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Improved management of cattle phosphorus status through applied physiology

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Abstract

A research program has investigated aspects of P nutrition in breeder cattle in circumstances where improved knowledge of P physiology and nutrition should lead to practical management manipulations for increased cost-effectiveness in northern Australian production systems.

Phosphorus (P) physiology and nutrition in tropical breeders were investigated to: (i) improve attraction of P supplements for P-deficient cattle, (ii) understand the circumstances wherein P is mobilized from body P reserves (particularly bone) allowing breeders to alleviate the adverse effects of diet P deficiencies during the pregnancy and lactation, and the circumstances for replenishment of the body P reserves, (iii) improve understanding of physiological control of body P mobilization and replenishment, and (iv) improve diagnosis of P deficiency.

Attraction of P-deficient cattle to bones was demonstrated to be a learned, not an innate behaviour. There are not likely to be specific attractants for cattle, but it may be possible to use this knowledge to better animals to achieve target supplement intakes.

Mature breeders calving in high P status and fed severely P-deficient diets during lactation mobilized body P and fat reserves to largely maintain lactation and early calf growth, but with rapid loss of cow liveweight (LW) and also bone P. Calving heifers (first-calf cows) exhibited a lesser capacity. Improved understanding in breeders of the physiological control of P and bone growth is leading to improved diagnosis and prediction of responses of tropical breeders to P supplementation through the annual cycle.

The basic recommendation that it is most effective to feed P supplements through the wet season is not changed and should be the most effective and efficient use of P supplements.

Executive summary

The importance of phosphorus nutrition and the adverse effects of widespread P deficiencies in cattle grazing the rangelands of northern Australia are well known. P deficiencies decrease growth and increase age of turnoff at slaughter. Productivity is adversely affected in both heifers and mature breeders with decreased fertility and weaning weight and increased mortality. Although there are large production benefits to addressing P deficiency only a small of cattle grazing P-deficient pastures across northern Australia are effectively supplemented to manage P deficiencies.

A research program investigated aspects of P nutrition in breeder cattle in circumstances where improved knowledge of P physiology and nutrition should lead to practical management manipulations for increased cost-effectiveness in northern Australian production systems. The project objectives were to (i) identify the constituents of old bones which are attractive specifically to P-deficient cattle so that these can be used as attractants in P supplements, (ii) determine the magnitude and diet x animal interactions affecting the deposition and mobilisation of body P reserves in breeder cattle in situations typical of northern Australia, and (iii) understand the physiological mechanisms controlling mobilization and deposition of body P reserves in breeder cattle. Also to use this knowledge to better manage P deficiency in breeders through improved estimation of current animal P supply from diet and body reserves and animal responses and by better diagnostic tools of P status.

For objective (i) the attraction of P-deficient cattle to ingest old bones was examined to identify the olfactory constituents causing attraction which might be used to increase intake of P supplements during the wet season. It was shown that the attraction was a learned response, not innate, and thus there are not likely to be any universal attractants. The understanding may be used to improve training of cattle to attract them to P supplements.

For objectives (ii) and (iii) five intensive experiments with Droughtmaster mature breeders or first-calf cows (FCC) investigated P mobilization and deposition. The animals were housed in individual pens for 3-8 months and accurate measurements of intake, excretion, P balance, milk production and LW changes in cows and growth of calves during late pregnancy and/or early lactation were measured. Cattle were fed semi-purified or molasses-straw diets to achieve appropriate metabolisable energy (ME) and P intakes while also measuring the effects on voluntary feed intake.

Mature breeder cows calving in high P status (from high P intakes during pregnancy) and fed severely P deficient diets in early lactation decreased intakes, extensively mobilized body P (5-10 g P/day) and maintained milk output and calf growth. Calf growth was not affected in one experiment and there was only a small decrease (from 0.92 to 0.80 kg/day) in a second experiment. The mobilised P provided up to about half the P requirements of these lactating cows. However this body P mobilization was associated with large losses in cow live weight (LW; e.g. 0.5 kg/day) as well as P in bone. A third experiment with FCC showed that these animals had a lesser capacity to mobilize body P reserves to maintain early lactation. Milk production in FCC and mature cows was comparable when ingesting adequate P diets. In FCC fed a P deficient diet during both late pregnancy and early lactation milk output was reduced by up to 44% and calf growth up to 0.57 kg/day. These adverse effects during lactation were reduced by P-adequate diets during pregnancy due to P mobilisation. Greater adverse effects in FCC were likely due to their need for P for continuing skeletal growth and with less P available for milk secretion.

Another experiment examined the effects diet P deficiency of heifers rapidly losing conceptus-free LW during late pregnancy as occurs routinely in harsh nutritional environments and where P deficiency is common. Regardless of the magnitude of the LW loss feeding a high P diet caused the heifers to store more body P with higher bone P at calving. When heifers were maintaining (or in another experiment gaining) conceptus-free LW during late pregnancy diet P deficiency reduced the voluntary intakes (VI) of dry matter (DM) and metabolisable energy (ME). Supplementation of P during the dry season in late-pregnant breeders as well as lactating breeders grazing low P pastures is recommended. Body P mobilized to support lactation must be replenished later in the annual cycle to prepare cows calving annually for the next lactation. An experiment showed that P depleted mature breeders could replenish both bone P and soft tissue P post-weaning when fed diets comparable with late wet season or early dry season pasture, and the replenishment was much greater when a diet high in both P and ME was fed. Thus breeders could respond to P supplementation and stored bone P during the dry season.

The experimental program improved understanding of key physiological mechanisms in beef breeder cows in P deficiency and P replenishment. Severely P deficient diets resulted in immediate and large decreases in blood P (PIP) concentrations (e.g. < 0.7 mmol/L), reduced intake, caused rapid loss of LW and often mobilisation of bone minerals. Bone P mobilisation was insufficient to normalise PIP in breeders but did maintain PIP in weaners. Substantial mobilisation of bone unexpectedly did not involve the key bone mobilising hormone, parathyroid hormone PTH). With low P diets there is also an increase in the active form of Vit D3 (1,25-diOH Vitamin D3) which would promote intestinal absorption of Ca and P. Overall diet P deficiency in cows was characterised by low PIP, high blood Ca, high blood Ca/P ratio, markedly increased concentration of the bone mobilisation marker carboxy-terminal telopeptides of type I collagen (CTX-1) despite low PTH, and increased active Vit D3 and bone alkaline phosphatase (BAP).

The project has advanced understanding in the physiology of P in northern breeder cattle. It has improved both prediction of P supplementation responses in breeders through the annual cycle and the diagnosis of P status from blood. The body P reserves in breeders can be mobilised to alleviate diet P deficiency during the wet season, and replenished slowly through the dry season when diet P is adequate. Breeders, particularly if mature, can tolerate intervals of P deficiency while lactating but with severe loss of body condition. The basic recommendation that it is most effective to feed P supplements through the wet season is not changed and should be the most effective and efficient use of P supplements. When feeding P supplements through the majority of the wet season is not possible then P supplements should be fed for the remainder of the wet season. Feeding some P supplements during the dry season to maintain and/or replenish bone P in some classes of breeders (lactating, late pregnant, depleted bone P) is recommended. The estimation of diet P adequacy should be based on blood P and Feacal NIRS. Because it is now clear that the relationship between concentrations of P in the diet and in faeces is inconsistent the FP:ME ratio should not continue to be recommended as a diagnostic.

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1 Background

1.1 Rationale for the project

The importance of phosphorus (P) nutrition and the problems of widespread P deficiencies in grazing cattle across northern Australia are well known and there are large productivity gains to cattle enterprises in addressing deficiencies in P nutrition. However, sales of P supplements and an abundance of anecdotal evidence indicate that only a low proportion of P-deficient cattle in northern Australia are effectively fed P supplements in accord with technical recommendations.

The main reasons for this appear to be:

1) lack of knowledge by managers of the P status of their land systems and herds;
2) lack of simple and effective diagnostic tools;
3) lack of confidence that management (and specifically P supplementation) provides high economic returns; and
4) the practical difficulties of feeding P supplements during the wet season as recommended (e.g. achieving target intakes of P supplements, physical access in boggy or flooded conditions, labour).

Current project work is addressing, in part at least, points (1), (2) and (3) through revising and updating the producer phosphorus manual for northern Australia (B.NBP.0540), through evaluating a faecal-based indicator of P the adequacy of P intake in steers and breeders (B.NBP.0537), and through assessing the feasibility of developing a decision support tool that assesses the benefit-cost of feeding P for various land types and animal classes (B.NBP.0594). However, P supplements are needed during the wet season, and supplying P at this time of year is often difficult, or impossible, on many commercial properties. Also, assessing the P status of breeders is problematic and simply assessing P intake may often be inadequate as breeding animals mobilise and replete P stores depending on P intake and P demand, both of which vary markedly through-out the year.

To overcome the widespread problem of feeding P in the wet season, this project will evaluate the natural capacity of breeder cows to store P in bone when diet P is in surplus (such as post-weaning or when P supplements are fed during the dry season) and to subsequently mobilise this P when diet P intake is inadequate (such as during lactation and the wet season when the P is most needed). It is well known that reproducing sheep, goats and dairy cattle store large amounts of P in body (bone) reserves, and that substantial amounts of this P can be mobilized during late pregnancy and lactation to substitute for current dietary deficiency of P. However, this mechanism of P mobilization of body reserves has not been widely known or utilized in the management of P nutrition of beef cattle. From published studies it is likely that at least 600 g P (and possibly up to 1200 g P) could be mobilized in a *Bos indicus* breeder cow during late pregnancy and early lactation. An amount of 600 g P would correspond to 4 kg dicalcium phosphate supplement, almost half of the annual requirements for P supplement by reproducing cows grazing very P-deficient soils. There is limited information about the mechanisms of P mobilization and deposition but it does appear that they occur in the cow during the reproductive cycle, but only to a limited extent in growing cattle.

Before management strategies dependent on deposition and mobilization can be recommended for the northern industry, it is important to understand the magnitude and limitations of using body P

reserves, how these can be manipulated, and be able to reliably predict when these processes occur. This includes an understanding of the physiological control mechanisms and associated biochemical markers which can be measured in blood or urine. Past research has shown that endocrinological mechanisms involved in P mobilization and deposition are complex, closely linked with those for calcium (Ca), and involve a number of hormones (e.g. parathyroid hormone, leptin). Research in human osteoporosis suggest a number biochemical markers indicative of bone turnover, including osteocalcin and alkaline phosphatase. This project will evaluate these markers as diagnostic tools to identify and estimate the magnitude of P deficiency, mobilization and deposition.

