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Mobilisation and replenishment of phosphorus reserves in *Bos indicus* cows. 1. Mid-pregnant mature cows post-weaning

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ABSTRACT

Context. Lactating beef cows grazing phosphorus (P)-deficient pastures often mobilise body P to alleviate a P deficiency. Knowledge of the circumstances when body P is mobilised, and later replenished, is necessary for optimal management of the P nutrition of breeder herds. **Aims.** To investigate mobilisation and replenishment of body P in mature *Bos indicus*-cross beef cows post-weaning. **Methods.** Cows (n = 40) in their second trimester of pregnancy were individually fed *ad libitum* low-P (LP) or high-P (HP) diets containing moderate or high metabolisable energy (ModE-LP, HighE-LP, ModE-HP and HighE-HP) for 14 weeks. **Key results.** Plasma inorganic P concentrations (Pi) in LP- or HP-diet cows (0.58 and 2.15 mmol/L respectively) indicated that diet P was deficient or adequate. Intakes of DM and metabolisable energy, liveweight gain, and P retention were higher ($P < 0.05$ to $P < 0.001$) in cows fed the HP diets, and were also increased in the high metabolisable-energy diets. Efficiency in use of metabolisable energy for slow growth was lower in the HighE-LP than the ModE-HP cows. The cows fed the LP diets mobilised 4–5 g body P/day. Cows fed the Mod-HP and HighE-HP diets retained 1.1 and 8.8 g body P/day, and those ModE-HP cows with low bone P reserves retained ~2.3 g P/day. Rib cortical bone P did not change in HP cows but decreased ($P < 0.05$) in LP cows. The HP diets increased the bone volume, mineralised bone, and the thickness of the struts in trabecular bone in the *tuber coxae*. Osteoid tissue decreased ($P < 0.05$) in HighE-HP cows. Changes in plasma concentrations of 1,25-dihydroxy vitamin D_3, carboxy-terminal telopeptides of type I collagen (CTX-1) and bone alkaline phosphatase (BAP) were in accord with substantial bone mobilisation in cows fed LP diets, and bone replenishment with HP diets. **Conclusions.** Cows that had ingested P-deficient diets during lactation were able to further mobilise body P when fed LP diets post-weaning, but cows replenished body P when fed HP diets. **Implications.** Mature cows with low body P reserves can replenish these reserves more rapidly when consuming diets high in both P and metabolisable energy.

Keywords: bone mineral replenishment, bone phosphorus, diet quality, energetic efficiency, liveweight change, P deficiency, plasma markers, plasma minerals.

Introduction

Many rangelands, particularly in northern Australia, South America, and Africa, have low soil phosphorus (P) concentrations that lead to low P in the pastures. Grazing beef cattle that are entirely dependent on such pastures are often P deficient, particularly during the rainy season when metabolisable energy and protein in pastures are highest and therefore when the demands of the animal for P are also highest (Winter 1988; Kerridge *et al.* 1990; Winks 1990). In herds grazing rangelands, signs of P deficiency are most often observed in lactating breeder cows because of their high demands for P during late pregnancy and lactation (Hart and Mitchell 1965; McCosker and Winks 1994). The most common symptom of P deficiency is reduced animal productivity, although acute and prolonged P deficiency in breeder cows causes osteophagea, poor body condition, low fertility, low bone mineralisation, bone breakages, high mortalities and osteomalacia (Dixon *et al.* 2020*a*; Jackson *et al.* 2023).

During dietary P deficiency in late pregnancy and lactation most ruminants, including beef-genotype cows, can mobilise a substantial proportion (e.g. often >30% and in at least some circumstances up to 45%) of their bone minerals (Benzie *et al.* 1959; Little 1983; Dixon *et al.* 2017, 2020*a*). Cows that mobilise a high proportion of bone minerals during late pregnancy and lactation need to replenish these reserves in preparation for the next reproductive cycle. When beef cows grazing P-deficient pastures are not able to replenish their body P, there are often severe adverse effects on liveweight, lactation, reconception, welfare, and mortality (Read *et al.* 1986*a*, 1986*b*, 1986*c*; Spangenberg 1997; Dixon *et al.* 2017; Schatz *et al.* 2023). Similar adverse outcomes also occur in severely P-deficient dairy cows (Valk and Sebek 1999; Knowlton and Herbein 2002). In seasonally dry rangeland environments where cows typically lactate during the rainy season, it will often be desirable to replenish cow body P reserves by feeding P supplements during the late rainy season and the early dry season when cows are in late lactation or after weaning. However, the effectiveness of P supplementation to replenish cow body P during these seasons is not known.

An experiment investigated the hypothesis that mature pregnant *Bos indicus*-cross breeder cows that had grazed P-deficient pastures during lactation, and were therefore expected to have low bone P reserves at weaning, could (i) continue to mobilise body P to maintain voluntary feed intake and plasma inorganic P (Pi) when fed P-deficient diets, and (ii) replenish body P reserves when consuming diets with either moderate or high metabolisable-energy (ME) content together with additional P surplus to immediate requirements. The experiment examined a high-ME diet (~9.5 MJ ME/kg DM) comparable with high-quality grass or grass–legume pastures during the mid- to late rainy season, versus a moderate-ME diet (~8 MJ ME/kg DM) comparable with grass pastures during the late rainy season and the transition into the dry season.

Materials and methods

Selection and housing of the cows

Mature *Bos indicus* × *Bos taurus*-cross cows (~5/8–3/4 *Bos indicus*, F_n, Droughtmaster type, 6–11 years of age) were selected from a mixed-age herd on the Queensland Department of Primary Industries Spyglass Cattle Research Station (100 km west of Townsville, Queensland, Australia) in the seasonally dry tropics. The herd had grazed tropical northern speargrass native pastures in an open *Eucalyptus* woodland paddock without any supplementation during the 6 months before selection of cows on the 19 June 2014. The paddock primarily comprised soils containing <4 and 4–6 mg Colwell P_B/kg DM (Bryant and Harms 2016) that are classed as 'acutely deficient', and 'deficient' in P respectively, for grazing cattle (Kerridge *et al.* 1990; McCosker and Winks 1994; Jackson *et al.* 2023). The cows had been mated annually from ~2 years of age, and most recently from 15 January to 5 May 2014. The cows were blood-sampled from the coccygeal vein on the 5 May 2014 for measurement of Pi. On the 18 June 2014, the herd was mustered, calves weaned and pregnancy evaluated by rectal palpation. Forty cows were selected as (i) previously lactating through the 2013–2014 rainy season, (ii) currently 2.0–3.5 months pregnant, (iii) in body condition score (BCS) 2.0–3.3 on a 5-point scale (CSIRO 2007), and (iv) temperament suitable for an intensive pen experiment. About one-third of the cows were in each of the Age groups of 5–6, 7–8 and 9–11 years. The experiment was approved by the Queensland Department of Primary Industries Animal Ethics Committee (SA2014/04/464 and SA2014/04/470).

The cows were transported by truck to the Brian Pastures Research Station, Gayndah, in the subtropics of Queensland. After arrival (20 June 2014), the cows were allowed to recover from transport, while held in yards and fed barley straw *ad libitum*, and were blood-sampled on the 23 June 2014. The cows were then fed a low-P diet (the ModE-LP diet described below). On the 26 June 2014, liveweight of the cows was measured, BCS evaluated, the cows (n = 40) were randomly allocated to 10 blocks, and the four cows within each block were randomly allocated to the four treatments. The cows were allocated to 40 individual pens in the animal shed, with the four cows in each block in adjoining pens. The cows were (mean ± s.d. (range)) 430 ± 45 (307–502) kg liveweight, 2.7 ± 0.35 (2.0–3.3) BCS, and fetal age was 12 ± 1.2 (9–14) weeks. Thus, the cows were generally in their second trimester of pregnancy during the experiment. Water was available in each pen. Half of each pen (~33 m^2) with the feed bunk was roofed and with a concrete floor, whereas the remainder was soil. Additional shade was provided with canvas sails.

Diets, total collections of faeces and measurements of liveweight and BCS

The four diet treatments in a 2 × 2 factorial design were as follows: (i) moderate ME, low P (ModE-LP), (ii) moderate ME, high P (ModE-HP), (iii) high ME, low P (HighE-LP), and (iv) high ME, high P (HighE-HP). The main effects of P concentration are referred to as LP or HP diets, whereas the main effects of ME content are referred to as ModE or HighE diets. The diets were prepared as total mixed rations based on coarsely chopped wheat straw, wheat flour and sugar. The HighE diets contained (g/kg as fed) 512 wheat straw, 282 wheat flour and 141 sugar, whereas the ModE diets contained 667 wheat straw, 186 wheat flour and 94 sugar (Table 1). The HP diets contained mono dicalcium phosphate (Kynophos, KK Animal Nutrition Pty Ltd, Umbogintwini, South Africa). The treatment diets were fed daily from the 26 June 2014 (Day 1) for 101 days, and cows were individually offered ~10% in excess of their previous intakes to achieve *ad libitum* intakes. Feed refusals were collected weekly. Cows

Table 1. The ingredients (g as-fed/kg) and the composition (g as-fed/kg for DM concentration and g/kg DM for the other constituents) of the four mixed diets containing low or high concentrations of P (LP and HP) and moderate or high contents of metabolisable energy (ModE and HighE) fed to the cows in pens during the second trimester of pregnancy.

Feed and composition	Treatment diet			
	ModE-LP	ModE-HP	HighE-LP	HighE-HP
Ingredients (g/kg as fed)				
Wheat straw	669	664	515	509
Wheat flour	186	185	283	280
Sugar	94	93	142	140
Canola oil	29	29	22.4	22.1
Urea	11.9	11.8	22.4	22.1
Calcium phosphate[A]	0	6.2	0	9.4
Limestone	3.1	3.1	4.8	4.7
Ammonium sulfate	3.1	3.1	4.8	4.7
Sodium chloride	3.1	3.1	4.8	4.7
Rumigro premix[B]	0.50	0.50	0.77	0.76
Elanco rumensin 100[C]	0.25	0.25	0.27	0.27
Composition				
Dry matter (g/kg as fed)	948	946	947	944
Organic matter (g/kg DM)	912 (5)	911 (3)	928 (4)	927 (6)
Crude protein	67.2 (5.0)	72.4 (2.3)	111 (7.1)	115 (5.8)
Neutral detergent fibre	593 (12)	577 (29)	432 (28)	427 (26)
Acid detergent fibre	374 (30)	372 (8)	284 (19)	279 (15)
Lignin	51 (10)	44 (11)	34 (5)	37 (6)
Starch	86 (9)	95 (6)	189 (13)	193 (8)
Crude fat	29 (1.9)	28 (2.2)	33 (2.9)	31 (2.7)
Calcium	3.02 (0.335)	4.30 (0.868)	2.96 (0.332)	4.74 (0.530)
Phosphorus	0.68 (0.071)	1.58 (0.356)	0.76 (0.057)	2.11 (0.282)
Magnesium	0.91 (0.05)	0.95 (0.06)	0.72 (0.06)	0.83 (0.01)
Sulfur	2.0 (0.36)	1.8 (0.32)	2.3 (0.33)	2.0 (0.16)
Calcium:phosphorus	4.5 (0.50)	2.9 (0.20)	3.9 (0.23)	2.2 (0.13)

Mean and s.d. (in parenthesis) of the constituents ($n = 5$).
[A]Mono dicalcium phosphate; Kynophos, KK Animal Nutrition Pty Ltd, Umbogintwini, South Africa).
[B]BEC, Brisbane Export Corporation, Brisbane, Qld, Australia.
[C]100 g/kg monensin as sodium monensin, Elanco, Sydney, NSW, Australia.

were introduced to the treatment diets over 4 days, during which additional straw was added to the diet to minimise the risk of acidosis. Water was freely available from troughs in each pen.

