$, Table 2). Importantly, mean CBT in P_{defic} cows was 30% less than in P_{adeq} cows indicating that rib CBT was much more responsive than BMD to prolonged diet P deficiency. In Experiment 3 tail-bone BMD was poorly correlated with rib-bone CBT ($P = 0.057$, $R^2 = 0.16$).).

Table 2. Experiment 3. Effect of dietary P status (treatments P_{defic} and P_{adeq}) of mature breeding cows on plasma inorganic phosphorus (PIP, mg/L) and tail-bone bone mineral density (BMD, g/cc) measured in September (late pregnancy), March (mid to late lactation) and May (post-weaning) and rib-bone cortical bone thickness (CBT, mm) measured post-weaning

Measurement	Treatment		s.e.m.	Probability
	P_{defic}	P_{adeq}		
PIP (September 1994)	42	59	5.5	0.089
PIP (March 1995)	18	45	3.8	0.008
PIP (May 1995)	20	52	0.8	<0.001
BMD (September 1994)	0.329	0.348	0.0095	0.234
BMD (March 1995)	0.303	0.351	0.0123	0.053
BMD (May 1995)	0.308	0.361	0.0106	0.024
BMD change (Sept.–March)	–0.026	+0.003	0.0079	0.064
BMD change (March–May)	+0.004	+0.008	0.0162	0.877
BMD change (Sept.–May)	–0.022	+0.012	0.0100	0.070
Rib CBT (June 1995)	2.58	3.69	0.22	0.021

Discussion

Based on previous research at the Lansdown, Springmount and other sites in northern Australia, the measurements of LW change, PIP and rib-bone CBT, and the differences between the P_{adeq} and P_{defic} cattle in each of the three experiments with respect to these attributes, indicated little likelihood that cattle in the P_{adeq} treatments would have responded to additional diet P, and also that cattle in the P_{defic} treatments were severely P deficient (Kerridge *et al*. 1990; Wadsworth *et al*. 1990; Winter *et al*. 1990; Coates 1994; Ternouth and Coates 1997). Furthermore the large effects of P supplementation observed in both growing steers in Experiment 1 and reproducing females in Experiments 2 and 3 are in accord with the liveweight responses expected when severely P-deficient cattle are given P supplements (McLean *et al*. 1990; Winks 1990; Winter *et al*. 1990). It was, therefore, against the background of diet treatments of acute P deficiency and adequate P intake that tail-bone BMD and BMM were evaluated in the three experiments.

Tail-bone BMD, BMM and diet P status

In Experiment 1 BMD and BMM were both responsive to diet P intake (Fig. 1*c*, *d*, respectively). The difference in BMD between P_{adeq} and P_{defic} steers of both age groups increased progressively during the 49 weeks when the P_{adeq} steers were fed P supplement. During that period BMD increased in P_{adeq} steers and decreased

in P_{defic} steers. Most of the decrease in BMD of P_{defic} steers occurred during the July–December interval with little change thereafter despite the continuing and severe dietary P deficiency. The lack of further change suggested that decreases in BMD had reached a physiological limit which prevented further decline in BMD. The measured decreases of 0.058 and 0.047 g/cc in Group 1 and Group 2 P_{defic} steers, respectively, suggested mobilisation of P from tail-bones. However, calculations of BMM_1 in July 1994 and BMM_2 in July 1995 revealed that no net mobilisation occurred because increased bone volume more than counteracted the decrease in BMD such that BMM_2 was 4% and 18% greater than BMM_1 for Group 1 and Group 2 steers, respectively. These calculations indicated that there was probably little if any net mobilisation of bone P at the proximal end of Cy9 in Group 1 P_{defic} steers, and that there was appreciable net deposition of bone mineral at the site in Group 2 P_{defic} steers.