This project will also investigate a novel solution to the long-standing problem for managers of achieving target intakes of P supplements. This is especially relevant in the wet season when the metabolic demands of the animal for P are high and responses of cattle to P supplements are greatest. During the wet season physical access to paddocks to feed out supplements is often difficult, cattle are dispersed, and with green pasture available cattle are often not attracted to consume typical dry lick or block supplements. The universal innate ('hard-wired') behaviour of P-deficient cattle to chew old bones is well known. There is strong evidence that there are constituents of old bones which are attractive specifically to P-deficient cattle and not to P-replete cattle. These past studies indicate that P deficient cows are attracted to old bones by olfactory cues which are produced by decay of the bone marrow. If the constituents causing the attraction to P-deficient cattle could be identified and synthesised there would be an opportunity to mix them into conventional supplements to make such supplements attractive to P-deficient cattle and to achieve high and target intakes of P specifically in P-deficient cattle.

This project examined key constraints to cost-effective and practical use of supplementary P by cattle in northern Australia by using natural physiological mechanisms to obtain benefits through:
(i) clever use of dry season rather than the wet season supplementation in breeder herds,
(ii) simpler and more informative diagnosis of P status, and
(iii) making P supplements attractive to P deficient cattle.

MLA is committed to investing in top quality scientific research, performed by suitably qualified, experienced and registered researchers and organisations. In experiments that involve livestock, MLA acknowledges that such research will first need to be assessed, and if deemed relevant, approved by a recognised Animal Care and Ethics Committee (AEC). The responsibility for obtaining AEC approval lies with the researcher. MLA has in the past not specifically asked for evidence that such AEC approval had indeed been obtained.

1.2 Reviews of the literature which developed the philosophy of the project

1.2.1 Dixon and Coates Review 2010

"A review of phosphorus nutrition of cattle in northern Australian grazing systems" by Rob Dixon and David Coates. 2010. This review was prepared as part of the MLA project to publish the first edition of the book "Phosphorus management of beef cattle in northern Australia" by Desiree Jackson, Joe Rolfe, Bernie English, Bill Holmes and Rebecca Mathews (QDPI), Rob Dixon (QAAFI, UQ), Peter Smith (WADA) and Neil MacDonald (NT DPIF).

1.2.2 Review Paper Northern Beef Research Update Conference 2011

A major paper at the 2011 Northern Beef Research Update Conference, 3 & 4 August 2011, Darwin: Dixon RM, Coates DB, Holmes W, English B, Rolfe J (2011) Phosphorus nutrition and management – overcoming constraints to wider adoption. Proceedings of the Northern Beef Research Update Conference, Darwin, Australia, 3-4 August 2011, p. 102.

2 Project objectives

2.1 General objectives

2.1.1 Objective 1.

Identified the constituents of old bones which are attractive specifically to P-deficient cattle so that these can be used as attractants in P supplements.

2.1.2 Objective 2

Determined the magnitude of, and diet by animal factors affecting, the deposition and mobilisation of body P reserves in breeder cattle in situations typical of northern Australia.

2.1.3 Objective 3

Understood the physiological mechanisms controlling mobilization and deposition of body P reserves in breeder cattle and used this knowledge to help better manage P deficiency in breeder cattle by:

(a) better estimating current animal P supply from diet and body reserves and predicting animal responses; and

(b) producing better diagnostic tools of P status.

2.2 Specific objectives

2.2.1 Objective 1. Task 1.

To identify the constituents of old bones which are attractive specifically to P-deficient cattle so that these can be used as attractants in P supplements. If these constituents can be identified then it should be possible to incorporate these “attractive” compounds into conventional P supplements to achieve target intakes of P by grazing cattle. The experimentation is intended to establish the compound(s) involved in the "attractive" mechanism.

The experimentation will first require identification of the constituent(s) of old bones which are attractive only to P-deficient cattle.

(i) Experimentation will establish a pen bioassay where P-deficient cattle can be offered old bones and supplements in various forms to confirm attractiveness as reported in previous experimentation. Test material will be offered in single or multiple choice situations with suitable replication following biometrical advice, and with scoring of the magnitude of responses on a devised scale.

(ii) A variety of bones (e.g. with various weathering), bone extracts and fractions will be tested and scored for attractiveness to narrow the search for attractive components.

(iii) Solid phase micro extraction (SPME) combined with gas chromatography-mass spectrometry (GC-MS) analysis has been used to identify components in volatiles from human bones as aldehydes (C6-C9), alcohols (C5-C9), aromatic hydrocarbons, ketones, acids and acid esters,

disulfides and halogen components (Hoffman et al., 2009). Similar techniques will be used in this study. Bone extracts and fractions will be analysed (GCMS and/or LCMS) to identify constituents which are potentially the effective attractants and which are most likely to cause the olfactory response in P-deficient cattle.

(iv) It is expected that single compounds, and mixtures thereof, will need to be tested in the cattle bioassay to identify individual attractants and likely synergistic effects in multi-component mixtures. The next step would be to mix synthetic attractant formulation(s) with conventional P supplements containing the P in forms such as dicalcium phosphate and to test in grazing cattle in a small-scale field situation.

As discussed above past experimentation has demonstrated that volatile constituents of old bones are highly attractive to P deficient cattle. This research will require exemption under Part 8 of Queensland Stock Regulations and such approval is expected to require disposal of animals allowed to chew on animal and animal-contaminated matter.

2.2.2 Objective 2. Task 2.

To determine the magnitude of, and diet x animal factors affecting, the deposition and mobilisation of body P reserves in breeder cattle in typical northern Australia grazing systems

Experimentation will be based around well-controlled pen experiments where specified intakes of known diets will be fed. Measurement of net deposition or mobilization will be based on measurement of the P balance (i.e. the difference between P intake and P excretion, and with adjustments for conceptus and milk outputs as appropriate). This is the experimental approach usually used in past comparable experimentation with dairy cows, sheep and goats. Experiments will be designed to examine net mobilization or deposition of P in cows in various stages of pregnancy and lactation and for a variety of diet x animal circumstances.

(i) It is proposed that the first major experiment (year 2) will be a 2x2 factorial design examining the effects of (a) diets very deficient or adequate in P and (b) during mid-late pregnancy and/or lactation in young cows. This is intended to determine the maximum rate and extent to which the young northern *Bos indicus* breeder cow is able to mobilize body reserves of P when the diet is deficient in P, and to deposit P to body reserves when the diet is in surplus in P. Knowledge of the maximum rates (g P/day) and the extent (total g P per lactation) of these processes under optimal conditions is necessary to understand the potential of this mobilization / deposition mechanism. The expectation is that in northern breeder herd systems the cow may need to mobilize P to meet demands in late pregnancy and early to mid-lactation when diet P is insufficient. However, clearly for such mobilization to be repeated in the next pregnancy / lactation the body P reserves would need to be repleted; thus understanding is also needed of the maximum deposition of P in late lactation or post-lactation, and especially during the dry season when P supplements may be fed. This knowledge is needed to understand the maximum extent to which body reserves of P can be used to meet short-term diet deficiencies, and to understand limitations of replenishment of P body reserves for the next parturition.

(ii) In years 3 and 4 experiments will be designed to examine during both pregnancy and lactation the effects of diet (metabolisable energy intake, macro-minerals particularly calcium, protein, mild metabolic acidity) on bone P mobilization and replenishment and the effects of cow

age on capacity to mobilise and replenish bone P reserve. The selection of treatments will depend on the results observed during year 2, and these will be discussed and agreed with MLA on an annual basis.

As discussed above similar experimental approaches have been successfully used to measure the extent of P mobilization and deposition in sheep, goats and dairy cows. However measurement of mineral balance is experimentally difficult and decisions on the details of experimentation will be reviewed annually.

2.2.3 Objective 3. Task 3.

To understand the physiological mechanisms controlling mobilization and deposition of body P reserves in breeder cattle. To use this knowledge to better estimate current animal P supply from diet and body reserves and predict animal responses, to develop better diagnostic tools of P status, and to better manage P deficiency in breeder cattle.

Generalized understanding of the circumstances and extent of P mobilization and deposition, and ability to predict these events across the variety of circumstances in northern breeder cattle herds, is clearly necessary. These depend on an understanding of the hormonal control mechanisms as they interact with diet and animal. In addition the identification of hormones and /or metabolites which can be used as markers (or indicators) of these events in other experimentation and in the commercial field situation would be extremely valuable. Experimentation will examine the relationship between likely markers of bone metabolism and bone-related minerals and the actual changes in P status determined by P balance in the Task 2 experiments. Prospective markers will be related to current diagnostic tools

3 Task 1. Bones_A experiment

Task 1 objective: To identify the constituents of old bones which are attractive specifically to P-deficient cattle so that these can be used as attractants in P supplements. **The Bones_A experiment addressed Task 1 of the project.**

3.1 Bones_A. Factors involved in attraction of phosphorus deficient cattle to ingest bones

3.1.1 Summary of Bones_A experiment

An experiment was undertaken to identify the olfactory constituents of old bones which are attractive to P-deficient cattle. Heifers naïve to P deficiency were either fed a P-deficient diet (LowP) or grazed P-adequate pasture (AdeqP), and preference tests examined their attraction to weathered bones or a control (wood). During phase 1 (d 1-145) the LowP heifers developed severe P deficiency and pica, but demonstrated little attraction to weathered bones. During phase 2 (d 146-155) heifers were allowed to interact with and to chew a variety of weathered bones. Following this experience LowP heifers were more attracted to bones during phase 3 (d 156-166) than during phase 1 (P<0.05), and more attracted than AdeqP heifers during either phase. Subsequently, in phase 4 (d 167-171) LowP heifers were more attracted than AdeqP heifers (P<0.01) to weathered bones than a control of wood, and in phase 5 (d 172-176) to bones with more extended weathering. During phase 6 (d 177-182) attraction was reduced when bones were placed inside a cloth bag. The olfactory constituents from weathered bones were dominated by aliphatic aldehydes and ketones, consistent with long chain fatty acid breakdown. It was concluded that attraction of P-deficient cattle to seek and ingest bones is primarily a learned behaviour as a post-ingestive feedback response where P deficient cattle obtain P from chewing old bones and this develops and reinforces an attraction to old bones. Smell, taste and visual appearance all appear to be important cues for attraction. Pica is likely important in causing P-deficient cattle to investigate unusual materials, including bones, resulting in cattle learning an association between bone chewing and P ingestion. The role of the olfactory constituents is likely to be to reinforce a learned behaviour response rather than being an essential an innate part of the attraction response. A consequence is that the olfactory cues are presumably those associated with the learning behaviour and will not necessarily be consistent.

3.1.2 Background to the Bones_A experiment

Based on knowledge of the industry, numerous anecdotal reports and supplement sales Niethe (2011) concluded that adoption of P supplementation is low with perhaps only 10% of the 6 million cattle in acutely P deficient regions of northern Australia are effectively supplemented during the wet season as recommended. Undoubtedly a number of factors contribute to this situation. However one important reason identified by the team preparing the MLA 2012 book "Phosphorus management of beef cattle in northern Australia" is the practical difficulty on large cattle enterprises of feeding P supplements during the wet season. These difficulties include achieving target intakes of low palatability P supplements when cattle are dispersed, and with green pasture are often not attracted to consume typical dry lick or block supplements unless show 'salt hunger'.