Cow liveweight was measured, and cow BCS evaluated by the same experienced person, weekly. Voluntary intake of feed was measured weekly from the amounts offered and refused. The feed offered was sampled when each batch of diet was mixed, and feed refusals were sampled weekly from each pen. During 5-day total collections the faeces were collected frequently from the concrete floors during Weeks 3, 8 and 14, while the animals were constrained to the section of the pen with concrete flooring. The feed offered and refused during the total collection intervals were bulked and sampled for each cow. A 10% subsample of the faeces excreted each day was stored at −20°C, and at the end of the collection interval, these samples were mixed and subsampled. Feed offered and refused during other intervals of the experiment were bulked monthly. Feed and faeces samples were dried at 60°C, ground through a 1 mm screen (Christie and Norris Mill, Chelmsford, UK) and retained for analysis. Hip height of the cows was measured at the start and at the end of the experiment. The depth of fat at the P8 and rib sites, and the eye-muscle area were measured at Weeks 1 and 14 by ultrasound (Esaote Pie Medical Aquila with a 3.5 MHz linear array transducer; Pie Medical Imaging, Maastricht, The Netherlands).

Sampling of blood, urine and bone

Blood samples were obtained on the 23 June 2014 (Day −3), on the 30 June 2014 (Day 5) and then at fortnightly intervals, by jugular venipuncture using vacutainers containing lithium–heparin (BD, Diagnostics, Plymouth, UK). Samples were chilled in iced water, plasma was separated by centrifugation (3000*g*, 15 min at ambient temperature), and stored at −20°C. Urine samples (~30–60 mL) collected by stimulation of the vulva on days immediately preceding and following the total collection at Week 14 were acidified (pH < 3) with 4 M sulfuric acid, and stored at −20°C for subsequent analysis of creatinine, purine derivatives and P. Biopsy samples of outer cortical bone from the 12th rib, and from the *tuber coxae* bone, were obtained at commencement (Days 2–4) and from the opposite side of the cow near the end of the experiment (Days 93–95) (Little 1972; Kidd *et al.* 2014; Silva *et al.* 2021). The procedures used to obtain the bone biopsies are described in Supplementary Appendix S1.

Laboratory procedures

Organic matter was determined as the DM minus the ash measured by incineration (550°C for 8 h). Concentrations of P, calcium (Ca), magnesium (Mg) and sulfur (S) in the feed offered, feed refusals and faeces from the total collection intervals, were analysed using an inductively coupled plasma spectrometer (Optima 7300 DV, Perkin Elmer, Waltham, MA, USA) after nitric-perchloric acid digestion. Samples of feed offered were analysed for total nitrogen, neutral detergent fibre, acid detergent fibre, lignin and crude fat by near-infrared spectroscopy (NIRS) and using in-house calibrations (Dairy One, Ithaca, New York, USA). Purine derivatives (allantoin and uric acid) and creatinine in urine were measured by high-performance liquid chromatography (George *et al.* 2006).

Samples of rib cortical bone were scraped to remove any trabecular bone. Cortical bone thickness was measured

using vernier callipers and specific gravity was measured gravimetrically. The concentration of P (mg P/mL) in rib cortical bone was calculated as follows (Dixon *et al.* 2019*a*):

$$\text{P concentration} = 229 \times (\text{specific gravity}) - 261.$$

The P in cortical rib bone per unit surface area of cortical bone (PSACB, μg P/mm^2) index was calculated as the product of P concentration and the thickness of the cortical bone (Dixon *et al.* 2019*a*). Samples of cortical rib bone and *tuber coxae* trabecular bone for histology were fixed in 10% neutral buffered formalin for at least 8 weeks, decalcified in 14% EDTA (pH 7.0) for at least 12 weeks (Callis and Sterchi 1998), and then embedded in paraffin. Sections (5 μm) were stained with Masson's trichrome, photographed on a microscope at ×2, ×10 and ×20 magnification. Measured attributes and changes were described following the nomenclature of Dempster *et al.* (2013). An image-analysis program (ImageJ; Doube *et al.* 2010; Schneider *et al.* 2012) was used to measure porosity (void volume:tissue volume; Po) in the rib cortical bone. The latter was expressed as (100-Po) so that increasing values corresponded with increasing amounts of bone in parallel with the measurements of P concentration in cortical rib bone. In *tuba coxae* bone, the measurements were the proportions of bone volume to total volume, osteoid to total bone volume, mineralised bone to total bone volume, mean trabecular strut thickness and maximum trabecular strut thickness. Mineralised bone and oestoid together represented the total bone volume. Changes were calculated from the measurements at the beginning and the end of the experiment.

Plasma samples were analysed calorimetrically for inorganic P (Pi), total Ca, and Mg by using a Beckman Coulter AU680 analyser (OSR6122, OSR 6189 and OSR6189 assay kits respectively), whereas the concentration of P in urine was analysed as described by Goodwin (1970). Plasma concentrations of a number of hormones and bone metabolism markers were determined using commercial assay kits as follows: parathyroid hormone (PTH; A11930, Beckman Coutler), 25-dihydroxy vitamin D_3 and 1,25-dihydroxy vitamin D_3 (AA-35F1 and AA-54F2, Immunodiagnostic Systems), insulin-like growth factor 1 (IGF-1; A15729, Beckman Coulter), carboxy-terminal telopeptides of type I collagen (CTX-1; Crosslaps AC-02F1, Immunodiagnostic Systems), bone alkaline phosphatase (BAP; MicroVue 8012, Quidel), and osteocalcin (OCN; MicroVue 8002, Quidel). Total alkaline phosphatase (ALP) and aspartate aminotransferase (AST) were analysed with kits (Beckman Coulter) on the basis of recommendations of the International Federation for Clinical Chemistry. Further details of these assays conducted in the same laboratory are given by Silva *et al.* (2021).

Calculations and statistical analyses

The age of the fetus at each measurement during the experiment was calculated from the actual date of parturition and assuming a 284 day gestation. The conceptus (gravid uterus) weight was calculated as described by O'Rourke *et al.* (1991) and the conceptus-free cow liveweight (CF-LW) was calculated by difference between the measured LW of the cow minus the estimated weight of the gravid uterus. The LW change of the cows was calculated as the difference between the initial and final LW.

The measured intakes of total P in each diet treatment were compared with the calculated P requirements of the cows (CSIRO 2007) (i) for the measured DM intakes, LWs and LW gains of the cows in the treatment, and also (ii) for the LP treatments, the P intakes were compared with the P requirement for the higher DM intakes and higher LW gains of the cows in the corresponding HP diet. The latter calculation allowed for the reduction in voluntary intakes during P deficiency, lower LW, and lower LW gain of cows fed the LP treatment diets. The amounts of absorbed P ingested in each of the diets were calculated as described by CSIRO (2007), (i) adopting their recommended true absorption coefficient of 0.70, and also (ii) when adopting a true absorption coefficient of 0.90 for the P as mono dicalcium phosphate included in the HP diets (Dixon *et al.* 2020*a*). Apparent digestibilities of DM and organic matter were calculated by conventional procedures. The ME contents of the diets (MJ ME/kg DM) were calculated from the organic-matter digestibility (OMD) measured during the total collections as follows (CSIRO 2007, p. 8):

$$\text{ME} = 0.169(\text{OMD}\%) - 1.986.$$

The volume of urine excreted at the Week 14 total collection was calculated from the creatinine concentration in urine and assuming a daily creatinine excretion of 0.91 mmol/kg $W^{0.75}$.day (Chen *et al.* 1995). Microbial crude protein synthesis was calculated from the excretion of purine derivatives (Chen and Gomes 1992), assuming an endogenous purine derivative excretion of 0.190 mmol/kg W^{075}.day (Bowen *et al.* 2006). The P retention was calculated as the difference between intake and faecal excretion of P during each of the total collection intervals.

Statistical analysis of the results was conducted by ANOVA using Genstat (release 16.1, VSN International, Hemel Hemstead, UK). Measurements of the animals at the commencement of the experiment (cow LW, BCS and age, fetal age, rib cortical bone thickness, P concentration and PSACB) were examined as potential covariates and were included in the statistical model when significant ($P < 0.05$). Repeated-measures ANOVA in a 2 × 2 factorial design with main effects of diet energy and diet P, time, and the interactions, was used for analyses of intakes, digestibilities, P retentions, blood minerals, hormones and bone metabolism markers. Measurements of the metabolites PTH, 25-dihydroxy vitamin D_3, 1,25-dihydroxy vitamin D_3, ALP, AST and IGF-1 were transformed ($\log(X + 1)_{10}$) to address heterogeneity of variance. The effects of the diet treatments at individual

time points were compared *post hoc* using Fishers protected least significant difference (l.s.d.) tests. In addition, a one-tailed *t*-test was used to examine whether changes in P retention, and in attributes of rib and *tuba coxae* bone, were greater than, or less than, zero in the HP and LP diets respectively.

Results

Initial status of the cows and the intakes of P provided by the treatment diets

The Pi measured on 5 May 2014 in the cows selected for the experiment when they were grazing late rainy–early dry transition-season native pastures was (mean ± s.d. (range)) 1.22 ± 0.48 (0.4–2.6) mmol/L. After arrival at Brian Pastures Research Station and having been fed barley straw for 3 days, the Pi was 1.78 ± 0.41 (1.02–2.73) mmol/L, plasma Ca 2.23 ± 0.41 mmol/L, and the ratio plasma Ca:Pi 0.81 ± 0.41. The diet treatments were commenced 3 days later. The initial cortical bone thickness was 3.73 ± 0.70 (2.70–4.99) mm, the P concentration was 149 ± 9.4 (130–162) mg P/mL, and the PSACB was 556 ± 116 (352–783) μg P/mm^2. In *tuber coxae* trabecular bone, the initial bone volume/total volume averaged 8.68 ± 2.60 (4.72–15.91)%, mineralised volume/total volume 7.08 ± 2.28 (4.16–14.60)%, osteoid volume/total volume 1.80 ± 1.27 (0.20–5.03)%, and trabecular strut thickness 107 ± 12.8 (91–156) μm.

The total P concentrations in the ModE-LP and HighE-LP diets were 0.68 and 0.76 g P/kg DM, and in the ModE-HP and HighE-HP diets 1.58 and 2.11 g P/kg DM respectively (Table 1). The crude protein (CP) to ME ratio, calculated from values in Tables 1 and 2, ranged from 7.9 to 12.2 g CP/MJ ME. Calculations of the P requirements based on an adopted true absorption coefficient of 0.70 indicated that the ModE-HP and HighE-HP diets provided 0.99 and 1.10 of P requirements respectively (Table 2). When a true absorption coefficient of 0.90 was adopted for mono dicalcium phosphate, the ModE-HP and HighE-HP diets provided 1.15 and 1.30 of the absorbed P requirements respectively. The ModE-LP and HighE-LP diets both provided 0.50 of both the total and absorbed P requirements for the measured LW gains, but provided only 0.36 and 0.27 of the P intakes required to achieve the higher intakes and LW gains measured in the respective HP diets, as limited by animal factors and diet other than P. Therefore, the two LP diets were acutely P deficient and the two HP diets provided in excess of the estimated requirements. Intakes of Ca ranged among the diets from 1.3 to 1.8 of the requirements.

Intake, diet digestibility, LW change and rumen microbial synthesis

The cows were all in good health and no signs of osteomalacia such as abnormal gait were observed through the experiment. The voluntary intakes of DM and organic matter by the cows were increased ($P < 0.001$) by both higher ME and higher P contents of the diets, and there was an ME × P interaction ($P < 0.001$) (Table 2). Voluntary intake of the moderate-ME diet was increased 11% by additional P in the diet (13.4 and 14.9 g DM/kg LW in the ModE-LP and ModE-HP diets respectively; $P < 0.05$), whereas voluntary intake of the high-ME diet was increased by 36% by additional P (15.8 and 21.5 g DM/kg LW.day in the HighE-LP and HighE-HP diets respectively; $P < 0.001$). The voluntary DM intakes of the ModE-HP and the HighE-LP diets were similar ($P > 0.05$). Voluntary DM intake decreased ($P < 0.001$) by, on average, 0.33 g DM/kg LW.week in each of the diets through the experiment.