The differences in the magnitude and direction of change between BMM and changes in BMD during severe P deficiency of the P_{defic} growing steers in Experiment 1 is important and was a consequence of continuing dimensional growth of the bones in these young animals even during severe P deficiency. The results indicated there is little scope for net mobilisation of bone mineral from the tail-bones of growing cattle to release P for other needs. Although dimensional bone growth is retarded and BMD decreases during P deficiency in growing cattle, net mobilisation of bone mineral from tail-bones is unlikely because dimensional bone growth will usually more than counteract decreases in BMD. In P_{adeq} young cattle the increases in BMM were proportionally much greater than the increases in BMD as BMM incorporated increases in both BMD and bone volume. For the interval July 1994–July 1995 the percentage increase in BMM was 65% for both Group 1 and Group 2 P_{adeq} steers compared with increases of 19% and 13% in BMD for Groups 1 and 2, respectively.

Tail-bone BMD of the first-calf cows in Experiment 2 was also responsive to dietary P status (Fig. 3*c*). In the P_{defic} treatment BMD declined from 0.344 g/cc in April 1994 to 0.240 g/cc in April 1995, a decrease of 30%, whereas there was a slight increase in BMD in the P_{adeq} treatment. However, in contrast to the steers in Experiment 1, there was no detectable change in TBT in either the P_{adeq} or P_{defic} treatments through Experiment 2. Therefore, the percentage changes in BMM did not require any adjustment for dimensional bone growth and mirrored relative changes in BMD. Thus, the 30% reduction in BMD of P_{defic} females from April 1994 to April 1995 indicated first that there was 30% net mobilisation of bone mineral at the measurement site over the same period and second that, unlike young growing animals, these first-calf cows were able to mobilise P from the tail-bones to partly offset the ongoing deficiency in diet P. The progressive decline in BMD in the P_{defic} females from mid-April 1994 to late April 1995 (Fig. 3*c*) indicated that net mobilisation of tail-bone mineral continued throughout that interval due to the P demands of the developing fetus and then throughout lactation. The increase in BMD and therefore of BMM during the final 8-week interval of the experiment was probably a response to the cessation of the lactation demand on cow P reserves when the calves were weaned on 11 May. Thus, net deposition of P into the tail-bones commenced after a long period of mobilisation of bone mineral. These results are in contrast to those observed in another similar experiment with first-calf cows of similar genotype where little mobilisation of tail-bone P occurred during extended deficiency (Dixon *et al*. 2016).

Results from Experiment 3 with mature-aged cows contrasted with Experiments 1 and 2 in that tail-bone BMD was much less responsive to dietary P status even though the measurements of LW, PIP and rib-bone CBT indicated that the P_{defic} cows suffered severe dietary P deficiency throughout the experiment. Measured BMD did not differ ($P = 0.324$) between the P_{defic} and P_{adeq} treatments in September, 3 months after the treatments were imposed. There were indications of a response to P deficiency by March with a close-to significant trend ($P = 0.053$) for BMD of P_{defic} cows to be lower than BMD of P_{adeq} cows (Table 2). The difference reached significance in May ($P = 0.024$) but BMD of P_{defic} cows was only 15% lower than that of P_{adeq} cows. Moreover the BMD for the P_{defic} treatment in May was only 6.5% lower than the September measurement despite the P_{defic} cows being subjected to acute P deficiency for 11 months, which included periods of high P demand during pregnancy and lactation. There was, in fact, a slightly larger reduction (8%) during the September–March interval and this indicated the possibility of some recovery in BMD after calves were weaned in mid-April. As we observed in the first-calf cows in Experiment 2, there was no increase in TBT through Experiment 3 so that percentage changes in BMM at the measurement site were the same as for BMD. Overall, the results indicated that mobilisation of bone mineral at the measurement site in the mature cows was substantially less than the 30% mobilisation observed in the younger first-calf cows in Experiment 2. Additionally, the observation that mean tail-bone BMD of the P_{defic} cows in Experiment 3 did not decrease to less than 0.303 g/cc, a value appreciably higher than the minimum BMD of 0.22–0.25 g/cc measured in the P_{defic} animals at the end of Experiments 1 and 2, suggested an age and/or maturity limitation to mobilisation of tail-bone mineral in mature breeding cows. These results are consistent with those of Ramberg *et al*. (1975) who reported large decreases in bone mineral deposition and mobilisation rates as dairy cattle increased in age from several months to the mature animal.