The importance of this management constraint led to the objective of Task 1 to investigate a known natural mechanism specific to P deficient cattle to make P supplements more attractive to P deficient cattle. It is well known that P deficient cattle will avidly seek out and chew old carcasses and bones, apparently to obtain P. Past research concluded that this behaviour of P deficient cattle is an innate ('hard-wired') behaviour and that there are constituents of old bones which are attractive specifically to P-deficient cattle but not to P-replete cattle (Blair-West et al. 1989, 1992). In behavioural choice assays, P deficient cows exhibited an avid appetite for old weathered bones, whole, crushed or powdered bones, and bone marrow but were not attracted to fresh bones, aged blood or fat, or phosphate salts (Blair-West *et al.*, 1990). In these bioassays a stereotypical behaviour of P deficient animals included immediate investigation of choice materials (Blair-West *et al*., 1990). The selection was evidently olfactory-based with choices frequently made from a distance of a metre or more, with covered attractive items detected and chewed. If the constituents causing the attraction to P-deficient cattle could be identified and synthesised there would be an opportunity to mix them into conventional supplements to make such supplements attractive to P-deficient cattle and to achieve high and target intakes of P specifically in P-deficient cattle. A second reason is that consumption of carcasses and bones may conflict with community and export market expectations for responsible and ethical cattle management and clean, healthy and wholesome meat products.

The rationale of the research was that if the constituents of old bones which caused attraction of P deficient cattle to bones could be identified then it should be possible to incorporate these “attractive” compounds into conventional P supplements based on calcium phosphates to achieve increased and target intakes of P by grazing cattle. Thus the first objective of the experimentation was to identify the constituent(s) of old bones which are attractive specifically to P-deficient cattle. The next objective was to use this knowledge to design and test novel P supplements more attractive to P deficient cattle.

The scientific paper describing this work is reported (Appendix 4).

3.1.3 Background to Bones_A experimental design

A substantial experiment was conducted at Brian Pastures Research Station, Gayndah, from September 2012 through to March 2013 to investigate the attraction of phosphorus (P) deficient cattle to the olfactory components present in old weathered bones. This experiment was the first step to investigate a novel solution to the problem of achieving adequate and target intakes of phosphorus supplements. The behaviour of phosphorus-deficient cattle to chew old bones is well known and documented.

There are numerous anecdotal reports that cattle exhibiting 'pica' behaviour will chew sticks, cables, plastic, general rubbish and lick soil, as well as eating old bones and carcasses if they are available. Such behaviour has been discussed in detail by authors such as Green (1925), Theiler and Green (1932) and McCosker and Winks (1994).

A series of experiments from the Howard Florey Institute, The University of Melbourne (Blair-West *et al.* 1989, 1992) concluded that such attraction of P-deficient cattle is innate, and the attraction is due to olfactory components given off by weathered old bones. The first objective of

Task 1 was to identify the chemical composition of the key attractive constituents of bones associated with this innate behaviour. It was planned that if such olfactory constituents causing attraction could be identified, then synthetic forms of these olfactory constituents could be mixed with conventional phosphorus supplements to increase their palatability and attractiveness to P-deficient cattle and to achieve high intakes of supplement.

Phase A of the experimental program was to confirm and validate the test of attraction of P-deficient cattle to old weathered bones as had been reported by Blair-West and colleagues. A reliable, consistent and functioning bioassay of this type suitable for cattle in a tropical and sub-tropical environment was required to be able to identify which classes and types of old bones (and extracts from old bones) are attractive to P-deficient cattle. The approach adopted was based on the procedure used in the Blair-West *et al.* (1989, 1992) experiments in which mature *Bos taurus* P-deficient cattle were offered bones (or various types, classes and extracts of bones) in single-animal attraction tests and in which the principal criterion was the behaviour of animals demonstrating attraction to the offered test materials. However the approach in the present project was more rigorous in that a formal experimental design was used, a much larger number of animals was tested, there were groups of P-deficient and P-adequate animals prepared from an initial herd, and the attraction test procedures used objective measurements rather than subjective observations. The present experimentation set out to measure the chemical constituents in the olfactory components reported by Blair-West *et al.* (1989, 1992) to cause the attraction of the P-deficient cattle to the weathered bones.

It was intended that a Phase B would use this bioassay to test in phosphorus-deficient cattle the attraction of various types and extracts of bones (e.g. aging, heating, aqueous extracts) and related materials (muscle, fat, collagen, amines) to identify those chemical constituents which are common in causing attraction. Sampling and chemical analyses of the olfactory (smell) components of bones and extracts would identify those chemical constituents which are associated with attractiveness of bones to phosphorus deficient cattle.

3.1.4 Bones_A Experimental

Weaner heifers (Belmont Red genotype, 160-180 kg) were sourced from Belmont Research station in July 2012 and relocated to Brian Pastures Research Station. These heifers were considered particularly appropriate for several reasons: (i) information was available about the history and management of these weaners, (ii) the heifers were docile and tractable and thus more suitable than most industry cattle for animal behaviour testing experimentation, and (iii) Information and experience over many decades (high-phosphorus fertile alluvial soils, high animal growth rates, no known observations of bone-chewing or other symptoms of P-deficiency) indicated that neither the weaners or their dams were likely to have been P-deficient or acquired any behavioural responses associated with P deficiency. Thus these animals were expected to be entirely naive to bone attraction responses associated with P-deficiency (permission was obtained from the Chief Inspector of Stock in Queensland to feed animal material (bones) to cattle).

The 20 weaner heifers were divided into two balanced groups: (i) to be reduced to a P-deficient status by feeding a P-deficient diet for an extended interval, or (ii) maintained as P-adequate

animals by grazing improved pasture on fertile soils expected to be P-adequate. The P-deficient heifers were fed in the yards as a group on a diet based on wheat straw, wheat flour, sugar, urea, and a mineral mix without P. Measurements of blood P (PIP, plasma inorganic P), faecal P, animals liveweight, and (for the yarded heifers) voluntary intake were made. Attraction of the heifers (i.e. heifers from both the P-adequate and P-deficient treatment groups) to an array of weathered old bones was tested on an individual animal basis using a standard procedure based on that reported by Blair-West *et al.* (1989, 1992) and colleagues. Heifers were introduced for 2 min into a small round yard (10 m diameter) with two feeders which contained either bones or wood as a control. The bones or wood were covered with mesh to prevent the heifers from actually making contact with or chewing the bones or wood during this phase. The bones comprised four types of older bones expected, on the basis of the previous experimentation, to be attractive to P-deficient cattle. The attraction of the heifers to the feeders was scored against criteria (e.g. the length of time during which a heifer was at or near a feeder, and a subjective score of degree of interest by the animal in the contents of the trough).

After being fed a severely P deficient diet for approximately three months heifers had reduced PIP concentrations from the initial 2-3 mmol/L to <1 mmol/L the reported olfactory attraction response should occur. The heifers were demonstrating 'pica' behaviour with chewing of cables, trough legs, poly pipe and weeds, and licking of soil. However, in the individual animal attraction tests none of these P-deficient heifers demonstrated consistent attraction to the weathered bones. Thus the attraction of P-deficient cattle to weathered bones appeared to involve a 'learning' behaviour, and was not a simple innate behaviour. The opportunity for learning by the heifers was provided by allowing access to a variety of bones. Most of the P-deficient heifers were immediately and strongly attracted to the bones presented in this manner, and ingested bone material and were consistent with the behavioural 'pica' already observed in the P-deficient heifers. No such attraction occurred with the control P-adequate heifers. Following this learning experience the P-deficient heifers exhibited attraction to the bones. Thus it was concluded that the attraction and chewing of bones by P-deficient cattle was a learned, not an innate response.

A hypothesis to explain the published results, and the results of the present experiment is as follows. It appears that the P-deficiency in cattle is associated with the development of 'pica' behaviour during which the animal seeks out, investigates, and ingests unusual materials in its environment. In a natural rangeland environment old bones and carcasses will be available and are one of the very few concentrated sources of phosphorus potentially available to grazing herbivores such as cattle. It appears that a positive feedback develops when a P-deficient animal ingests bones. This provides a learning experience with positive outcomes, and the P-deficient animal then develops a strong attraction for old bones. The importance of the olfactory components associated with the weathered bones is that they provide a cue to the P-deficient animal that is associated with a positive outcome, and therefore the olfactory components can appear to be the cause of the attraction of P-deficient cattle to weathered bones when in fact they are simply helping to reinforce a learned behaviour.

If this latter hypothesis is correct a fundamental premise for the Task 1 objectives is also not correct. This clearly has major implications for the continuation of research in the Task 1 area of the project.

3.1.5 Conclusions

This finding that the attraction of P deficient cattle to old bones is a learned positive feedback response and not an innate response had major implications for the Task 1. It was in conflict with the hypothesis that specific olfactory compounds or groups of compounds could identified which could be included in P supplements to make them more attractive, and specifically attractive to all -deficient cattle. The olfactory constituents providing the attraction cues are likely to vary among herds and there may be any specific attractant(s) which can be used to make P supplements more palatable.

Following discussion with MLA it was decided not to continue this Task 1 area of research in the present project.

3.1.6 References specific to Task 1

(Additional references are given in the scientific paper under review and is an attached document – Appendix 4)

Blair-West JR, Denton DA, Nelson JF, McKinley MJ, Radden BG, Ramshaw EH (1989) Recent studies of bone appetite in cattle. *Acta Physiol Scand* 136, *Supplementum* 583, 53-58.

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4 Tasks 2 and 3 – Pens_A to Pens_E

4.1 Experimental section 2. Pens_A experiment

Pens_A. Capacity of first-calf cows to mobilize and deposit body reserves when fed severely P deficient diets through pregnancy and/or lactation, and during post-weaning recovery.

Objective Tasks 2 and 3.