Organic matter digestibility was consistently higher in the high-ME diets than in the moderate-ME diets, averaging 676 and 596 g/kg respectively ($P < 0.001$) (Table 2). There was an ME × P interaction effect on the OMD ($P < 0.01$); OMD of the ModE-HP diet was lower ($P < 0.05$) than of the ModE-LP diet (572 and 619 g/kg respectively, and the OMDs of both of the moderate-ME diets were lower than those of the high-ME diets (mean 676 g/kg). Also the OMD was lower at the total collection at Week 3 than at Weeks 8 and 14 ($P < 0.001$). Voluntary intakes of ME through the 14 weeks averaged 112 and 115 kJ ME/kg LW.day for the ModE-LP and ModE-HP treatments, and increased ($P < 0.001$) to 148 and to 204 kJ ME/kg LW.day for the HighE-LP and HighE-HP diets respectively (Table 2). Microbial CP (MCP) synthesis per day was similar in the ModE-LP, ModE-HP and HighE-LP diets (354–387 g MCP/day), and was higher in the HighE-HP diet (573 g MCP/day; $P < 0.05$), in association with the higher ME intake. However, the efficiency of microbial synthesis was higher for the moderate-ME diets than for the high-ME diets (means 8.04 and 6.56 g MCP/MJ ME; $P < 0.05$).

Phosphorus intakes, excretion and retention

The average intakes of P during the three total collection intervals were 2.9 and 3.7 g P/day (7.1 and 9.0 mg P/kg LW.day) for the LP diets, and 10.0 and 20.2 g P/day (22.8 and 43.5 mg P/kg LW.day) for the HP diets (Table 3). Mean faecal P excretion ranged from 6.8 to 12.0 g P/day. Urinary P excretion at the total collection in Week 14 was negligible (≤0.1 g P/day; data not shown) and, therefore, the differences between intake and faecal excretion were used as the measure of P retention. The P retention was not significantly affected by the total collection interval and averaged −4.1 and −4.7 g P/day (−9.3 and −11.2 mg P/kg LW.day) in the ModE-LP and HighE-LP diets respectively, indicating that these amounts of body P were being mobilised. In contrast, cows fed the ModE-HP diet tended to be in a small positive P retention (+1.1 g P/day; +2.5 mg P/kg LW.day), although this was not different ($P > 0.05$) from zero. The cows fed the HighE-HP diet retained 8.8 g P/day (18.4 mg P/kg LW.day).

Table 2. Measurements of the intakes of total P and estimates of absorbed P in relation to the P requirements of the cows. In addition, measurements are given of the voluntary intakes of DM, diet in vivo organic-matter digestibility (OMD) at three total collection intervals (Weeks 3, 8 and 14), estimated metabolisable energy (ME) content of the diet and estimated ME intake, and microbial crude protein (CP) synthesis in mature cows fed four diets for 14 weeks during mid-pregnancy ($n = 10$). The fat depth measured at the rump and rib sites and eye muscle area (EMA) and the changes in these measurements are also given.The diets were moderate-ME (ModE) or high-ME (HE) diets containing low (LP) or high (HP) concentrations of phosphorus (P). The fat depth measured at the rump and rib sites and eye-muscle area (EMA), and the changes in these measurements, are also given. P intake/requirement-A, P intake/requirement-B, the P required to provide for the cows at their measured liveweight gain (A), or for the LP diet treatments at the measured growth rate for the HP diets where liveweight gain was not constrained by the availability of P (B).

Measurement	Diet treatment				s.e.m. (df 19)	Cov	Probability			
	ModE-LP	ModE-HP	HighE-LP	HighE-HP			Energy (ME)	Phos (P)	ME × P	Time (T)
P intake and requirements										
Total P intake (g P/day)	3.7	10.3	5.2	21.4	–	–	–	–	–	–
Total P requirement (g P/day)	7.5	10.4	10.4	19.5	–	–	–	–	–	–
Total P intake/requirement-A	0.50	0.99	0.50	1.10	–	–	–	–	–	–
Total P intake/requirement-B	0.36	–	0.27	–	–	–	–	–	–	–
Absorbed P intake (g P/day)	2.6	8.4	3.6	17.7	–	–	–	–	–	–
Absorbed P requirement (g P/day)	5.3	7.3	7.3	13.6	–	–	–	–	–	–
Absorbed P intake/requirement-A	0.50	1.15	0.50	1.30	–	–	–	–	–	–
Absorbed P intake/requirement-B	0.36	–	0.27	–	–	–	–	–	–	–
Voluntary intakes										
DM intake (kg/day)	5.47c	6.54b	6.83b	10.14a	0.15	1	<0.001	<0.001	<0.001	<0.001
DM intake (g/kg LW.day)	13.4c	14.9b	15.8b	21.5a	0.30	1	<0.001	<0.001	<0.001	<0.001
OMD Week 3 (g/kg)	618	516	676	622	13	–	<0.001	<0.001	n.s.	–
OMD Week 8 (g/kg)	629	622	695	725	9	–	<0.001	n.s.	n.s.	–
OMD Week 14 (g/kg)	610c	579d	645b	690a	7	–	<0.001	n.s.	<0.001	–
OMD Mean (g/kg)	619b	572c	672a	680a	7	–	<0.001	0.06	<0.01	<0.001
ME content (MJ/kg)	8.47b	7.68c	9.37a	9.46a	0.12	–	<0.001	n.s.	<0.05	–
ME intake (MJ/day)	45.8c	50.7c	64.2b	96.0a	1.42	1	<0.001	<0.001	<0.001	–
ME intake (kJME/kg LW.day)	112c	115c	148b	204a	2.7	2	<0.001	<0.001	<0.001	–
Microbial CP (g/day)	354b	358b	387b	573a	17.4	–	<0.001	<0.001	<0.001	–
Microbial CP/ME (g/MJ ME.day)	7.77	8.30	6.78	6.34	0.40	1	<0.05	n.s.	n.s.	–
Liveweight and BCS										–
Initial LW (kg)	434	409	414	421	8.9	–	n.s	n.s.	n.s.	–
LW change (kg)	+6c	+29b	+26b	+97a	3.4	–	<0.001	<0.001	<0.001	–
LW change (kg/day)	0.07c	0.32b	0.29b	1.05a	0.037	–	<0.001	<0.001	<0.001	–
Initial BCS	2.6	2.7	2.7	2.7	0.07	–	n.s.	n.s.	n.s.	–
BCS change	−0.10c	0.25b	0.27b	1.25a	0.08	–	<0.001	<0.001	<0.01	–
Ultrasound measurements										–
Initial rump fat (mm)	1.5	2.3	2.0	1.7	0.25	–	n.s.	n.s.	n.s.	–
Change in rump fat (mm)	0.5c	0.5c	2.3b	8.7a	0.32	2	<0.001	<0.001	<0.001	–
Initial rib fat (mm)	1.4	1.5	1.6	1.3	0.15	–	n.s.	n.s.	n.s.	–
Change in rib fat (mm)	−0.2c	0.2bc	0.9b	4.4a	0.23	–	<0.001	<0.001	<0.001	–
Initial EMA (mm^2)	50.5	53.5	53.5	56.4	1.83	–	n.s.	n.s.	n.s.	–
Change in EMA (mm^2)	−3.2b	0.9b	0.7b	14.3a	1.01	–	<0.001	<0.001	<0.01	–

Means in the same row with different lower-case letters are significantly different (at $P \leq 0.05$), and the means in the same column, within the same measurement, with different upper-case letters are significantly different (at $P \leq 0.05$).
LW, liveweight; BCS, body condition score; cov, covariates: 1, cow initial liveweight; 2, cow age. ME × P, The interaction between metabolisable energy and phosphorus in the diet.

Table 3. Measurements of the intakes of P, faecal excretion of P and P retention (intake – faecal excretion) during three total collection intervals (Weeks 3, 8 and 14) and their mean. Urinary P excretion was negligible. The mature cows were fed moderate-ME (ModE) or high-ME (HE) containing low (LP) or high (HP) concentrations of phosphorus (P) for 14 weeks during their second trimester of pregnancy ($n = 10$).

Measurement	Diet treatment				cov	s.e.m. (d.f. 19)	Probability			
	ModE-LP	ModE-HP	HighE-LP	HighE-HP			Energy (ME)	Phos (P)	ME × P	Time (T)
P intake										
Week 3 (g/day)	3.1a	9.4bA	4.0c	17.3a	1, 2	0.36	<0.001	<0.001	<0.001	–
Week 8 (g/day)	3.4a	9.8bAB	3.0a	21.2cB	1, 2	0.51	<0.001	<0.001	<0.001	–
Weel 14 (g/day)	2.3a	11.0cB	4.1b	22.8d	1	0.32	<0.001	<0.001	<0.001	–
Mean (g/day)	2.9c	10.0b	3.7c	20.2a	1, 2	0.51	<0.001	<0.001	<0.001	<0.001
Faecal P excretion										
Week 3 (g/day)	5.7a	7.3a	6.8aA	10.6bA	–	0.60	<0.05	<0.01	<0.05	–
Week 8 (g/day)	7.2a	8.6ab	10.3bcB	11.7cAB	–	1.25	n.s.	n.s.	<0.05	–
Week 14 (g/day)	7.6a	9.9a	8.4aAB	13.7bB	–	1.30	n.s.	0.06	<0.05	–
Mean (g/day)	6.8a	8.6a	8.5a	12.0b	3	1.05	<0.05	<0.05	<0.05	<0.05
P retention										
Week 3 (g/day)	−2.8a	+1.7b	−2.8a	+7.2c	–	0.60	<0.01	<0.001	<0.01	–
Week 8 (g/day)	−3.9ab	+0.8b	−7.2a	+9.9c	–	1.32	n.s.	<0.001	<0.01	–
Week 14 (g/day)	−5.5a	+0.7b	−4.2a	+9.5c	–	1.69	<0.01	<0.001	0.05	–
Mean (g/day)	−4.1a	+1.1b	−4.7a	+8.8c	2	1.21	<0.01	<0.001	<0.01	n.s.
P intake, mean (mg/kg LW.day)	7.1a	22.8b	9.0a	43.5c	2	1.07	<0.001	<0.001	<0.001	<0.001
Faecal P, mean (mg/kg LW.day)	16.1	20.6	20.3	25.1	–	1.87	n.s.	0.09	n.s.	<0.001
P retention, mean (mg/kg LW.day)	−9.3a	+2.5b	−11.2a	+18.4c	–	2.67	<0.01	<0.001	<0.01	n.s.

Means in the same row with different lower-case letters are significantly different (at $P < 0.05$), and the means in the same column, within the same measurement, with different upper-case letters are significantly different (at $P < 0.05$).
LW, liveweight; cov, covariates: 1, cow age; 2, cow initial liveweight; 3, cow initial BCS; ME × P, the interaction between metabolisable energy and phosphorus in the diet.

The rates of LW gain of cows in each of the treatments were consistent throughout the 14 weeks. The ModE-LP diet cows were at about LW maintenance (+0.07 kg LW/day) (Table 2). The inclusion of P (ModE-HP diet) significantly ($P < 0.05$) increased LW gain to 0.32 kg LW/day, similar to that of cows fed HighE-LP (0.29 kg LW/day). The HighE-HP diet resulted in a large increase ($P < 0.05$) in growth, to 1.05 kg LW/day. Thus, provision of adequate diet P increased the LW gain much more in cows fed the high-ME diet, as described above. The ME intake was 29% higher for the HighE-LP diet than for the ModE-HP (148 and 115 kJ ME/kg LW.day; $P < 0.05$), even though the LW gains were similar (Table 2). Therefore, ingested ME was used with higher efficiency in cows fed the P-adequate diet than in those fed the P-deficient diet. Because the conceptus was estimated to grow, on average, from 4 to 16 kg during the experiment, the gains in total LW of the cows were ~0.13 kg/day higher than were the gains in CF-LW.

There were few changes during the experiment, or differences between the ModE-HP and the two LP treatments in BCS, fat depth at the rump and rib sites, and eye-muscle area (EMA, Table 2), although each of these attributes increased ($P < 0.05$) in cows fed the HighE-HP diet. Hip height of the cows (initially 1380 ± 43 mm) did not change ($P > 0.05$).