In contrast to the relative unresponsiveness of tail-bone BMD, it was evident that rib-bone CBT was responsive to dietary P status in the mature breeders in Experiment 3. Little (1984) argued that P concentration in total fresh ribs of cattle is a sensitive criterion of skeletal P reserves and that it may be estimated from CBT. Similar claims have been made that the tail-bones of cattle are representative of the skeleton as a whole (e.g. Ternouth 1990). On the basis of the contrasting responsiveness in rib-bone CBT and tail-bone BMD to P deficiency and the poor correlation between rib-bone CBT and tail-bone BMD at the end of Experiment 3, we have to conclude that rib-bone CBT is much more sensitive than tail-bone BMD as an indicator of skeletal P reserves in mature breeders. This was in direct contrast to the results for the growing steers in Experiment 1 where rib-bone CBT and tail-bone BMD were both responsive to diet P status and where there was a close correlation between these attributes at the end of the experiment.

We therefore suggest that tail-bone BMD and rib-bone CBT may both be useful criteria of skeletal P reserves in young, growing cattle.

It is of interest that the BMD of the 30-month-old pregnant females at the start of Experiment 2 (overall mean BMD of 0.333 g/cc) was similar to the final BMD of the Group 1 P_{adeq} steers in Experiment 1 when they were of comparable age and similar LW. Mean BMD of the P_{adeq} mature cows at the end of Experiment 3 at 0.361 g/cc was higher than BMD of the P_{adeq} steers and first-calf cows at the end of Experiments 1 and 2, despite final LW of the mature cows in Experiment 3 being somewhat less than the final LW of the P_{adeq} steers and first-calf cows. This supports our previous hypothesis that tail-bone BMD of P_{adeq} cattle increases with age and/or maturity as well as LW.

Three scenarios were identified in the three experiments with regard to mobilisation of P from tail-bones when cattle undergo severe and prolonged P deficiency. In growing cattle there was a relatively rapid reduction in tail-bone BMD in response to diet P deficiency but dimensional bone growth continued so that there was little if any scope for net mobilisation of tail-bone P to alleviate the adverse effects of dietary P deficiency on feed intake and growth rate. In young breeding females calving at 3 years of age there was also a rapid and marked reduction in tail-bone BMD in response to severe P deficiency but, unlike young growing animals, dimensional growth in bones was complete, or close to complete. Therefore, decreases in tail-bone BMD equated with mobilisation of bone mineral, which would have alleviated to some extent the adverse effects of dietary P deficiency on liveweight and milk production. In both growing cattle and young breeding females there appeared to be physiological limits to decreases in tail-bone BMD. In mature breeders (>4 years old), reductions in tail-bone BMD in response to P deficiency were markedly less than in growing cattle and young breeding females. Because there is no dimensional bone growth in mature breeders any decrease in tail-bone BMD necessarily equates with mobilisation of bone mineral. However, because decreases in tail-bone BMD in response to even severe P deficiency were relatively small, any beneficial effect from mobilisation of bone mineral from tail-bones would be limited.

Potential of tail-bone BMD for diagnosing P deficiency

Data from the three experiments were combined to investigate the relationship between tail-bone BMD and LW in P-replete cattle and to assess BMD as a diagnostic tool for identifying P deficiency in cattle. There was a strong linear relationship between tail-bone BMD and LW of P_{adeq} steers in Experiment 1 (Fig. 5) and there was good separation between BMD of P_{adeq} and P_{defic} steers. This indicated that tail-bone BMD may be useful for diagnosing chronic P deficiency in growing cattle provided due allowance is made for the relationship between BMD and LW in cattle with adequate P and provided other factors such as genotype and pasture attributes not related to P do not confound the relationship between LW and BMD. The relationship between BMD and LW of P_{adeq} cattle becomes less robust with breeding cows because large decreases in LW occur at calving but with little reduction in BMD post calving.