4.1.1 Summary of experiment

A pen experiment was undertaken to determine the magnitude of the deposition and mobilisation, and the physiological mechanisms controlling, body P reserves in first-calf *Bos indicus* cows (FCC). Heifers were fed semi purified diets severely P deficient (LP) or P adequate (HP) during late pregnancy and/or early lactation in a 2x2 factorial design where the main effects were the LP or HP diets imposed during pregnancy and/or lactation (i.e. 4 treatment diets LP-LP, HP-LP, LP-HP and HP-HP). The cows were weaned after 12 weeks and during a 6 week recovery period were all fed the HP diet also fed during lactation. Measurements were made of voluntary intake (VI), DM digestibility (DMD), P balance (as P intake minus P faecal excretion, and during lactation minus also milk P), liveweight (LW) change, milk production and calf growth. The concentrations of a range of metabolites in blood as markers of P metabolism were measured. During pregnancy the DMI and ME by the LP FCC was only 78% and 76%, respectively, of the HP diet; FCC fed the LP diet lost 10 kg conceptus-free LW (CF-LW), while the FCC fed the HP diet gained 26 kg CF-LW (i.e. 37 kg difference). During lactation the VI of the FCC fed the LP diet was lower than for the HP diet, but also depended on whether animals had been fed LP or HP in pregnancy. VI of LP-LP treatment animals was 78% and 45% of the VI of the HP-LP and the HP-HP treatment animals, respectively. VI of the HP-LP treatments animals was 58% of the HP-HP animals. Thus VI during early lactation was increased by feeding HP during the last 4 months of pregnancy. This occurred even though the plasma inorganic phosphorus (PIP) concentrations during the 12 weeks of lactation were similar and low and were not affected by the diet P concentration during pregnancy. Diet DM digestibility (DMD) was not affected by diet P and the estimated ME intakes and animal LW change reflected voluntary DM intakes. There were large differences among diet treatments in milk production (range 4.9 – 8.7 kg/day; 13.9-24.1 MJ/day) and calf growth (0.57- 0.93 kg/day). These effects of diet P concentration on intake and LW change were generally explained as main effects of diet P concentration during the pregnancy or lactation; the interactions were generally not statistically significant. The exception was for P balance during lactation; the FCC fed LP during lactation (LP-LP and HP-LP) or HP during both pregnancy and lactation (HP-HP) exhibited an average net mobilisation of 5.6 – 7.5 g P/day (17.2 – 19.7 mg P/kg LW.day) but the HP-LP treatment FCC mobilised only 1.6 g P/day (4.4 mg P/kg LW.day). Also in the HP-HP diet P balance was similar throughout lactation, but for the other three treatment diets declined as lactation progressed. In FCC fed LP the concentrations of PIP were always low (<0.8 mmol/L), while increased plasma calcium (Ca), and large increases in plasma Ca/P ratio and carboxy-terminal telopeptides of type 1 collagen (CTX-1) concentrations indicated substantial bone mobilization. Changes in bone volume and structure in rib and hip (tuber coxae) bone biopsies also showed that there was substantial bone mobilization. In LP diets parathyroid hormone (PTH) was suppressed and was thus unlikely to be the main hormone stimulating bone resorption in these diets. Active 1,25diOH Vitamin D3 (active vit D3) was increased by LP diets. Osteocalcin (OCN), a

marker of bone deposition, was high in all treatments indicating continuing bone growth in these young cows, but differences in osteoid tissue indicated that less of this bone was mineralized in the LP diets during lactation. Overall the results indicated that the animals fed the LP diets during lactation were severely P deficient and were rapidly losing LW. These FCC mobilized bone during lactation regardless of diet P intake, but the loss was greater in animals fed the LP diet. There was a carryover effect of feeding HP during pregnancy on VI and milk production in early lactation, and VI and milk production were lower than that when the HP diet was provided during lactation. The HP-LP cows were 34 kg heavier at parturition, and 42 kg heavier at weaning. During the replenishment phase the HP-LP treatment animals increased intake and recovered LW, but there was a lesser recovery of the LP-LP animals. Feeding a HP diet and P replenishment during 6 weeks post-weaning was sufficient to normalise many markers and to partially replenish bone P in the HP-LP and LP-HP treatments, but to a lesser extent in the LP-LP treatment. In conclusion there were large effects of LP diets fed in late pregnancy or lactation to reduce FCC productivity and these were associated with large losses in LW.

4.1.2 Background

The Pens_A experiment was the first in the series to investigate the occurrence and magnitude of the net mobilization of P from, and net deposition of P into, body reserves in the breeder cow through pregnancy and lactation. The experiment investigated the first-calf cow (FCC) as an important cohort of the breeder herd which is particularly sensitive to under-nutrition. Current P recommendations and the need for supplement P are based on calculation of the amounts of P required and the amounts ingested without any quantitative consideration of net mobilization or deposition. If the rates of mobilization of body P are sufficient to alleviate diet P deficiency under at least some circumstances, then it is likely that management will be able to use this mechanism to change the seasons of the year when P supplements are fed. It may be possible to provide P supplements during some intervals (such as the dry season) when P intake is in excess of immediate P requirements, and to use this additional P during the wet season to at least partially meet P needs when the diet is deficient in P relative to ME and N contents. It appears likely that the magnitude of P mobilization and deposition will depend on many factors such as current body P reserves, cow age, and current intakes of metabolisable energy, protein and calcium.

The project investigated such nutritional factors and attempted to define the magnitude of mobilization and deposition of P and of changes in LW associated with P deficiency, and the circumstances where they are likely to occur. The most obvious use of such information in management of commercial breeder herds in P-deficient rangelands will be to feed P supplements in the late wet and dry season (when it is practical and convenient to feed supplements) to meet the nutritional needs for P through the annual cycle. The issues include whether such a change in timing of P supplementation can alleviate, or effectively avoid, the adverse effects of P deficiency, and if so whether inefficiencies are introduced in the utilization of P supplements.

4.1.3 Material and methods

Animals, diets and experimental design

Fifty pregnant Droughtmaster heifers (*ca.* 2.5 years of age) from the commercial herd on the DAF Spyglass Research Station were relocated to the DAF Brian Pastures Research Facility in June 2013.

Stage of pregnancy was estimated by ultrasound scanning. After recovery from transport on pasture, and following vaccination and anthelminthic treatment, 40 heifers were selected on the basis of temperament, VI and their adjustment to individual pens (*ca.* 33 m^2). During this adjustment phase they were fed a low P diet for 2 weeks to mimic dry season pasture. The animals remained in the individual pens for 8-9 months through the experiment which comprised an introductory interval (4-6 weeks) when all animals were fed a low P diet, and then fed low P (LP) or high P (HP) diets during the last 14 weeks of pregnancy, 12 weeks of lactation and then for 6 weeks post-weaning. During pregnancy and lactation four diet treatments were imposed in a 2x2 factorial design, the factors being LP or HP diets fed *ad libitum* during pregnancy and/or lactation. Thus there were 2 diet treatments during pregnancy and 4 diet treatments in lactation. The calves were weaned after 12 weeks of lactation, and following weaning all the cows were offered the lactation phase HP diet *ad libitum* for 6 weeks.

Through pregnancy the cows were offered a total mixed diet without (LP) or with (HP) added P as calcium phosphate (Kynophos®) to provide diets deficient (1.0 g P/kg DM) or adequate (1.80 g P/kg DM) in P. The diets were based on wheat straw, flour, sugar, canola oil, urea and limestone and contained about 90 g CP/kg DM and 560 g NDF/kg DM) (Table A-1). At parturition half of each pregnancy treatment group was allocated to either a P deficient (0.84 g P/kg DM) (LP) or a P adequate (2.1 g P/kg DM) (HP) diets. For these lactation diets a proportion of the straw was replaced with flour and sugar to increase ME content. These diets contained about 120 g CP/kg DM and 440 g NDF/kg DM and were intended to provide ME intakes representative of high quality wet season pasture. Thus during lactation there were 4 dietary treatments: high P in pregnancy/high P in lactation (HP-HP); high P in pregnancy/low P in lactation (HP-LP); low P in pregnancy/high P in lactation (LP-HP) and low P in pregnancy/low P in lactation (LP-LP). During the replenishment phase post-weaning all cows remained in their individual pens and were fed the HP lactation diet to examine the carryover effects of the previous diet treatments imposed during pregnancy and lactation.

The samples and measurements obtained during the three phases of the experiment were as follows:

Phase A pregnancy

Two treatments (LP and HP) were imposed. The procedures were:

(i) Heifers were fed individually in pens. VI of feed and LW were measured weekly.
(ii) Total collections of faeces were conducted over 5-day intervals during the week before the diet treatments were imposed (TC-01), and then at approximately 8 and 2 weeks before parturition (TC-02 and TC-03, respectively).
(iii) Spot samples of urine were obtained at the beginning and end of each total faecal collection period,
(iv) Jugular blood samples were obtained fortnightly,
(v) Bone biopsies from the 12th rib were obtained at the beginning of the diet treatments and at calving.
(vi) At calving the calf birth weight and cow liveweight (LW) were measured and blood, urine, and faecal samples obtained.

Table A-1. Pens_A. The ingredients (g as-fed/kg) and the composition (g as-fed/kg DM for DM content; g/kg DM for other constituents) of the mixed diets containing low or high concentrations of P and fed to cows in late pregnancy, early lactation and during recovery post-weaning. Mean and SD (in parenthesis) of the constituents

Attribute	Treatment diet offered			
	Pregnancy		Lactation	
	Low-P	High-P	Low-P	High-P
Ingredient (as fed, g/kg)				
Wheat straw	668	664	514	509
Wheat flour	187	186	283	280
Sugar	93.2	92.6	141	140
Canola oil	29.6	29.4	22.3	22.1
Urea	11.9	11.8	22.3	22.1
Calcium phosphate[A]	0.0	6.2	9.5	9.4
Limestone	3.1	3.1	4.7	4.7
Ammonium sulphate	3.1	3.1	4.7	4.7
Sodium chloride	3.1	3.1	4.7	4.7
Rumigro premix	0.51	0.50	0.77	0.76
Elanco rumensin 100	0.25	0.25	0.27	0.27
Composition				
Dry matter (g/kg as fed)	940 (7)	941 (2)	942 (6)	942 (6)
Organic matter (g/kg DM)	920 (5)	922 (10)	920 (5)	922 (6)
Crude protein	88 (6)	91 (2.5)	124 (6)	118 (6)
Neutral detergent fibre	560 (26)	557 (17)	435 (29)	447 (23)
Acid detergent fibre	380 (15)	370 (17)	279 (28)	281 (19)
Lignin	48 (5.1)	42 (3.2)	34 (5.7)	34 (3.6)
Starch	131 (20)	129 (22)	203 (21)	194 (10)
Crude fat	40 (1.5)	40 (2.2)	31 (3.0)	30 (2.6)
Ca	2.8 (0.24)	3.6 (0.17)	3.2 (0.3)	4.2 (0.49)
P	1.0 (0.06)	1.80 (0.08)	0.84 (0.14)	2.1 (0.35)
Mg	0.8 (0.05)	0.9 (0.0)	1.8 (0.17)	1.7 (0.11)
Ca/P	2.9 (0.43)	2.0 (1.9)	3.9 (0.45)	2.0 (0.27)

[A,] Kynophos.

Phase B lactation

The four treatments were imposed during the first 12 weeks of lactation with the following sampling and measurement procedures:

(i) Cows with their calves were continued in individual pens. VI of feed and LW of both cows and calves were measured weekly,
(ii) Total collection of faeces were conducted during the first, third and last fortnights of lactation (TC-04, TC-05 and TC-06, respectively),
(iii) Urine was sampled at the beginning and end of each total faecal collection period,
(iv) Milk production was estimated fortnightly by machine milking and milk samples were retained.
(v) Jugular blood was sampled fortnightly,
(vi) Bone biopsies from the 11th rib and hip (*tuber coxae*) were obtained during the last fortnight of lactation.