Changes in rib and *tuber coxae* bone

The cortical thickness of rib bone was maintained in cows fed the HP diets, but decreased, on average, by 12% (−0.44 mm) in cows fed LP diets ($P < 0.05$) (Table 4). The P concentration in the cortical bone was not affected by diet P, but decreased in the cows fed the high-ME diets by 4% (10 mg P/cc; $P < 0.01$). The PSACB, which estimates the amount of P in cortical rib bone, did not change through the experiment in cows fed the high-ME diet ($P > 0.05$). However, the PSACB decreased by 14% (77 mg P/mm^2) in LP-diet cows ($P < 0.05$), in association with changes in cortical bone thickness rather than in P concentration. Furthermore, across all the treatments, the change in PSACB was correlated with the initial PSACB (Fig. 1*a*), indicating that cows with initial higher PSACB mobilised more bone. Rib cortical bone volume expressed as (100-Po) indicated that the HP-diet cows gained bone, whereas the LP-diet cows lost bone (1.57% increase and 1.86% decrease respectively; $P < 0.05$) (Table 4), with these increases and decreases both different from zero ($P < 0.05$). Across all the treatments, the change in 100-Po was correlated with the initial (100-Po) (Fig. 1*b*), indicating that cows with initial higher (100-Po) mobilised more bone. The relationships for the PSACB (Y1 and X1) and for (100-Po) (Y2 and X2) were as follows:

Table 4. Measurements in cortical rib bone of the bone thickness, P concentration, phosphorus per unit surface area of cortical bone (PSACB) and the cortical bone volume (100-porosity of cortical bone; 100-Po), and the changes in these measurements during the experiment.

Measurement	Diet treatment				s.e.m. (df 19)	Cov	Probability		
	ModE-LP	ModE-HP	HighE-LP	HighE-HP			Energy (ME)	Phos (P)	ME × P
n	10	10	9	8	–	–	–	–	–
Cortical bone thickness (mm)									
Initial	3.76	3.98	3.54	3.66	0.146	–	n.s.	n.s.	n.s.
Final	3.33	3.61	3.28	3.83	0.135	1	n.s.	<0.05	n.s.
Change	−0.41	−0.13	−0.46	+0.10	0.135	1	n.s.	<0.05	n.s.
P concentration (μg P/mL)									
Initial	150	148	149	148	2.4	–	n.s.	n.s.	n.s.
Final	149	152	137	142	2.3	2	<0.01	n.s.	n.s.
Change	−1	+4	−12	−7	2.3	2	<0.01	n.s.	n.s.
PSACB (μg P/mm^2)									
Initial	565	591	527	533	–	–	–	–	–
Final	502	572	436	549	22	3	n.s.	<0.05	n.s.
Change	−63	−19	−91	+16	20	3	n.s.	<0.05	n.s.
Cortical bone volume (100-Po) (%)									
n	9	8	8	6	–	–	–	–	–
Initial	97.0	96.3	97.0	95.4	–	–	–	–	–
Final	96.6	98.1	93.8	96.6	0.57	4	<0.05	<0.05	n.s.
Change	−0.4	+1.8	−3.2	+1.2	0.58	4	0.09	<0.001	n.s.

Covariates (cov): 1, initial cow cortical bone thickness; 2, initial P concentration; 3, initial PSACB; 4, cow age. ME × P, the interaction between metabolisable energy and phosphorus in the diet.

(a) Y1 = 132–0.321 (X1) (n = 36, r = 0.38, P < 0.05). (b) Y2 = 79.3 – 0.825 (X2) (n = 30, r = 0.49, P < 0.01). In addition, there was a negative correlation between the change in (100-Po) and cow age (r = −0.39, n = 32, P < 0.05), indicating that bone replenishment occurred more slowly in older cows.

In *tuber coxae* trabecular bone, there was, as given above, high variation in the initial bone volume, mineralised bone volume and osteoid tissue in proportion to total bone volume. The variation in average trabecular strut thickness (107 ± 13 μm) was much lower than the variation in the maximum trabecular strut thickness (263 ± 65 μm). Examples of the histology of trabecular bone in representative cows from each diet treatment after 14 weeks are shown in Fig. 2. None of these attributes of trabecular bone was correlated with the PSACB of rib cortical bone. Changes in the attributes of trabecular bone were due to diet P rather than diet ME (Table 5). The bone volume:total volume increased by 36% in HP-diet cows (P < 0.05), but there was no change in LP-diet cows. Cows initially with low trabecular bone volume:total volume replenished more bone, whereas those initially with high bone volume:total volume mobilised more bone. Mineralised bone increased (P < 0.01) by 39% by the HP diets, but was not affected by diet ME. There were large changes in the amount of osteoid tissue and there was a diet ME × P interaction (P < 0.01). Osteoid volume increased by 51% in the HighE-LP diet cows and by 40–41% in the ModE-LP and ModE-HP diet cows, but was decreased by 55% in the HighE-HP diet cows. The cows fed the HighE-HP diet had thicker and more numerous trabecular struts than did those fed the ModE-LP diet (Fig. 2). The high proportion of osteoid and, therefore, poor bone mineralisation in the cows fed the LP diets (Table 5) occurred to a lesser extent in the cows fed the ModE-HP diet. Only in the cows fed the HighE-HP diet was there sufficient P substrate to allow mineralisation of the new bone formation. The average thickness of trabecular struts was increased by HP (P < 0.05) by 24%, and there was also a tendency (P = 0.07) for an increase in the maximum thickness of these struts. Furthermore, across all the treatments, the change in bone volume:total volume (Y3) was related to the initial bone volume:total volume (X3) (Fig. 1*c*). The initial mean thickness of trabecular struts (Y4) was related to the change in the mean thickness (X4) (Fig. 1*d*), and also the initial maximum thickness of trabecular struts was related to correlated the change in the maximum thickness (Fig. 1*e*) These regression relationships were as follows: Fig. 1*c*: (c) Y3 = 10.7 – 1.07 (X3) (n = 36, r = 0.64, P < 0.001); Fig. 1*d*: Y4 = 92 – 0.729 (X4) (n = 36, r = 0.34, P < 0.05); Fig. 1*e*: Y5 = 337 – 1.08 (X5) (n = 36, r = 0.65, P < 0.001).

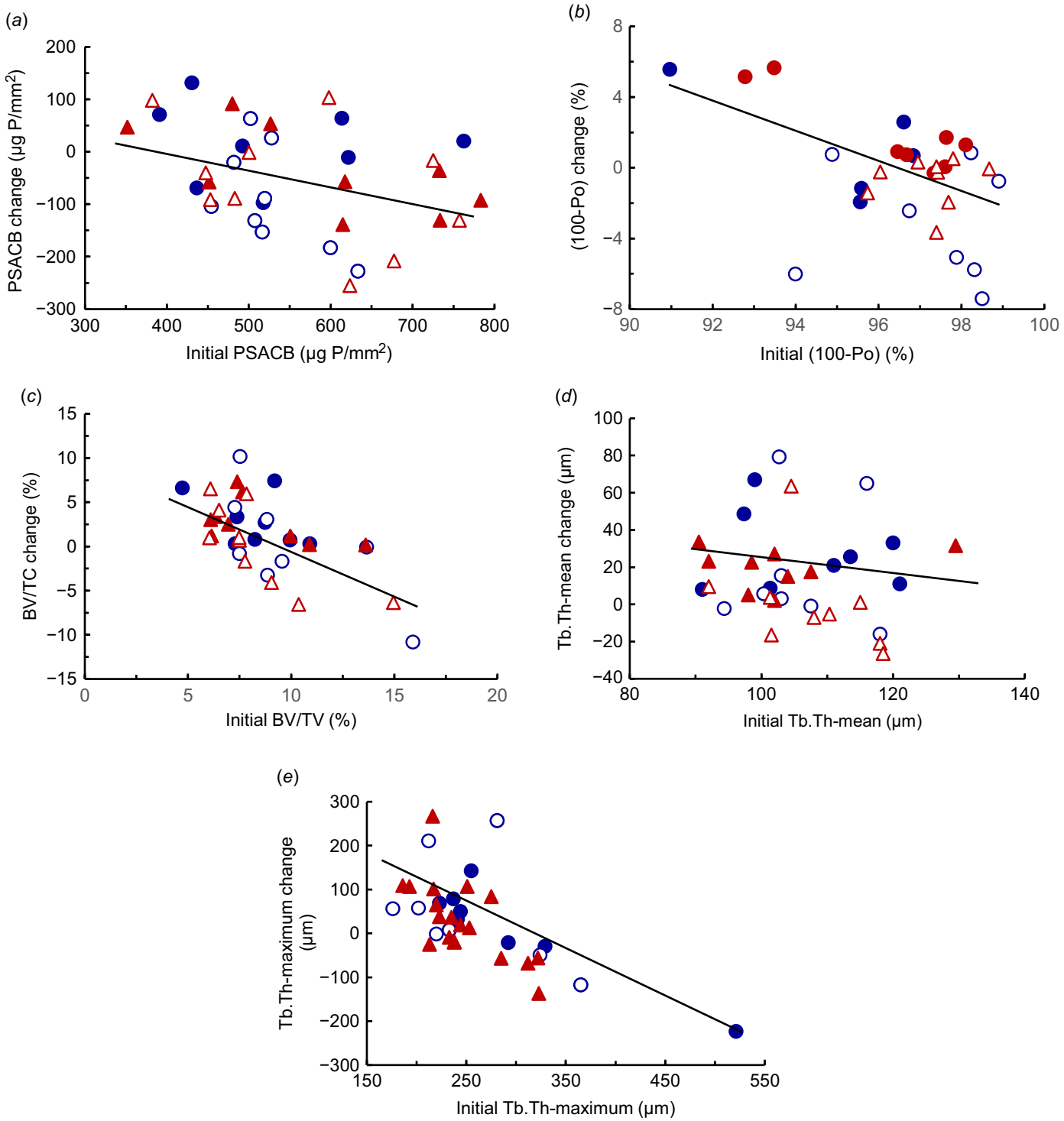

Fig. 1. (*a*) The relationship between the initial P per unit surface area of cortical bone (PSACB) and the change in PSACB in cortical rib bone, and (*b*) the relationship between the initial (100-Po) and the change in (100-Po). (*c*) Measurements in trabecular bone *tuber coxae* of the initial bone volume:total volume (BV:TV) and the change in BV:TV, (*d*) the initial mean thickness of trabecular struts and the change in this mean thickness, and (*e*) the initial maximum thickness of trabecular struts and the average change in this maximum thickness, during the experiment are shown. Cows were fed four diets comprising ModE-LP (△), ModE-HP (▲), HighE-LP (○), and HighE-HP (●).

The measurements showed that HP diets led to large increases in formation of trabecular bone, and that the gains or losses in individual cows were closely related to the amounts of bone at the commencement of the experiment.

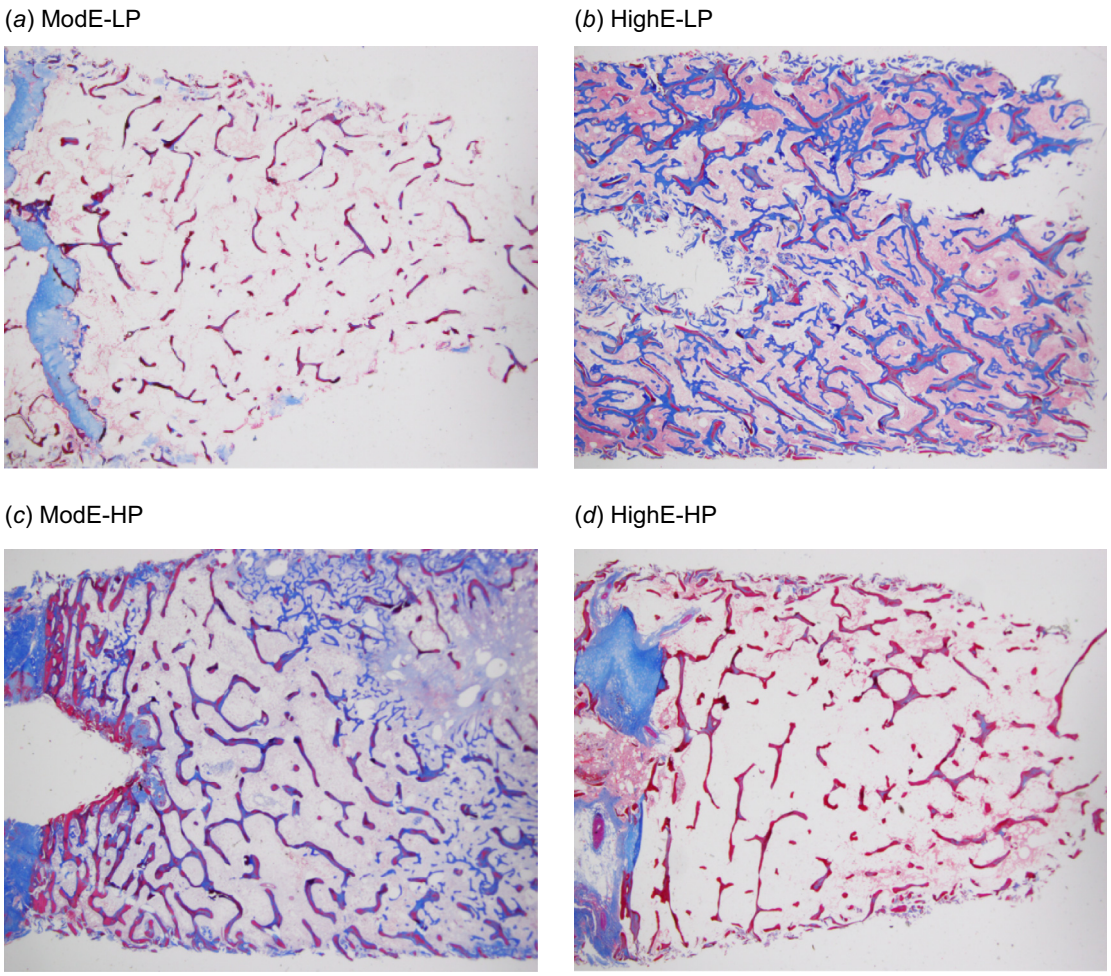

Fig. 2. Images of stained trabecular bone from the *tuber coxae* in the mature cows fed the diet treatments. The mineralised bone is shown in red, the unmineralised bone and osteoid are shown in blue. The cows were fed four diets either moderate or high in metabolisable energy and either with low or high P concentration in a 2 × 2 factorial design. (*a*) ModE-LP; (*b*) HighE-LP; (*c*) ModE-HP; and (*d*) HighE-HP.