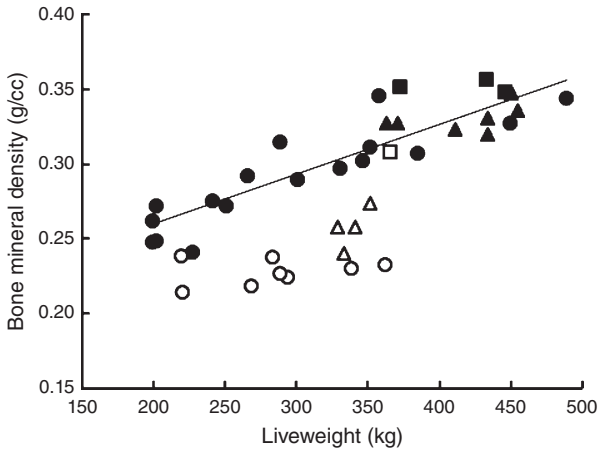

Fig. 5. The relationship (filled symbols) between the treatment means for bone mineral density (BMD) and liveweight (LW) measured at intervals for cattle ingesting diets adequate in P for 12–17 months in Experiments 1 (growing steers, ●), 2 (young breeding females, ▲) and 3 (mature breeding cows, ■). BMD measurements were made at the proximal end of the ninth coccygeal vertebra using single photon absorptiometry. In Experiment 1 the group mean data points were calculated separately for Group 1 (older) and Group 2 (younger) steers. The relationship was as follows: BMD = 0.0003 (LW) + 0.1935 (n = 27; r = 0.90). In addition data points from animals in the same experiments ingesting P-deficient diets are shown (○, △ and □) and were not included in the regression. The data points for the P-deficient diets were restricted to measurements made after the animals had been on the P-deficient diets for at least 4 months and represent the last four measurements in Experiment 1 (○) and in Experiment 2 (Δ), and the final measurement in Experiment 3 (□).

Moreover, as noted in the mature cows in Experiment 3, BMD of P_{adeq} cows was higher than BMD of steers of similar LW due possibly to an effect of age and maturity on BMD. The BMD results presented in Fig. 5 indicated good discrimination between P_{adeq} and P_{defic} groups for steers and first-calf cows where P_{defic} data points fell well below the regression line relating BMD to LW for P_{adeq} cattle. This contrasted with the data point that indicated the unresponsiveness of tail-bone BMD in mature cows in Experiment 3 to chronic, severe P deficiency. Because of the above considerations we suggest that tail-bone BMD is unlikely to provide a reliable tool for diagnosing P deficiency in breeding cows. However, measurements of tail-bone BMD could be made on a sentinel herd of growing steers as a guide to paddock P status.

In conclusion the present study indicated that prolonged severe P deficiency in growing cattle and in young breeder cows could be reliably diagnosed from SPA measurements of tail-bone BMD provided that threshold values indicative of P deficiency are related to LW. Serial measurements of tail-bone BMD would indicate increases or decreases in BMD and improve diagnostic reliability. Based on the results of this study tail-bone BMD as a diagnostic for P deficiency in mature breeders cannot be recommended. Thus we conclude that tail-bone BMD as a diagnostic for identifying P deficiency in cattle should be restricted at this stage to use on growing steers or heifers, and that different threshold values need to be applied to different LW brackets. Furthermore we recommend that diagnosis should be based on a series of measurements

made on a group of cattle during the growing season rather than on a single measurement. The present study indicated that SPA measurement of tail-bone BMD in cattle could provide a valuable non-invasive on-farm tool for investigation of the P status of cattle. Further research and development is warranted to refine and improve the technique.

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