Phase C replenishment

The HP lactation diet was fed to all animals for 6 weeks post-weaning with the following sampling and measurement procedures:

(i) Voluntary Intake of feed and LW of cows were measured weekly,
(ii) Total collection of faeces was conducted during the last week of the phase (TC-07),
(iii) Urine sampling at the beginning and end of the total faecal collection period,
(iv) Jugular blood was sampled fortnightly,
(v) Bone biopsy at the end of the phase from the 11th rib and the tuber coxae,

Voluntary intake of DM, P and Ca, and digestibility, were calculated by classical procedures. The differences between P intake and faecal excretion of P (I-F) was used as the estimate of P balamce during pregnancy and the post-weaning recovery interval. During lactation the estimated milk P secretion was also subtracted from the P intake. The P requirements of the animals was calculated as described by CSIRO (2007) except that the true absorption coefficient was assumed to be 0.8 rather than 0.7; this value was assumed as that observed for tropical forages (Ternouth and Coates 1992) and for MDCP which provided the majority of the P in the HP diets. Also the intake, liveweight and milk production of the HP or HP-HP diet were used since this is the amount of P required when the animal is not limited by P availability.

4.1.4 Results

PREGNANCY. Animal production measurements

The composition of the diet fed is given in Table A-1. The actual intake of P by animal fed the HP diet was 12.9 g P/day which was 111% of the calculated requirements (Table A-2). However the actual P intake of the LP animals was 43% of that required for the VI, LW and LW gain measured for the HP animals.

Table A-2. Pens_A. PREGNANCY. The DM intake and digestibility, intake and balance of P (intake minus faecal P; (I-F), and LW change during late pregnancy of heifers fed Low P or high P diets. The calculated P requirement is that for heifers fed the HighP diet where diet P did not constrain voluntary intake

Measurement	Diet treatments		sem	Probability
	LowP	HighP		
n	19	18	--	--
P required (g P/day)	10.9	10.9	--	--
P intake (g P/day)	4.7	12.1	--	--
P (Intake/Required)%	43	111	--	--
T-LW. Week -14 from parturition (kg)	382	393	5.79	0.177
T-LW. Week -1 from parturition (kg)	409	458	6.32	<0.001
T-LW change (kg/day)	0.248	0.651	0.0270	<0.001
CF-LW. Week -14 from parturition (kg)	365	376	5.79	0.177
CF-LW. Week -1 from parturition (kg)	355	402	3.03	<0.001
CF-LW change (kg/day)	-0.138	+0.295	0.0300	<0.001
Calf birth weight (kg)	31.4	32.5	1.009	0.454
Voluntary intake (kg DM/day)	4.971	6.707	0.1191	<0.001
Voluntary intake (g DM/kg T-LW.day)	13.3	17.0	0.0273	<0.001
ME content (MJ ME/kg DM)	9.01	8.75	0.174	0.277
ME intake (MJ ME/day)	51.9	68.4	2.4	<0.001
ME intake (est kJ ME/kgLW.day)	127	156	5.4	<0.001
DM digestibility(g/kg)_TC02	626	607	17.5	0.418
DM digestibility(g/kg)_TC03	620	609	6.94	0.265
DM digestibility(g/kg)_mean	623	608	10.1	0.277
DM intake (kg/day)_TC02	6.40	7.87	0.261	<0.001
DM intake (kg/day)_TC03	5.12	7.64	0.248	<0.001
DM intake (kg/day)_mean	5.76	7.76	0.214	<0.001
P intake (g P/day)_TC02	5.83	16.44	0.445	<0.001
P intake (g P/day)_TC03	6.52	12.60	0.354	<0.001
P intake (g P/day)_mean	6.18	14.52	0.326	<0.001
P faeces (g P/day)_TC02	5.12	11.35	0.491	<0.001
P faeces (g P/day)_TC03	4.05	10.57	0.252	<0.001
P faeces (g P/day)_mean	4.58	10.96	0.260	<0.001
P (I-F) (g P/day)_TC02	0.71	5.09	0.457	<0.001
P (I-F) (g P/day)_TC03	2.48	2.02	0.277	0.250
P (I-F) g P/day)_mean	1.59	3.56	0.263	<0.001
P intake (mg P/kg LW.day)_mean	15.1	33.3	0.66	<0.001
P faeces (mg P/kg LW.day)_mean	10.4	24.9	0.59	<0.001
P (I-F) (mg P/kg LW.day)_mean	3.9	8.2	0.61	<0.001

At the commencement of the experiment and when heifers were in mid-pregnancy their average LW was 376 kg (Table A-2) and body condition score 3 (5-point scale). During the last 14 weeks of pregnancy to immediately after calving the total LW of the heifers increased by 27 and 65 kg (from 0.25 to 0.65 kg/day), while the conceptus-free LW (CF.LW) decreased by 10 kg and increased by 26 kg (from -0.14 to +0.30 kg/day) in those animals fed the LP and the HP diets, respectively. This was associated with lower VI in the LP heifers but no change in DM digestibility (mean 616 g DM/kg and 8.9 MJ ME/kg DM). VI of ME of the LP animals were 76% and 81% of the HP animals on a MJ ME/day and on a kJ ME/kg LW.day, respectively (Table A-2). The VI of the animals during the 14 weeks is shown in Figure A-1a and the CF.LW change in Figure A-1b.

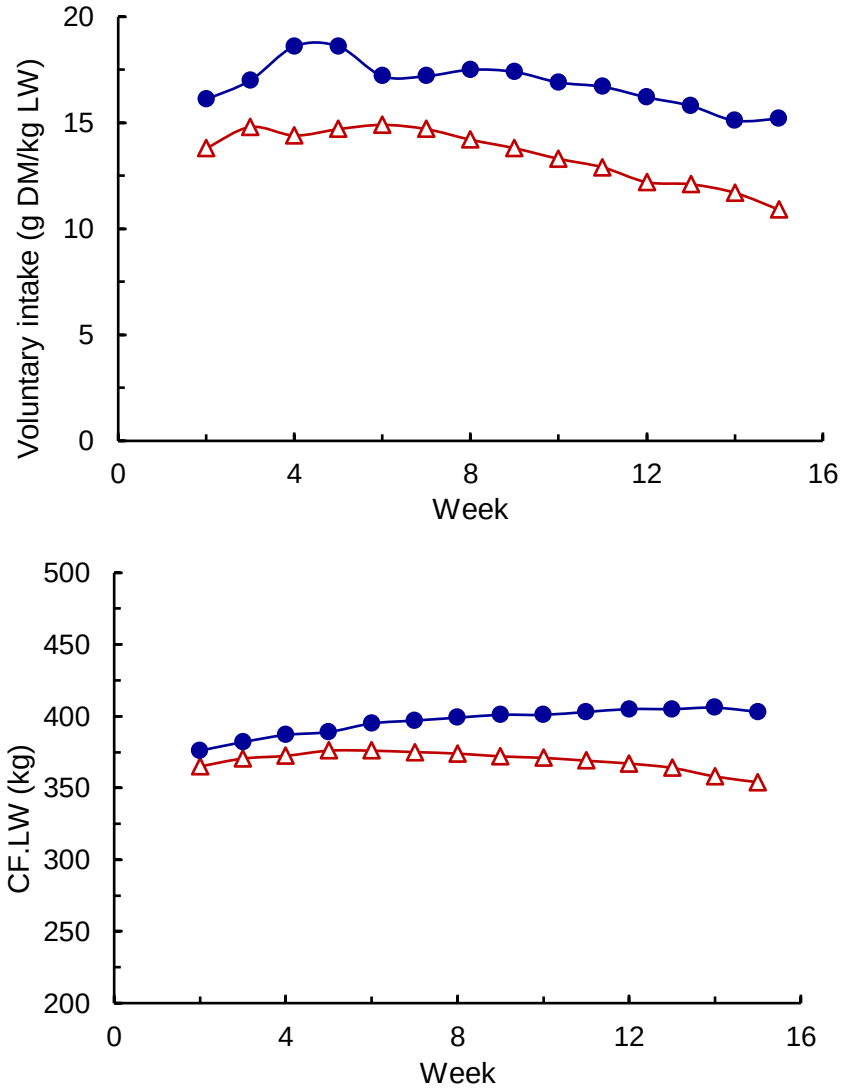


Figure A-2a and 2b. Voluntary intake (VI) of feed (g DM/kg LW.day) (Fig 1a) and conceptus-free liveweight (CF.LW)(kg) (Fig 1b) in FCC during the last 14 weeks of pregnancy. The heifers were fed low P (LP) (Δ) or high P (●) diets in late pregnancy.

Table A-3. Pens_A. PREGNANCY. The mean concentrations of minerals and endocrine markers in plasma of heifers from mid-pregnancy to parturition when fed Low P or high P diets. In addition measurements of rib bone comprising the cortical bone thickness (CBT), P concentration in cortical bone (P conc) and the index phosphorus per unit surface area of cortical bone (PSACB) are given

Measurement	Diet treatments		s.e.m.	Probability
	LowP	HighP		
n	19	18	-	-
Metabolites and markers in plasma				
Plasma PIP (mmol/L)	0.91	1.78	0.042	<0.001
Plasma Ca (mmol/L)	2.63	2.36	0.0276	<0.001
Plasma Ca/PIP ratio	2.89	1.33		
Plasma Mg (mmol/L)	0.835	0.778	0.01996	0.051
Plasma Log PTH (pg/mL)	3.15	5.28	0.247	<0.001
Plasma CTX-1 (ng/mL)	3.50	1.24	0.209	<0.001
Plasma OCN (ng/mL)	46.2	58.7	3.19	0.010
Plasma BAP (UI/L)	45.2	23.5	2.88	<0.001
Rib bone				
Initial CBT (mm)	3.58	3.61	-	-
Calving CBT (mm)	4.23	4.63	0.141	0.058
Change in CBT to calving	0.66	1.03	0.134	0.065
Initial Pconc (mg/cc)	118	124	-	-
Calving Pconc (mg/cc)	120	135	3.3	0.002
Change in Pconc to calving (mg/cc)	2	11	4.5	0.093
Initial PSACB (mg/mm^2)	0.421	0.446	-	-
Calving PSACB (mg/mm^2)	0.510	0.626	0.0248	0.002
Change in PSACB to calving (mg/mm^2)	0.089	0.177	0.0194	0.003

LACTATION. Animal production measurements

The actual intake of P by FCC fed the HP diets through both pregnancy and lactation was 91% of the calculated requirement based on the actual LW, intakes, milk production and LW changes of these animals (Table A-4). The FCC fed the LP-HP diet ingested only 80% of the calculated P requirement and this was associated with a lower intake of diet DM. However because these FCC were lower in LW and had lower DM intakes, but the P requirement was calculated on the basis of the LW and intake of the animals fed the HP-HP during lactation, the actual P intake of the LP-HP animals would have approached the calculated requirement. The FCC fed the LP diet (LP-LP and HP-LP treatments) were severely P deficient and the calculations indicted that these animals were consuming only about 20% of their calculated P requirement.