Plasma P, Ca and Mg concentrations

The Pi decreased rapidly when the LP diets were fed, and by Day 5, was lower ($P < 0.05$) in the LP than in the HP-diet cows (0.83 and 1.40 mmol/L respectively; $P < 0.001$) (Fig. 3*a*). There were effects of diet P over time on Pi ($P < 0.001$). The Pi in both LP diets continued to decrease ($P < 0.05$) and averaged 0.58 mmol/L during Weeks 10–14, whereas in the HP diets, Pi gradually increased and averaged 2.15 mmol/L during these weeks. The decrease in Pi at Week 8 in the HP diets coincided with a total faecal collection. There was no effect of the ME content of the diet on Pi ($P > 0.05$).

Mean plasma Ca concentrations were 2.23 ± 0.20 mmol/L before treatments commenced and the LP and HP diets generally showed opposite responses to the Pi, although through only a narrower range (Fig. 3*b*). The diet $P \times$ diet ME interaction approached significance ($P = 0.06$). By Week 3, the plasma Ca was higher in the LP- than in the HP-diet cows (means 2.26 and 2.00 mmol/L respectively; $P < 0.001$), and was higher at all sampling times thereafter. In cows fed the LP diets, the plasma Ca averaged 2.38 mmol/L during Weeks 10–14. There was no effect ($P > 0.10$) of diet ME on plasma Ca. Plasma Mg concentrations (data not shown) followed the same pattern as plasma Ca, with similar trends and differences between the LP and HP diets. The plasma Ca:Pi ratio averaged 0.81 ± 0.24 before treatments commenced and by Day 5 was higher ($P < 0.001$) in the LP than in the HP diets (Fig. 3*c*). There were diet P effects over time on the Ca:Pi ratio ($P < 0.001$). The plasma Ca:Pi ratio during Weeks 10–14 averaged 3.5 and 4.3 in cows fed the ModE-LP and HighE-LP diets respectively, compared with 1.1 in the two HP diets.

Endocrine and bone markers

Plasma parathyroid hormone (PTH) concentrations were highly variable within treatments, particularly before and shortly after the diet treatments commenced (Fig. 4). Three

Table 5. Measurements in *tuber coxae* biopsies of bone volume to total volume (BV:TV), osteoid as a proportion of total volume (OV:TV), mineralised bone as a proportion of total bone (Mb.V:TV) trabecular strut thickness (Tb.Th), maximum trabecular strut thickness (Tb.Th, max), and the changes in these measurements during the experiment. ME × P, the interaction between metabolisable energy and phosphorus in the diet.

Measurement	Diet treatment				s.e.m. (df 19)	Probability		
	ModE-LP	ModE-HP	HighE-LP	HighE-HP		Energy (ME)	Phos (P)	ME × P
n	10	9	9	8	–	–	–	
Bone volume to total volume (BV:TV) (%)								
Initial	8.36	8.34	9.79	8.30	–	–	–	
Final	8.40	11.36	10.17	11.23	0.73	n.s.	0.07	n.s.
Change	+0.04	+3.02	+0.38	+2.93	1.01	n.s.	<0.05	n.s.
Osteoid as a proportion of total volume (OV:TV) (%)								
Initial	1.64	1.35	2.12	2.02	–	–	–	–
Final	2.30b	1.90b	3.20c	0.90a	0.24	n.s.	<0.001	<0.01
Change	+0.66	+0.55	+1.08	−1.12	0.38	n.s.	<0.05	0.07
Mineralised bone as a proportion of total bone (Md.V:TV) (%)								
Initial	7.1	7.3	7.2	6.5	–	–	–	–
Final	6.4	9.1	6.8	10.3	0.54	n.s.	<0.001	n.s.
Change	−0.7	+1.8	−0.4	+3.8	0.082	n.s.	<0.01	n.s.
Mean trabecular strut thickness (Tb.Th) (μm)								
Initial	113	103	107	107	–	–	–	–
Final	108	127	127	135	3.8	0.06	0.06	n.s.
Change	−4	+23	+19	+27	5.1	n.s.	<0.05	n.s.
Maximum trabecular strut thickness (Tb.Th-max) (μm)								
Initial	253	238	262	293	–	–	–	–
Final	254	316	306	303	13.8	n.s.	n.s.	n.s.
Change	+4	+35	+26	+12	14.2	n.s.	n.s.	0.07

Means in the same row with different lower-case letters are significantly different (at $P < 0.05$).

days before the treatments commenced, the mean PTH concentration averaged 147 pg/mL. From Week 3 to Week 14, the PTH concentrations were much higher ($P < 0.05$) in the HP- than the LP-diet cows (means 184 and 30 pg/mL respectively), but there was no effect ($P > 0.05$) of the ME content of the diet. At Week 14, the plasma PTH concentrations were negatively correlated with plasma Ca concentrations, as follows:

$$(\log_{10}\mathrm{PTH}) = 7.1 - 2.2(\mathrm{Ca})$$
$$(r = 0.70,\ P < 0.001,\ n = 32)$$

Plasma concentrations of the precursor 25-hydroxy vitamin D_3 (data not shown) were not affected by the diets, averaging 27 ± 8 ng/mL at Day 5 and 33 ± 9 ng/mL at Week 14. The plasma concentrations of active 1,25-dihydroxy vitamin D_3 (data also not shown) averaged 155 ± 82 ng/mL on Day 5. After 14 weeks, the 1,25-dihydroxy vitamin D_3 concentrations in HP-diet cows (mean 118 pg/mL) did not differ from those at Day 5. However, in cows fed the ModE-LP and HighE-LP diets, the 1,25-dihydroxy vitamin D_3 concentrations were increased ($P < 0.001$) to 252 pg/mL and 332 pg/mL respectively. The ratio of active to precursor vitamin D_3 was higher ($P < 0.05$) in cows fed the HighE-LP diet (0.120) than in those fed the ModE-LP diet (0.084), and both were higher than that in the HP-diet cows (0.035 and 0.042; $P < 0.05$).

Plasma CTX-1 concentrations before treatments commenced averaged 0.57 ± 0.31 ng/mL, on Day 5 averaged 0.93 ng/mL, and did not differ among the diets (Fig. 5*a*). Thereafter, plasma CTX-1 concentrations were affected by diet P ($P < 0.001$) and diet ME ($P < 0.05$), and there was a diet P × time interaction ($P < 0.001$). From Week 3 to Week 14, the CTX-1 concentrations were higher ($P < 0.001$) in the LP- than in the HP-diet cows (2.27 and 0.64 ng/mL respectively). Also, during Weeks 10 and 14, the CTX-1 concentrations in the ModE-LP diet were higher than those in the HighE-LP diet (3.40 and 2.40 ng/mL respectively, $P < 0.05$). The CTX-1 (ng/mL) measured after 14 weeks was negatively correlated with Pi (mmol/L) and positively correlated with both the plasma Ca (mmol/L), and the plasma Ca:Pi ratio, as follows:

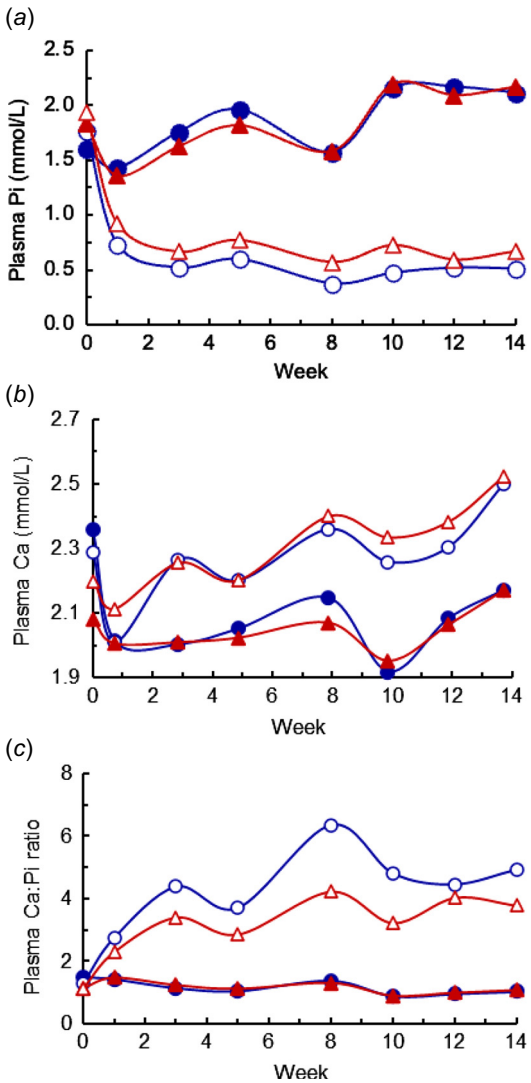

Fig. 3 (*a*) The plasma P concentration (Pi), (*b*) plasma Ca concentration, and (*c*) plasma Ca:Pi ratio of cows fed four diets comprising ModE-LP (△), ModE-HP (▲), HighE-LP (○), and HighE-HP (●). In (*a–c*), the effects of diet P × time were significant ($P < 0.001$) and the least significant differences (l.s.d.) (5%) were 0.19, 0.11 and 0.86 for Pi, Ca and Ca:Pi respectively.

$$(\text{CTX-1}) = 3.54 - 1.36(\text{Pi}) \quad (n = 40,\ r = 0.79,\ P < 0.001).$$

$$(\text{CTX-1}) = -7.90 + 1.01(\text{Ca})$$
$$(n = 40,\ r = 0.65,\ P < 0.001).$$

$$(\text{CTX-1}) = 0.61 + 0.37(\text{Ca:Pi})$$
$$(n = 40,\ r = 0.61,\ P < 0.01).$$

Plasma OCN concentrations were affected by time and the diet P × time interaction (both $P < 0.001$) (Fig. 5*b*). On Day 5 and Week 3, plasma OCN concentrations averaged 44 ng/mL, and then decreased ($P < 0.05$) to an average of 36 ng/mL from

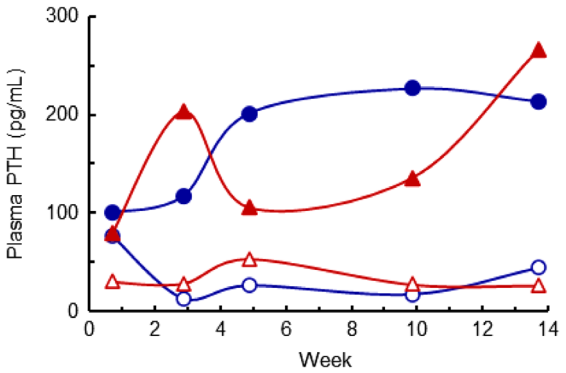

Fig. 4. The plasma parathyroid hormone (PTH) concentrations of cows fed four diets comprising ModE-LP (△), ModE-HP (▲), HighE-LP (○), and HighE-HP (●). The effects of diet P × time were significant ($P < 0.05$) and the l.s.d. (5%) to compare the mean LP and HP diets was 0.31. The values presented have been back-transformed.