During lactation the FCC fed the LP diet lost substantial LW (-65 kg or -0.77 kg/day (irrespective of their diet during late pregnancy (Table A-4, Figure 2b). However the HP-LP cows were 45 kg heavier than the LP-LP at calving and had 29% higher voluntary intakes during lactation (6.27 versus 4.95 kg DM/day). The VI (Figure 2a) of both treatment groups of FCC fed HP during lactation was higher (9.23 and 10.51 kg DM/day; 24.2 and 25.1 g DM/kg LW/day) than for the FCC fed the LP diet, and their LW gains during lactation (0.45 and 0.40 kg/day) was similar regardless of their diet P concentration during late pregnancy and thus their P status at parturition. Through both pregnancy

and lactation the LP-HP cows consumed 92% more DM, and were 106 kg heavier at weaning, than the LP-LP heifers. Furthermore the HP-HP cows consumed 122% more DM and were 141 kg heavier at weaning than the LP-LP cows.

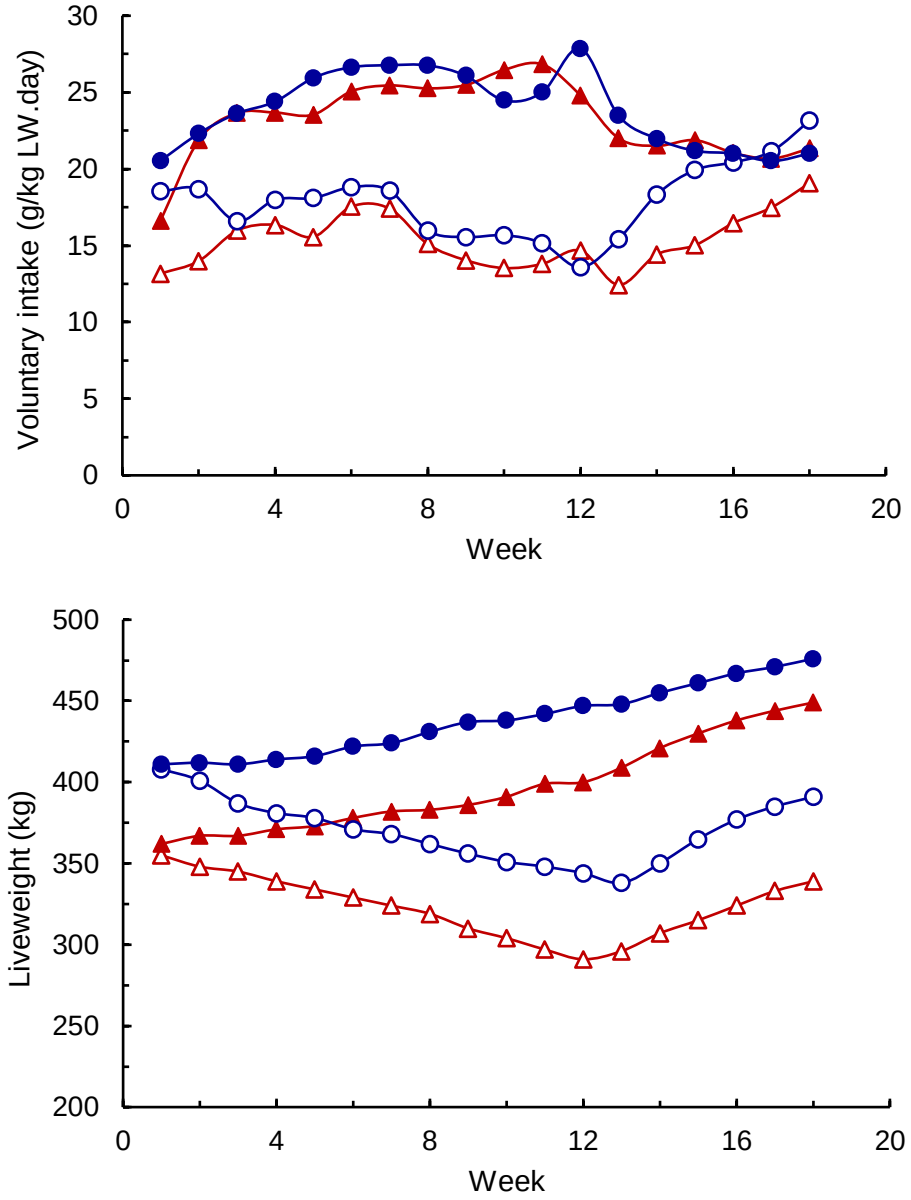


Figure A-2a and 2b. Voluntary intake (VI) of feed (g DM/kg LW.day) (Fig 1a) and liveweight (kg) (Fig 1b) in FCC during weeks 1-12 lactation and then post-weaning during replenishment (weeks 13-18). Cows were fed (LP-LP) (Δ), HP-LP (○), LP-HP (▲) or HP-HP (●) diet in pregnancy and lactation.

The measurements from the total collection of faeces at the three intervals of lactation indicated that the excretion of P and Ca in faeces approached intake in each of the diets except the LP-HP (Tables A-5 and A-6). The measurement of the secretion of P and Ca in milk (Table A-7) allowed calculation of the P balance (Table A-8). These results indicated that there was net mobilisation of

5.6 – 7.5 g P/day in the FCC fed the LP-LP, HP-LP and HP-HP treatments, but mobilisation of only 1.6 g P/day for the HP-LP diet. The low net mobilisation of body P in the latter treatment occurred during all three total collection intervals and did not appear to be an experimental aberration. In the FCC fed the LP-LP and the HP-LP diets there appeared to be a decrease in the negative P balance (i.e. in the body P mobilisation) as lactation progressed (means -8.9, -7.0 and -3.5 g P/day, respectively for TC04, TC05 and TC06). However there was no such trend for the HP-HP diet with P balance (i.e. body P mobilisation) increasing from -3.8 to -9.2 (mean -7.5) g P/day as lactation progressed from the TC04 to the TC06 interval.

The composition of milk was not generally affected by the diet treatments, the exception being for lactose concentration which was increased slightly by the LP diet in both pregnancy and lactation (Table A-7). The milk production, both kg/day and as MJ energy/day (Table A-7), was reduced by feeding LP in either (or both) pregnancy and in lactation ($P<0.05$, $P<0.001$), but there was apparently no interaction effect. Milk production ranged from 4.90 – 8.69 kg/day and 13.9 – 24.1 MJ energy/day. Calf growth reflected the differences in milk production and ranged from 0.54 – 0.96 kg/day (Table A-4), and was correlated with the milk production measured at the fortnightly intervals. This latter relationship was as expected and importantly provides additional evidence that the milk energy output varied through a wide range as a consequence of the P nutrition of the cows.

Table A-4. Pens_A. LACTATION. The LW and changes in LW, calf birth weight and LW gain, DM digestibility and estimated ME intakes of the FCC given four treatments (fed LowP or HighP diets during late pregnancy and lactation in a 2x2 factorial design). The measurements in the present table were made during the 12 weeks of early lactation. Thus diet treatments were LowP-LowP (LP-LP), HighP-LowP (HP-LP), LowP-HighP (LP-HP) and HighP-HighP (HP-HP). The calculated P requirement is for given for FCC fed the HighP diet through both pregnancy and lactation (HighP-HighP diet) where diet P did not constrain voluntary intake.

Measurement	Low P in lactation		High P in lactation		s.e.m.			Probability		
	LowP-LowP	HighP-LowP	LowP-HighP	HighP-HighP	Preg	Lact	PxL	Preg	Lact	PxL
n	10	9	9	9	--	--	--	--	--	--
P required (g P/day)	22.6	22.6	22.6	22.6	--	--	--	--	--	--
P intake (g P/day)	3.9	4.9	18.1	20.6	--	--	--	--	--	--
P (Intake/Required)(%)	17	22	80	91	--	--	--	--	--	--
LW. Start of lactation (kg)	362	407	362	411	6.64	ns	ns	<0.001	0.839	0.841
LW. Wk 12 of lactation	297	342	400	445	8.2	8.2	11.6	<0.001	<0.001	0.959
LW change (kg)	-65	-65	+38	+34					<0.001	
LW change (kg/day)	-0.77	-0.77	+0.45	+0.40	0.074	0.072	0.105	0.454	<0.001	0.181
Calf LW gain Wks 1-12 (kg)	0.54	0.72	0.81	0.96	0.033	0.033	0.062	0.004	<0.001	0.883
Calf LW at 12 weeks (kg)	81	91	101	112	1.7	1.7	2.5	<0.001	<0.001	0.749
DM intake (kg/d)_(12 week mean)	4.95	6.27	9.23	10.51	0.264	0.264	0.385	<0.001	<0.001	0.741
DM intake (g/kg LW.d) (12 week mean)	15.1	17.0	24.2	25.1						
DM digestibility (g/kg)_TC-4	659	644	666	663	9.6	9.6	13.6	0.512	0.334	0.637
DM digestibility (g/kg)_TC-5	665	630	668	654	13.7	13.7	19.3	0.219	0.484	0.599
DM digestibility (g/kg)_TC-6	667	665	678	668	8.4	8.4	11.8	0.615	0.554	0.716
DM digestibility (g/kg)_mean	664	646	669	661	7.5	7.5	10.6	0.246	0.371	0.683
ME content (MJ ME/kg DM)_mean	9.71	9.41	9.79	9.66	0.13	0.13	0.18	0.246	0.371	0.663
ME intake (MJ ME/day)_mean	50	63	96	112	3.0	3.0	4.2	<0.001	<0.001	0.616
ME intake (kJ ME/kgLW.day)_mean	157	169	254	266	6.5	6.5	9.2	0.114	<0.001	0.780

The lower LW of the LP-LP, HP-LP and LP-HP treatment animals would have reduced the calculated P requirement by 5-8 g P/day.

Table A-5. Pens_A. LACTATION. The intakes of DM and of P, excretion of P in faeces, balance of P between intake and faeces (I-F), during three total collection intervals during lactation (TC04, TC05 and TC06) in FCC that ingested Low P or high P diets during mid-late pregnancy and/or for 12 weeks during early lactation in a 2x2 factorial design. Thus diet treatments were LowP-LowP (LP-LP), HighP-LowP (HP-LP), LowP-HighP (LP-HP) and HighP-HighP (HP-HP)

Measurement	Low P in lactation		High P in lactation		s.e.m.			Probability		
	LowP-LowP	HighP-LowP	LowP-HighP	HighP-HighP	Preg	Lact	PxL	Preg	Lact	PxL
n					--	--	--	--	--	--
DM intake (kg/day)_TC04	5.33	7.56	8.80	10.20	0.364	0.364	0.515	<0.001	<0.001	0.421
DM intake (kg/day)_TC05	5.39	6.13	10.00	12.01	0.402	0.402	0.568	0.012	<0.001	0.262
DM intake (kg/day)_TC06	4.74	6.07	10.88	11.12	0.650	0.650	0.919	0.304	<0.001	0.555
DM intake (kg/day)_mean	5.13	6.59	9.81	11.09	0.34	0.34	0.48	0.003	<0.001	0.748
P intake (g P/day)_TC04	3.2	5.0	18.1	20.9	0.96	0.96	1.36	0.061	<0.001	0.717
P intake (g P/day)_TC05	3.5	4.2	21.0	20.3	1.72	1.72	2.43	0.853	<0.001	0.788
P intake (g P/day)_TC06	4.7	6.2	18.8	17.4	1.27	1.27	1.80	0.801	<0.001	0.434
P intake (g P/day)_mean	3.8	5.1	19.2	19.1	0.99	0.99	1.41	0.466	<0.001	0.562
P faeces (g P/day)_TC04	4.2	6.7	9.9	15.6	0.44	0.44	0.62	<0.001	<0.001	0.014
P faeces (g P/day)_TC05	4.4[c]	5.8[c]	13.1[b]	19.5[a]	0.70	0.70	1.00	<0.001	<0.001	0.017
P faeces (g P/day)_TC06	4.1	5.4	14.3	17.5	1.08	1.08	1.53	0.107	<0.001	0.540
P faeces (g P/day)_mean	4.3	6.0	12.4	17.4	0.55	0.55	0.78	<0.001	<0.001	0.039
P (I-F) (g P/day)_TC04	-1.0	-1.8	+8.3	+5.3	0.93	0.93	1.31	0.212	<0.001	0.401
P (I-F) (g P/day)_TC05	-0.8[a]	-1.6[b]	+7.9[a]	+0.8[b]	1.59	1.59	2.25	0.105	0.015	0.169
P (I-F) (g P/day)_TC06	+0.6b	+0.8[b]	+4.5[a]	-0.1[b]	0.75	0.75	1.06	0.059	0.147	0.033
P (I-F) g P/day)_mean	-0.5	-0.9	+6.8	+1.8	0.60	0.60	0.85	0.005	<0.001	0.009
DM intake (g DM/kg LW.day)_mean	15.1	17.0	24.2	25.1				0.003	<0.001	0.748
P intake (mg P/kg LW.day)_mean	12.1	13.9	50.2	46.8	2.2	2.2	3.1	0.939	<0.001	0.360
P faeces (mg P/kg LW.day)_mean	13.5	16.1	32.8	42.6	1.1	1.1	1.5	<0.001	<0.001	0.018
P (I-F) (mg P/kg LW.day)_mean	-1.4	-2.1	+17.5	+4.2	1.5	1.5	2.1	0.004	<0.001	0.005

sem and probability calculated from the 2x2 factorial of low or high diet P during pregnancy and lactation. Lower case superscript letters indicate differences between means when the interaction was significant. LW, liveweight.

Table A-6. Pens_A. LACTATION. The intakes of Ca, excretion of Ca in faeces, balabce of Ca between intake and faeces (I-F), during three total collection intervals during lactation (TC04, TC05 and TC06) in FCC that ingested Low P or high P diets during mid-late pregnancy and/or for 12 weeks during early lactation in a 2x2 factorial design. Thus diet treatments were LowP-LowP (LP-LP), HighP-LowP (HP-LP), LowP-HighP (LP-HP) and HighP-HighP (HP-HP)

Measurement	Low P in lactation		High P in lactation		s.e.m.			Probability		
	LowP-LowP	HighP-LowP	LowP-HighP	HighP-HighP	Preg	Lact	PxL	Preg	Lact	PxL
n	10	9	9	9	--	--	--	--	--	--
Ca intake (g Ca/day)_TC04	11.6	20.3	35.0	40.3	1.87	1.87	2.65	0.007	<0.001	0.519
Ca intake (g Ca/day)_TC05	15.3	17.5	40.4	47.0	2.7	2.7	3.8	0.185	<0.001	0.560
Ca intake (g Ca/day)_TC06	15.3	20.1	46.3	47.9	2.8	2.8	3.9	0.307	<0.001	0.678
Ca intake (g Ca/day)_mean	14.3	19.3	40.4	44.6	1.8	1.8	2.5	0.043	<0.001	0.742
Ca faeces (g Ca/day)_TC04	11.9	23.1	20.5	32.7	1.4	1.4	2.0	<0.001	<0.001	0.806
Ca faeces (g Ca/day)_TC05	12.2^{c}	17.6^{c}	29.9^{b}	41.1^{a}	1.6	1.6	2.2	<0.001	<0.001	0.193
Ca faeces (g Ca/day)_TC06	11.8	16.3	35.7	38.1	2.1	2.1	3.0	0.175	<0.001	0.726
Ca faeces (g Ca/day)_mean	12.1	19.0	28.8	37.3	1.1	1.1	1.6	<0.001	<0.001	0.553
Ca (I-F) (g Ca/day)_TC04	-0.3	-2.8	+14.5	+7.5	1.56	1.56	2.21	0.056	<0.001	0.323
Ca (I-F) (g Ca/day)_TC05	+3.1	-0.1	+10.5	+5.9	2.3	2.3	3.2	0.260	<0.001	0.831
Ca (I-F) (g Ca/day)_TC06	+3.5	+3.8	+10.6	+9.7	1.2	1.2	1.6	0.948	<0.001	0.727
Ca (I-F) (g Ca/day)_mean	+2.2	+0.3	+11.7	+7.3	1.3	1.3	1.8	0.105	<0.001	0.462
Ca intake (mg Ca/kg LW.day)_mean	46	52	105	111	4.1	4.1	5.8	0.212	<0.001	0.776
Ca faeces (mg Ca/kg LW.day)_mean	39	51	76	93	2.7	2.7	3.8	<0.001	<0.001	0.479
Ca (I-F) (mg Ca/kg LW.day)_mean	+7.3	+1.4	+29.8	+17.9	3.0	3.0	4.2	0.049	0.005	0.901

Table A-7. Pens_A. LACTATION. The mean concentrations and production of milk constituents during the first 12 weeks of lactation by FCC that ingested Low P or high P diets during mid-late pregnancy and/or for 12 weeks during early lactation in a 2x2 factorial design. Thus the diet treatments were LowP-LowP (LP-LP), HighP-LowP (HP-LP), LowP-HighP (LP-HP) and HighP-HighP (HP-HP). The FCC were milked on six occasions at approximately fortnightly intervals. sem, standard error of the mean

Measurement	Diet treatments				s.e.m.			Probability		
	Low P in lactation		High P in lactation							
	LowP-LowP	HighP-LowP	LowP-HighP	HighP-HighP	Preg	Lact	PxL	Preg	Lact	PxL
n					--	--	--	--	--	--
Milk production (kg/day)	4.90	6.49	7.90	8.69	0.26	0.26	0.37	0.003	<0.001	0.284
Milk composition										
Fat (g/kg)	35.4	36.0	36.4	36.3	1.00	1.00	0.25	0.867	0.653	0.812
Protein (g/kg)	25.0	24.8	25.0	24.8	0.60	0.60	0.85	0.754	0.989	0.981
Lactose (g/kg)	52.9	52.0	53.4	52.2	0.30	0.30	0.43	0.015	0.452	0.701
Ca (g/kg)	1.38	1.33	1.38	1.36	0.018	0.018	0.025	0.210	0.596	0.439
P (g/kg)	1.01	1.00	1.02	1.03	0.016	0.016	0.023	0.885	0.490	0.526
Mg (g/kg)	0.11	0.11	0.11	0.11	0.001	0.001	0.002	0.208	0.852	0.935
Energy (MJ/kg)	2.84	2.84	2.88	2.85	0.05	0.05	0.06	0.813	0.638	0.797
Milk energy (MJ/day)	13.9	18.4	22.8	24.1	0.84	0.84	1.19	0.022	<0.001	0.188
Milk Ca (g/day)	6.8	8.6	10.9	11.5	0.40	0.40	0.56	0.037	<0.001	0.332
Milk P (g/day)	5.0	6.4	8.0	8.7	0.30	0.30	0.43	0.023	<0.001	0.374

Table A-8. Pens_A. LACTATION. The secretion of P in milk, and the balance of P between intake and P lost in faeces and milk (I-F-M), during three total collection intervals during lactation (TC04, TC05 and TC06) in FCC that ingested Low P or high P diets during mid-late pregnancy and/or for 12 weeks during early lactation in a 2x2 factorial design. Thus diet treatments were LowP-LowP (LP-LP), HighP-LowP (HP-LP), LowP-HighP (LP-HP) and HighP-HighP (HP-HP)

Measurement	Low P in lactation		High P in lactation		s.e.m.			Probability		
	LowP-LowP	HighP-LowP	LowP-HighP	HighP-HighP	Preg	Lact	PxL	Preg	Lact	PxL
n	10	9	9	9	--	--	--	--	--	--
Milk P (g P/day)_TC04	6.6	8.2	8.9	9.00	0.46	0.46	0.65	0.184	0.017	0.242
Milk P (g P/day)_TC05	5.2	6.3	7.7	8.9	0.36	0.36	0.52	0.021	<0.001	0.857
Milk P (g P/day)_TC06	3.0	5.2	7.4	9.6	0.36	0.36	0.52	<0.001	<0.001	0.955
Milk P (g P/day)_mean	4.9	6.6	8.2	9.1	0.33	0.33	0.47	0.007	<0.001	0.404
P (I-F-M) (g P/day)_TC04	-7.9	-9.9	-1.3	-3.8	1.11	1.11	1.57	0.197	<0.001	0.891
P (I-F-M) (g P/day)_TC05	-5.9	-8.0	+0.3	-8.1	1.56	1.56	2.21	0.028	0.160	0.155
P (I-F-M) (g P/day)_TC06	-2.6[a]	-4.4[a]	-2.9[a]	-9.2[b]	0.66	0.66	0.93	<0.001	0.012	0.024
P (I-F-M) g P/day)_mean	-5.6	-7.4	-1.6	-7.5	0.55	0.55	0.78	<0.001	0.013	0.014
Milk P (mg P/kg LW.day)_mean	15.3	17.6	21.6	21.6	0.96	0.96	1.35	0.329	<0.001	0.417
P (I-F-M) (mg P/kg LW.day)_mean	-17.2	-19.7	-4.4	-17.5	1.5	1.5	2.1	0.001	<0.001	0.016

Lower case superscript letters indicate differences between means when the interaction between treatments was significant.