Week 5 to Week 14. After 3 weeks, the OCN concentrations were higher in the HP- than LP-diet cows ($P < 0.05$). In addition, higher diet ME tended ($P = 0.06$) to increase the OCN concentrations. The CTX-1:OCN ratios over time (Fig. 5*c*) showed a similar pattern to that of the CTX-1 concentrations. In addition, because the CTX-1 increased and the OCN tended to decrease owing to the treatment effects, the differences owing to P and to higher ME were greater than those for the CTX-1 concentrations. However, there appeared to be little advantage to using the CTX-1:OCN ratio rather than the CTX-1 concentration as an indicator of net bone P mobilisation.

Three days before the treatments commenced, the plasma BAP concentrations averaged 19 ± 7 U/L. The BAP concentrations were affected by the diet P × time and diet ME × time interaction ($P < 0.001$ and $P < 0.05$ respectively), and the diet P × diet ME × time interaction also approached significance ($P = 0.10$) (Fig. 5*d*). In each of the diets, the BAP concentration increased, or tended to increase, until Week 3. In the two HP diets, BAP then decreased progressively during the treatment period. In the HighE-LP diet, BAP increased markedly, whereas there was little change in the ModE-LP diet beyond Week 3. Average BAP concentrations from Week 10 to Week 14 were lowest in the two HP diets (mean 14 U/L), were increased (35 U/L) in the LowE-LP diet, and highest in the HighE-LP diet (58 U/L). ALP concentrations in plasma (data not shown) were generally consistent within each treatment from Week 5 to Week 14, and from Weeks 9 and 14 were higher ($P < 0.05$) in the LP-diet cows than in the HP-diet cows (means 145 and 76 U/L respectively). However, six cows had persistent high ALP concentrations (>200 U/L). Plasma concentrations of BAP were poorly correlated with those of ALP ($r = +0.14$, $P = 0.41$). Plasma AST concentrations were not affected by diet treatment ($P > 0.05$) and were, except for one sample in one animal, <170 U/L. This indicated that all the cows had normal liver

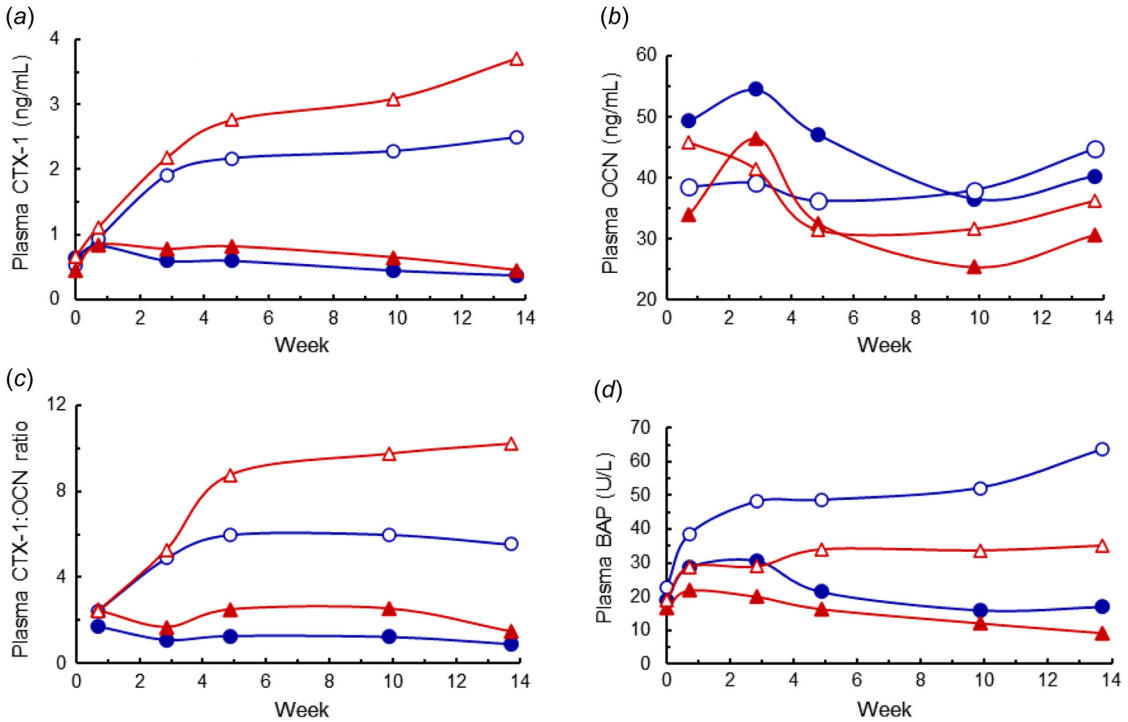

Fig. 5. (*a*) The plasma CTX-1 concentration, (*b*) plasma OCN concentration, (*c*) plasma CTX-1:OCN ratio ands (*d*) plasma bone alkaline phosphatase (BAP) concentrations of cows fed four diets comprising ModE-LP (△), ModE-HP (▲), HighE-LP (○), and HighE-HP (●). In (*a*), the effects of diet P, diet metabolisable energy (ME), time, and the diet P × time interaction were significant ($P < 0.05$ or lower), and the l.s.d. (5%) of the diet P × time interaction = 0.63. In (*b*), the diet P × time interaction was significant ($P < 0.001$), and the effects of diet ME and the diet P × diet ME × time interaction both approached significance ($P = 0.06$, $P = 0.09$). The l.s.d. (5%) of the latter interaction = 12.6. In (*c*), the main effects and interactions, including the diet P × diet ME × time interaction, were significant ($P = 0.01$; l.s.d. (5%) = 0.020). In (*d*), the effects of diet P, diet ME, time, diet ME × time and the diet P × time interaction were significant ($P < 0.05$ or lower), and the diet P × diet ME × time interaction approached significance ($P = 0.10$, l.s.d. (5%) = 15.6).

function during the experiment and notably AST did not aid in diagnosis of the high ALP concentrations in the six cows.

Plasma IGF-1 concentrations were affected by the diet P × time and the diet ME × time interactions ($P < 0.001$ and $P < 0.01$ respectively) (Fig. 6). The IGF-1 concentrations were generally stable within treatments from Week 3 to Week 14, and during this interval, the higher diet P increased IGF-1 from 55 to 121 ng/mL, and higher diet ME increased IGF-1 from 63 to 126 ng/mL respectively. The mean IGF-1 concentrations at 10 and 14 weeks were correlated with the average ME intake ($n = 40$, $r = +0.65$, $P < 0.01$).

Prediction of P retention, plasma markers and bone PSACB from other measurements

There were linear relationships between the P retention and the measured P intake during each of the total collections at Weeks 3, 8 and 14 ($R^2 = 0.76$, 0.71 and 0.59 respectively) and for the mean P retention and intake ($R^2 = 0.75$) (Table 6). No other measured variable improved prediction of P retention in multiple-regression models, and the prediction of P retention was also not improved when expressed as mg P/kg LW.day (data not shown). Phosphorus retention was related to Pi as follows:

$$Y = 11.5(\text{Pi}) - 11.1 \quad (n = 38,\ R^2 = 0.58,\ P < 0.001).$$

Plasma Pi was related to P intake at each of the total collection intervals ($R^2 = 0.62$, 0.45 and 0.58 respectively) and the mean of these intervals ($R^2 = 0.59$) (Table 6). The prediction of mean Pi was improved in multiple-regression models that included both P intake and the initial PSACB as independent variables ($R^2 = 0.70$). It was also improved by inclusion of both the measured initial cow LW, and the ME intake during the experiment, as independent variables ($R^2 = 0.86$). The initial PSACB was correlated with ME intake ($r = 0.33$, $P < 0.05$), but not with the initial cow LW. There was no correlation between the initial cow LW and the ME intake ($P > 0.05$).

Plasma CTX-1 and the Ca:Pi ratio were both related to the P intake ($R^2 = 0.52$ and 0.42, $P < 0.001$ for both) (Table 6). The prediction of CTX-1 was improved by inclusion of the initial cow LW and ME intake as additional variables ($R^2 = 0.70$). Similarly, the prediction of the Ca:Pi ratio was improved by

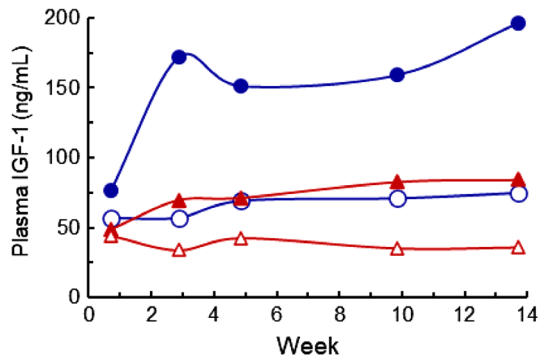

Fig. 6. The plasma IGF-1 concentrations in cows fed four diets comprising ModE-LP (△), ModE-HP (▲), HighE-LP (○), and HighE-HP (●). The diet P × time and the diet metabolisable energy × time interactions were significant ($P < 0.01$ or lower), and the l.s.d. (5%) of the $\log(X + 1)_{10}$ transformed data was 0.14. The values presented have been back-transformed.

inclusion of PSACB ($R^2 = 0.70$) and by inclusion of both PSACB and cow LW change, or of PSACB and ME intake, as additional variables ($R^2 = 0.70$) (Table 6).

The high variability of the PSACB of the cows at the commencement of the experiment had significant effects on the responses of the cows as plasma Pi, plasma Ca:Pi and P retention. Inclusion of the initial PSACB as an independent variable in a multiple-regression model improved ($P < 0.001$) the prediction of Pi both at the Week 3 total collection and, on average, over the 14 weeks by 0.062 and 0.053 mmol/L for each 100 μg P/mm^2 increase in initial PSACB respectively. Thus, the Pi at the Week 3 total collection, and the mean Pi over the entire experiment increased by 0.22 and 0.18 mmol/L respectively, as the initial PSACB of the cows increased from 350 to 750 μg P/mm^2. In contrast, the P retention at the Week 3 total collection was increased 0.84 g P/day (1.75 mg P/kg LW.day) for each 100 μg P/mm^2 decrease in the initial PSACB. It follows that at Week 3 the cows fed the ModE-HP diet with the lowest initial PSACB would have retained ~2.3 g P/day, whereas those with the highest initial PSACB would have retained ~1.1 g P/day; these are the highest and lowest P retentions respectively, compared with the mean of 1.7 g P/day during the Week 3 total collection (Table 3). There were similar effects of the initial PSACB (in Week 3; $P < 0.001$), in the Week 8 and Week 14 total collections, and for the average for the three total collection intervals ($P < 0.10$) on the plasma Ca:Pi, which increased as the initial PSACB decreased (Table 6).

Discussion

This study has clearly shown that mature cows in their second trimester of pregnancy fed acutely P-deficient diets mobilise substantial body P (~4–5 g P/day) to alleviate the effects of the P deficiency. Because mobilised P is presumably entirely

Table 6. Multiple-regression models describing the P retention (during total collection, Week 3 and the mean of the three total collection intervals), plasma Pi, plasma CTX-1 concentration and the ratio of plasma Ca:Pi in the cows.

Dependent variable	Constant	Independent variable					R^2	s.e.
		P intake (g P/day)	PSACB (μg P/mm^2)	MEI (kJ ME/kg LW.day)	Cow initial LW (kg)	Cow LW change (kg/day)		
P retention (g P/day), Week 3								
Y_1	−5.196***	0.726***	–	–	–	–	75.7	2.41
P retention (g P/day), mean								
Y_2	−7.14***	0.829***	–	–	–	–	75.0	3.41
Plasma Pi (mmol/L), mean								
Y_3	0.563***	0.0746***	–	–	–	–	58.7	0.44
Y_3	−0.461$^{n.s.}$	0.128***	0.00159**	–	–	−0.874**	69.7	0.381
Y_3	3.151***	0.129***	–	−0.0131***	−0.00286*	–	85.7	0.261
Plasma CTX-1 (ng/mL), mean								
Y_4	2.825***	−0.128***	–	–	–	–	52.4	0.865
Y_4	−2.930*	−0.194***	–	0.0153**	0.00989**	–	70.0	0.686
Plasma Ca:Pi ratio, mean								
Y_5	4.673***	−0.195***	–	–	–	–	42.3	1.60
Y_5	8.230***	−0.208***	−0.0062**	–	–	–	52.7	1.45
Y_5	8.920***	−0.418***	−0.0066***	–	–	3.630**	63.9	1.26
Y_5	3.000$^{(-)}$	−0.338***	−0.00337$^{(-)}$	0.0339***	–	–	69.5	1.16

LW, liveweight; PSACB, phosphorus per unit area of outer cortical bone; MEI, metabolisable energy intake.
n.s. not significant; (–), $P < 0.10$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

available to the animal, this mobilisation would have corresponded to ~6–7 g of dietary P (CSIRO 2007). However, these LP cows were still P deficient because their voluntary intakes and their efficiency in utilisation of ingested ME were substantally lower than in the HP cows. The measured changes in rib and *tuber coxae* bone, and in plasma minerals and hormones (Pi, Pi:Ca ratio, 1,25-dihydroxy vitamin D_3, CTX-1 and BAP) were informative of the changes in P metabolism in the cows.

Because the cows were previously lactating, and grazing native pastures growing on soils that were classed as deficient or acutely deficient through a rainy season (December 2013 – June 2014), it was expected that they would be P deficient and have low bone mineral reserves at the commencement of the experiment (McCosker and Winks 1994; Jackson *et al.* 2023). This was supported by the low Pi of the cows on 5 May 2014 (1.22 ± 0.48 mmol/L). However, at the commencement of the experiment, the bone measurements (PSACB in rib cortical bone and bone volume:total volume in *tuber coxae* rib bone) in the cows were highly variable. The coefficient of variation of the PSACB, bone volume:total volume and mineralised bone volume:total volume ranged from 21% to 32%. This was much higher than the coefficient of variation that we have observed in the PSACB of other groups of mature cows (10% and 15%) or in the bone volume:total volume of pregnant heifers (8%) (Dixon *et al.* 2020*b*). In addition, based on rib bone measurements in mature cattle fed P-adequate or P-deficient diets for extended periods, PSACB classes of <550, 550–650 and >650 μg P/mm^2 are indicative of deficient, marginal and adequate bone P status respectively (Dixon *et al.* 2019*a*). By these criteria the PSACB measurements at the commencement of the present study indicated that only 57% of the cows were in deficient bone status, whereas 27% and 16% of the cows were in marginal and adequate bone P-status classes respectively. Furthermore, it is clear from the relationships shown in Fig. 1 that the initial bone mineral reserves, as measured by PSACB of rib bone and bone volume:total volume and thickness of the struts of trabecular bone, substantially affected the responses of the cows to the diet treatments in the present study.

Nutrient intakes and LW change of the cows

The MedE-HP and HighE-HP diets provided for slow and rapid LW gains (0.32 and 1.05 kg/day respectively) that were representative of low and high post-weaning performance of breeder cows grazing pasture ranging from moderate to high quality during the late-rainy into the early dry seasons in seasonally dry tropical rangelands (Holroyd *et al.* 1983, 1988; Dixon *et al.* 2011*a*, 2011*b*). The low Pi, voluntary intakes, P retentions, metabolites in plasma and LW gain in the LP-diet cows, and the responses to additional diet P, provided unequivocal evidence that the cows fed the LP diets were acutely P deficient (Wadsworth *et al.* 1990; Winks 1990; Dixon *et al.* 2020*a*). Furthermore, the diet P concentrations and P intakes of the LP-diet cows (0.68 and 0.76 g P/kg DM, 7–9 mg P/kg LW.day) fed in pens were comparable with P intakes measured in field experiments with growing cattle and breeder cows grazing native pastures classed as 'acutely deficient' or 'deficient' for northern Australia (McLean *et al.* 1990; Hendricksen *et al.* 1994; Ternouth and Coates 1997; Miller *et al.* 1998) and South Africa (Read *et al.* 1986*a*, 1986*b*, 1986*c*). In contrast, from the P intakes relative to the calculated requirements, and the high Pi, the supply of absorbed P was not likely to have limited either LW gain or body P replenishment in the HP-diet cows. Thus, the LP and HP diets did represent the P intakes of pregnant cows shortly after weaning during the late rainy season and the early dry season grazing P-deficient pastures of at least moderate quality, or such pasture supplemented with P, as classed in the P-management packages described by McCosker and Winks (1994) and Jackson *et al.* (2023). However, in some circumstances cows may be losing LW in the late rainy and early dry seasons because of poor seasonal conditions and/or when weaning is delayed until the mid- to late dry season (Holroyd *et al.* 1983, 1988; Dixon *et al.* 2007). When nutrient intake is insufficient for LW maintenance, the cow responses to supplementary P may well be lower than observed for the cows fed the moderate-ME diets in the present experiment.

The large responses to providing HP that we observed on voluntary intakes of DM and ME, and LW gain, are in general agreement with pen and grazing experiments reported for growing cattle ingesting P-deficient diets (Winks 1990; Bortolussi *et al.* 1996; Quigley *et al.* 2015). The observed responses were also consistent with reduced LW loss, and large increases in LW gain and reproductive performance, in field experiments with beef breeder cows where the benefits of additional diet P were associated with increases in voluntary intake and cow LW (Winks 1990; Winter *et al.* 1990; Ternouth and Coates 1997; Coates *et al.* 2019; Schatz *et al.* 2023). We are not aware of any previous research under pen conditions in non-lactating pregnant beef cows where the diet was closely controlled and the responses by P-deficient cows to additional diet P could be accurately measured.

The gradual decrease in voluntary intake of each of the diets by the cows during the present experiment was most likely to be a consequence of weaning shortly before the experiment commenced. In *Bos indicus* cows fed forage diets, the voluntary intake is usually increased by 20–30% during lactation (Penzhorn and Meintjies 1972; Hunter and Siebert 1986), and following weaning, gradually decreases over several months (Foote and Russel 1979). A carry-over effect of lactation on intake also likely contributed to the high voluntary intake (mean 21.5 g DM/kg LW.day) and high LW gain (mean 1.05 kg/day) of the cows fed the HighE-HP diets.

The observed higher efficiency in the utilisation of ingested ME in cows fed the ModE-HP diet than in those fed the ModE-LP diet was unexpected, but is the only explanation for the similar LW gains of the cows in these two treatments

(0.32 and 0.29 kg/day), even though the HighE-LP diet cows consumed 29% more ME (Table 2). This result is in accord with slower growth over 40 weeks in P-deficient than in P-adequate heifers with similar ME intakes reported by Gartner *et al.* (1982). Severe P deficiency has been associated with a large decrease (~30%) in the P concentration of soft tissues in growing lambs (Ternouth and Sevilla 1990) and there is evidence that cellular metabolism, including of energy, is impaired by severe P deficiency (Henry *et al.* 1979; Wang *et al.* 1985; Ternouth 1989). Adverse effects on cellular metabolism may explain both the usual decreases in voluntary intake (Winks 1990), and a reduced efficiency in utilisation of ME, in severely P-deficient animals. A 30% increase in efficiency in utilisation of ME for maintenance and growth is clearly a very important production benefit if it does occur generally when P-deficient cattle are fed adequate diet P. Further information is needed to test this hypothesis.

Mobilisation of body P by the cows fed low-P diets

Even though some of the cows had partially depleted body P reserves at the start of the experiment, the LP-diet cows were still able to mobilise, on average, 4.1 and 4.7 g P/day (9.3 and 11.2 mg P/kg LW) of body P in the ModE-LP and HighE-LP diets respectively). This P corresponded to 55% and 45% of their calculated P requirements when the cows were ingesting <50% of their P requirements. Furthermore, over the 14-week experimental interval this corresponded to the mobilisation of ~12–14% of the body P reserves that was in addition to the mobilisation of body P that is likely to have occurred during the preceding lactation in these cows. The P mobilisation we observed was consistent with the decreases in PSACB, changes in the porosity of rib cortical bone, and changes in trabecular bone and osteoid in *tuber coxae* bone (Tables 4, 5) in the LP-diet cows. The much larger proportional changes in trabecular than cortical bone were consistent with the principle that during bone mobilisation and accretion, the changes in bone are much greater in trabecular bone with a large surface area, than in cortical bone (Seeman 2013; Winter *et al.* 2020). However, because cortical bone and trabecular bone represent about 80% and 20% respectively, of the total bone, a small change in cortical bone may be more important than a large change in trabecular bone on the total bone in the animal. Furthermore, the cortical thickness is a linear measure whereas the change in total rib bone in the animal is a volume. Hence, in the present study the small percentage changes in cortical bone thickness or PSACB represented greater changes in the total bone of the cows than the same percentage changes in the trabecular bone. A further consideration is that, in the present study, the diet treatments were imposed for only 14 weeks, which is only a short interval during the annual cycle for substantial changes to occur in bone.

The relationships between the amounts of bone initially present in the rib cortical bone and in *tuber coxae* trabecular bone, and the changes in both cortical and trabecular bone during the experiment (Fig. 1*a*–*e*) provided unequivocal evidence that the extent bone is gained or lost during P deficiency or P adequacy is highly dependent on the bone mineral status of the animals. The multiple regression modelling (Table 6) showed that the bone mineral status, measured as PSACB, contributed significantly to the prediction of plasma Pi and plasma Ca:Pi and is in accord with the important role of the bone minerals status. The hypothesis was also supported by the observation that inclusion of the initial PSACB as a covariate significantly ($P < 0.001$) increased the estimated Pi both at the total collection at Week 3, and, on average, over the 14 weeks, by 0.062 and 0.053 mmol/L for each 100 μg P/mm^2 increase in initial PSACB respectively. Thus, the Pi at the Week 3 total collection, and the mean Pi over the entire experiment, increased by 0.22 and 0.18 mmol/L respectively, as the initial PSACB of the cows increased from 350 to 750 μg P/mm^2. However, clearly, there must be limits to the rates and extent of bone mineral mobilisation as the bone reserves decline.

The cows were expected to have been in P-replete status in December 2013. A 450 kg P-replete cow is expected to have body reserves of ~8.9 g P/kg empty bodyweight (ARC 1980) (~3.4 kg P with 2.7 kg in bone and 0.7 kg in soft tissues). Since P-replete mature cows are expected to have a PSACB of 700–750 μg P/mm^2 (Dixon *et al.* 2019*a*), the average 546 μg P/mm^2 PSACB in June 2014 suggested that these cows had lost ~20–25% of their bone P reserves from December 2013 to June 2014, corresponding to mobilising ~4–5 g body P/day. This estimate is remarkedly similar to the mobilisation of P in the LP-diet cows measured as P retention during the experiment. Moreover, these calculations are consistent with the conclusion that cattle lose up to ~30% of their bone P reserves during part of their annual cycle, and in some circumstances over 40% of their bone P reserves, before becoming overtly P deficient and showing clinical signs of osteomalacia (Little 1983; Read *et al.* 1986*a*, 1986*b*, 1986*c*; Dixon *et al.* 2017, 2020*a*). The P-mobilisation rates in the LP-diet cows in the present experiment compare with 7–9 mg P/kg LW.day of P mobilisation in steers fed acutely P-deficient diets (Tuen *et al.* 1984; Bortolussi *et al.* 1996; Ternouth *et al.* 1996). More generally, it appears that in mature non-lactating beef cows, the maximum body P mobilisation is ~5 g P/day (11 mg P/kg LW), which will correspond to ~7 g diet P.

Replenishment of body P by the cows fed P-adequate diets

The histological changes and the changes in *tuber coxae* bone as increased bone volume (~36%), mineralised bone volume (~42%) and thickening of trabecular struts (~24%) (Table 5) clearly showed that there was increased trabecular bone

formation when the cows were fed HP diets. The importance of availability of P as a metabolic substrate for this bone formation was shown by the response to the HighE-LP diet where trabecular thickness increased but was poorly mineralised. The large (55%) decrease in osteoid tissue in the HighE-HP diet cows was presumably because only this diet provided sufficient P to allow a large increase in bone mineralisation. The increase in mineralisation when ModE-HP was fed (25%) was much lower than the 58% increase with the HighE-HP diet. These measurements indicated that trabecular bone growth and mineralisation were proceeding in the cows fed the higher-ME or high-P diets, but occurred much more rapidly in the HighE-HP diet cows. This was in accord with the much lower P retention in the ModE-HP than the HighE-HP diet cows.

The changes in the rib cortical bone owing to the diet treatments were, as expected, smaller than those observed in trabecular bone in the *tuber coxae*. High diet P increased the cortical bone thickness, whereas high diet ME led to a small decrease in the P concentration of the cortical bone. As in *tuber coxae* bone, the larger effects were due to providing adequate diet P rather than to increasing the ME content of the diet. Also, the changes in PSACB were a consequence of the changes in thickness rather than in P concentration of cortical bone. This is consistent with the paradigm (Shupe *et al*. 1988; Breves and Prokop 1990; Coates *et al*. 2018) that loss of cortical rib bone in mature cows during extended P deficiency is associated with thinning of cortical bone rather than decrease in its mineral concentration. The increase in PSACB in cows fed the HighE-HP diet (16 μg P/mm^2) was small compared with the decrease (mean 77 μg P/mm^2) in the LP-diet cows (Table 4). The benefit of the HP diets on rib cortical bone was to maintain rather than to increase this bone. The small response may have been associated with a time lag for cows to change from net mobilisation during P deficiency to net replenishment in rib cortical bone when the HP diets are fed; 14 weeks is a short interval in the annual cycle. Nevertheless, the substantial increase in (100-Po) (i.e. the decrease in porosity) of the rib cortical bone in the HP diets, and the changes in the *tuber coxae* bone, established that there was increased formation as 'infilling' of porotic spaces in cortical bone concurrently with the increased growth and mineralisation of trabecular bone in the *tuber coxae*.

The high P retention (+8.8 g P/day) in cows fed the HighE-HP diet (Table 2) was greater than the amount of absorbed P (6.4 g P/day) calculated to be required for their 1.05 kg/day LW gain (CSIRO 2007). Hence, ~2.4 g absorbed P/day should have been available for replenishment of P concentration in soft body tissues and bones. The small positive average P retention in cows fed the ModE-HP diet (+1.1 g P/day) showed that there was a slow increase in body P reserves, even when ME intake limited the cows to only slow LW gain. As the difference between two large flows as P intake and faecal P excretion, the measurement of P retention is inevitably subject to substantial error. However, the error associated with measurements of P retention in the present experiment (s.e.m. 2.7 mg P/kg LW.day) was lower than that often reported previously, ranging from 1 to 8 mg P/kg LW.day (Tuen *et al*. 1984; Bortolussi *et al*. 1992, 1996; Knowlton and Herbein 2002).

The modest average replenishment of the P reserves in the cows fed the ModE-HP diet in the present experiment was likely to be associated with only about half (57%) of the cows being deficient in bone minerals at the commencement of the experiment as discussed above, and also the mature ages of the cows (Table 6). In addition, there may also have been limitations to bone mineral replenishment associated with the principle that the structural strength of bones and bone minerals change as needed to support the increasing or decreasing weight of the animal (Siemon and Moodie 1973). Because the cows fed the ModE-HP diet were in slow growth, whereas the HighE-HP cows gained LW rapidly during the experiment, there was likely to be lesser endocrine signalling in the former cows to increase bone mass (Iwaniec and Turner 2016). Large decreases in LW in growing steers during extended dry seasons have been associated with decreased rib bone minerals (Winter 1988). Furthermore, as would be expected, and observed in the present experiment, the rate and extent of body P replenishment will be affected by the magnitude of a depletion in body P reserves as well as intakes of P and other nutrients. This is supported by the study of Schild *et al*. (2023) where beef breeder cows with clinical osteomalacia from grazing acutely P-deficient pastures largely recovered from osteomalacia over 3 months when fed 8 g supplementary P/day. During recovery, the P-supplemented pasture was able to support 0.3 kg LW gain/day, the P supplementation increased Pi from 1.0 to 1.9 mmol/L, rib cortical bone P concentration increased from 85 to 131 mg/mL, and serum Ca:Pi decreased from 2.8 to 1.1. The bone mineral status of the cows was much lower than that of the cows in the present experiment, but, nevertheless, 90% of the cows recovered from clinical signs of osteomalacia. More generally, replenishment of 1.1–2.3 g/day body P (as measured in the present experiment) for 8 months from the mid-rainy season and through the dry season would contribute 260–550 g P to substantially replenish body P reserves. Even if insufficient to completely replenish the body P reserves of a 450 kg cow following mobilisation of 30% of body P reserves (~1 kg P) during pregnancy and lactation, this would make a substantial contribution to P status of cows.

Markers of the bone mineral mobilisation and replenishment and endocrine control

The plasma Pi in the HP-diet cows were high and within the range reported for growing cattle and non-lactating cows consuming P-adequate diets (Wadsworth *et al*. 1990; Anderson *et al*. 2017; Dixon *et al*. 2020*a*). The large decreases in Pi to 0.5–0.8 mmol/L when the LP diets were fed to the cows

were expected and were comparable to those reported in severely P-deficient ruminants and following large abrupt decreases in diet P (Wadsworth *et al.* 1990; Rodehutscord *et al.* 1994; Dixon *et al.* 2020*b*). The rapid decreases in plasma Pi in the cows in the present experiment contrast with delays for many weeks or months for Pi to decrease in growing cattle fed P-deficient diets in some experiments (Gartner *et al.* 1982; Quigley *et al.* 2015; Dixon *et al.* 2019*b*), presumably owing to sufficient mobilisation of body P reserves to maintain Pi for extended intervals. In the present experiment, the concentrations of Ca and Mg in plasma were within the normal ranges for these minerals, and increases were consistent with their release into circulation with mobilisation of bone during P deficiency. The gradual increases in these minerals in the LP-diet cows as the experiment progressed were consistent with time lags and requirements for adaptation to the LP diets. Observed changes in plasma CTX-1, CTX-1:OCN ratio, and BAP concentrations support a hypothesis that mobilisation of bone increased gradually to provide P during severe P deficiency (Anderson *et al.* 2017). Although there was a rapid decrease within a week in plasma Pi, following the introduction of the LP diets, there were much more gradual increases in Ca and the other bone biomarkers during Weeks 2–5. It is likely that this delay reflected the time lag needed to recruit osteoclasts to enable bone mobilisation. This provides further evidence that the plasma Ca, CTX-1 and BAP provide reliable markers of bone mobilisation.

Interestingly, in the LP-diet cows, there was a large increase in active 1,25-dihydroxy vitamin D_3 in plasma at Week 14 that was likely to be a direct effect of low Pi rather than a response to PTH; the concentrations of PTH were reduced in LP-diet cows. Furthermore, low plasma PTH concentrations in LP-diet cows were associated with high plasma Ca in accord with tight regulatory control between Ca and PTH concentrations. Increased plasma Ca in LP-diet cows may have been associated with increased bone mineral mobilisation and increased absorption from the gut in association with the adequate Ca content of the LP diets. Nevertheless, unlike during diet deficiencies of Ca, PTH appears not to have had a central role in the control of bone mobilisation in P-deficient cows. Rather, the evidence supports the hypothesis that increased active 1,25-dihydroxy vitamin D_3 and/or other mediators are controlling P homeostasis and driving bone resorption in P deficiency.

It is well established that CTX-1, as a breakdown product of bone and cartilage during turnover, provides a marker of bone mobilisation (Anderson *et al.* 2017). The increases in CTX-1 in the LP-diet cows were in accord with these diets providing less than half of the calculated absorbed P requirements of the cows, with low Pi, with the substantial negative P retentions, and that these LP-diet cows were mobilising bone P. The CTX-1 concentrations measured in the current study, ranging from ~2.5 to 3.5 ng/mL after 10–14 weeks in the LP-diet cows, were much higher than those in the HP-diet cows, but were quite low compared with those in P-deficient beef and dairy cows in early to mid-lactation where CTX-1 concentrations are up to 6 ng/mL (Ekelund *et al.* 2006; Puggaard *et al.* 2014; Anderson *et al.* 2017; Dixon *et al.* 2020*b*). This supports a hypothesis that non-lactating cows have a lower potential than lactating cows to mobilise body P during P deficiency.

A potential simple alternative to CTX-1 as a marker of bone mineral mobilisation is the plasma Ca:Pi ratio. In mature lactating beef cows, there is a positive correlation between plasma CTX-1 concentrations and the plasma Ca:Pi ratio during P deficiency (Anderson *et al.* 2017). In the non-lactating cows in the present experiment, this relationship between plasma CTX-1 and the plasma Ca:Pi ratio was similar to that reported by Anderson *et al.* (2017), but through a smaller range and with a lower R^2 (0.78 and 0.37 respectively). The present experiment supports the hypothesis that both plasma CTX-1 and Ca:Pi ratio can be used as markers of net bone mobilisation during P deficiency.

Both OCN and BAP are potential markers for bone growth, although the latter is indicative primarily of bone mineralisation (Naito *et al.* 1990; Anderson *et al.* 2017). In the present study, the concentrations of OCN were increased by the two HP diets, but they also tended to increase with higher diet ME content. Unfortunately, there was not a clear difference between the HighE-HP and the ModE-HP diets in OCN concentrations, even though P retention was much higher in the former diet. In contrast, there were large diet effects on BAP concentration. The increases in BAP as a bone formation marker during diet P deficiency appear contradictory. However, Anderson *et al.* (2017) argued that in the absence of sufficient P substrate in P-deficient animals, the formation of bone cannot proceed, and BAP (i.e. alkaline phosphatase isoform in bone) is upregulated to drive localised bone mineralisation. Therefore, high BAP concentrations appear to be primarily an indicator of defective mineralisation in P-deficient cows. In the present experiment, there were differences among diets in 'total' ALP concentration, but because this is a measure of alkaline phosphatases derived mainly from both bone and liver, there does not appear to be any advantage in use of ALP rather than BAP as a marker. The changes and differences among diets in OCN and in the CTX-1:OCN ratio were generally small and differences among treatment diets were often not clear. Therefore CTX-1 concentration alone appears to be the more useful biomarker for P deficiency. The increases in 1,25-dihydroxy vitamin D_3 in plasma with P deficiency suggests that this also may provide a valuable hormonal marker. In summary, the concentrations of Pi, Ca, Ca:Pi, CTX-1, BAP and 1,25-dihydroxy vitamin D_3 in plasma are all useful markers of P status, including in non-lactating beef breeder cows.

Conclusions

Mature *Bos indicus*-cross breeder cows fed acutely P-deficient diets mobilised substantial body P (~4–5 g P/day, equivalent

to ~6–7 g diet P/day) to alleviate the effects of the deficiency, but both voluntary intake and the efficiency in utilisation of ingested ME were substantially reduced. Providing adequate diet P increased voluntary intake and LW gain, initiated growth of trabecular bone, and commenced replenishment of low body P reserves. The retention of P by cows fed a high-P diet but ingesting sufficient energy and protein for only slow LW gain was, on average, 1.1 g P/day, but was much higher in previously P-deficient cows with low bone mineral reserves. Responses by cows to adequate diet P were much greater when a higher ME-content diet allowed high voluntary intake and LW gain, increased P retention, replenishment of body P reserves, and increased bone growth. A number of metabolites (plasma Pi, Pi:Ca ratio, CTX-1, BAP and 1,25-dihydroxy vitamin D_3) and measurements of rib and *tuber coxae* bone were informative of P metabolism in the cows, although testing over a wider range of circumstances is needed for their general application as diagnostics in P-deficient cows.

Supplementary material

Supplementary material is available online.

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Data availability. The data that support this study may be shared upon reasonable request to the corresponding author.

Conflicts of interest. Dr Rob Dixon is an Associate Editor of *Animal Production Science*. To mitigate this potential conflict of interest he had no editor-level access to this manuscript during peer review. The authors declare that they have no further conflicts of interest.

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