Table A-9. Pens_A. LACTATION. The mean concentrations of minerals and endocrine markers in plasma of lactating FCC that ingested Low P or high P diets during mid-late pregnancy and/or for 12 weeks during early lactation in a 2x2 factorial design. In addition measurements of rib bone comprising the cortical bone thickness (CBT), P concentration in cortical bone (P conc) and the index phosphorus per unit surface area of cortical bone (PSACB) are given. Thus diet treatments were LowP-LowP (LP-LP), HighP-LowP (HP-LP), LowP-HighP (LP-HP) and HighP-HighP (HP-HP)

Measurement	Low P in lactation		High P in lactation		s.e.m.			Probability		
	LowP-LowP	HighP-LowP	LowP-HighP	HighP-HighP	Preg	Lact	PxL	Preg	Lact	PxL
n	10	9	9	9	--	--	--	--	--	--
Plasma minerals and endocrine markers										
Plasma PIP (mmol/L)	0.54	0.63	2.12	2.03	0.0385	0.0382	0.0558	<0.001	<0.001	0.066
Plasma Ca (mmol/L)	2.77	2.50	2.51	2.38	0.0257	0.0255	0.0373	<0.001	<0.001	0.084
Plasma Ca/PIP ratio	5.14	4.00	1.19	1.17						
Plasma Mg (mmol/L)	0.958	0.847	0.811	0.853	0.0153	0.0152	0.0222	0.088	0.002	0.003
Plasma Log PTH (pg/mL)	2.39	2.80	4.94	4.97	0.24	0.25	0.35	0.501	<0.001	0.573
Plasma CTX-1 (ng/mL)	4.60	3.94	1.51	1.95	0.24	0.25	0.35	0.688	<0.001	0.184
Plasma OCN (ng/mL)	71	70	81	65	3.4	3.5	6.3	0.103	0.616	0.128
Plasma BAP (UI/L)	58	32	33	33	3.1	3.2	4.6	0.004	0.008	0.007
Rib bone measurements										
Calving CBT (mm)	4.01	4.62	4.29	4.67	-	-	-	-	-	-
Weaning CBT (mm)	2.51	3.45	3.29	3.69	0.158	0.154	0.224	0.006	0.030	0.233
Change in CBT to weaning	-1.50	-1.16	-1.00	-0.98 ok	0.179	0.179	0.253	0.501	0.194	0.535
Calving Pconc (mg/cc)	123	136	133	133	-	-	-	-	-	-
Weaning Pconc (mg/cc)	128	129	127	146	3.80	3.80	5.38	0.059	0.143	0.106
Change in Pconc to weaning (mg/cc)	5	-7	6	13	6.4	6.4	9.1	0.782	0.259	0.296
Calving PSACB (mg/mm^2)	0.489	0.630	0.525	606	-	-	-	-	-	-
Weaning PSACB (mg/mm^2)	0.313	0.440	0.419	0.524	0.0205	0.0205	0.0290	<0.001	0.001	0.929
Change in PSACB to weaning (mg/mm^2)	-0.176	-0.190	-0.106	-0.082	0.0291	0.0291	0.0412	0.908	0.040	0.653

Table A-10. RECOVERY (post-lactation). The LW and LW change, intakes of DM and of P during the 6 week recovery interval in FCC following the imposition of diet treatments where the FCC ingested Low P or high P diets during mid-late pregnancy and/or for 12 weeks during early lactation in a 2x2 factorial design. In addition the intake of P, excretion of P in faeces and balance of P between intake and faeces (I-F), during a total collection interval (TC07) during the last week of the recovery interval is given. The diet treatments were LowP-LowP (LP-LP), HighP-LowP (HP-LP), LowP-HighP (LP-HP) and HighP-HighP (HP-HP). All the FCC were fed a high P diet ad libitum during the recovery interval

Measurement	Low P in lactation		High P in lactation		s.e.m.			Cov[A]	Prob.		
	LowP-LowP	HighP-LowP	LowP-HighP	HighP-HighP	Preg	Lact	PxL		Preg	Lact	PxL
n	10	9	9	9	--	--	--	--	--	--	--
LW. Wk 1 (kg)	296	338	409	448	8.24	8.24	12.01	--	<0.001	<0.001	0.761
LW. Wk 6 (kg)	332	397	453	475	7.2	7.0	10.6	1,2	<0.001	<0.001	0.051
LW change (kg)	36	59	44	27	0.11	0.13	0.148	n.s.	0.957	0.151	0.372
LW change (kg/day)	1.06	1.19	1.00	0.84	0.11	0.13	0.148	n.s.	0.957	0.151	0.372
DM intake (kg/d)	4.94	7.25	9.07	10.07	0.251	0.242	0.368	1, 2	<0.001	<0.001	0.080
DM intake (g/kg LW.d)	15.8	19.7	21.1	21.2	0.55	0.55	0.80	--	0.008	<0.001	0.019
DM digestibility (g/kg)_TC07	671	677	674	686	7.2	7.2	10.5	--	0.380	0.588	0.800
ME content (MJ ME/kg DM)	9.83	9.94	9.88	10.09	0.124	0.124	0.181	--	0.380	0.588	0.800
ME intake (MJ ME/day)	61.9	80.3	93.2	101.9	3.3	3.3	4.6	--	0.004	<0.001	0.297
ME intake (kJ ME/kgLW.day)	184	207	209	217	6.9	6.9	9.6	--	0.092	0.076	0.406
DM intake (kg DM/day)_TC07	6.34	8.07	9.47	10.08	0.33	0.33	0.466	--	0.011	<0.001	0.229
P intake (g P/day)_TC07	7.60	12.50	17.19	13.82	1.38	1.38	1.909	--	0.594	0.006	0.036
P faeces (g P/day)_TC07	6.53	10.03	14.47	16.22	0.539	0.539	0.746	--	0.594	0.006	0.036
P (I-F) (g P/day)_TC07	+1.07	+2.46	+2.72	-2.39	1.129	1.129	1.561	--	0.247	0.332	0.043
P intake (mg P/kgLW.day)_TC07	22.3	32.3	38.3	29.4	3.04	3.04	4.21	--	0.836	0.132	0.039
P faeces (mg P/kgLW.day)_TC07	19.3	25.8	32.2	34.4	0.92	0.92	1.27	--	0.001	<0.001	0.112
P (I-F) (mg P/kgLW.day)_TC07	+3.1	+6.5	+6.1	-5.1	2.63	2.63	3.64	--	0.322	0.293	0.065

A, covariates of the initial liveweight and weeks fed the treatment diets during pregnancy were significant at P<0.05. Lower case superscript letters indicate differences between treatments when the interaction was significant. LW, liveweight.

Table A-11. RECOVERY (Post-lactation). The concentrations of minerals and endocrine markers in plasma of FCC at the end of a 6 week recovery interval following the imposition of diet treatments where the FCC ingested Low P or high P diets during mid-late pregnancy and/or for 12 weeks during early lactation in a 2x2 factorial design. The diet treatments were LowP-LowP (LP-LP), HighP-LowP (HP-LP), LowP-HighP (LP-HP) and HighP-HighP (HP-HP). All the FCC were fed a high P diet ad libitum during the recovery interval.

Measurement	Low P in lactation		High P in lactation		s.e.m.			Prob.		
	LowP-LowP	HighP-LowP	LowP-HighP	HighP-HighP	Preg	Lact	PxL	Preg	Lact	PxL
n	9	9	8	9	--	--	--	--	--	--
Plasma PIP (mmol/L)	1.79	2.24	1.55	1.97	0.044	0.044	0.062	0.936	<0.001	0.281
Plasma Ca (mmol/L)	2.71	2.52	2.45	2.46	0.037	0.037	0.053	0.023	<0.001	0.018
Plasma Ca/PIP ratio	1.51	1.12	1.58	1.24						
Plasma Mg (mmol/L)	0.93	0.82	0.80	0.88	0.015	0.015	0.022	0.316	0.059	<0.001
Plasma Log PTH (pg/mL)	3.38	4.12	4.74	5.58	0.277	0.285	0.403	0.053	0.001	0.894
Plasma CTX-1 (ng/mL)	3.36	1.88	1.14	1.40	0.215	0.222	0.314	0.037	<0.001	0.008
Plasma OCN (ng/mL)	105.4	91.5	79.7	73.9	4.53	4.67	6.61	0.125	0.002	0.529
Plasma BAP (UI/L)	109.2	49.5	48.9	48.6	6.82	7.03	9.95	0.003	0.004	0.0040

REPLENISHMENT. Animal production measurements

During the six week recovery interval the DMI and of ME intake (as kg or MJ/day) were increased by the previous imposition of the LP diet treatments during both pregnancy and lactation (P<0.01 to P<0.001)(Table A-10). However when expressed on the basis of g DM/kg LW.day there was also an interaction such that the of the LP-HP and HP-HP treatments were similar (21.1 and 21.2 g DM/kg LW.day) and higher than the HP-LP treatment (19.7 g DM/kg LW.day), which was higher (P<0.05) than the LP-LP treatment (15.8 g DM/kg LW.day). This interaction effect on DMI decreased during the replenishment interval and it was not observed during the total collection interval during the last week. Nevertheless an interaction effect was observed on the P balance (measured as the P(I-F)) calculated as g P/day (P=0.043) and also tended (P=0.065) to occur when the results were expressed on the basis of mg P/kg LW.day) (Table A-10).

PREGNANCY. Plasma P, Ca, Mg, endocrine and bone markers

At the start of the experiment (4 months before calving) plasma mineral concentrations (mean ± sd) in these young cows indicated adequate Ca and Mg status (Ca 2.64 ± 0.02 mmol/L, Mg 0.76 ± 0.02 mmol/L), but marginal P status (PIP 1.15 ± 0.04 mmol/L) (Data not shown). This PIP was associated with the low P diet fed for several weeks preceding the first blood sample when animals were being adapted and evaluated for their suitability to the pens. The PIP concentration was low given these were young, still growing, cows. Thus overall the animals at the start of the experiment could be classified as hypophosphatemic, but normocalcemic.

Throughout the experiment PIP concentrations were very responsive to the changes in dietary P. During pregnancy the PIP concentrations in cows fed the LP diet decreased progressively to 0.7 - 0.8 mmol/L, whilst PIP concentrations increased in cows fed the HP diet to 1.9 – 2.0 mmol/L (Table A-3; Figure A-3). These results indicate P deficiency and P adequacy on LP and HP diets, respectively. During pregnancy there was a decrease in plasma total Ca in both HP and LP diets in late pregnancy, with half the cows on HP diets exhibiting mild hypocalcemia (Ca range 1.6 to 2.1 mmol/L). In contrast, only one cow on LP diet had a Ca concentration less than 2.2 mmol/L. Instead the majority (56%) cows on the LP diet exhibited hypercalcemia with Ca > 2.8 mmol/L. Correspondingly the plasma Ca/P ratio increased with LP diet (> 3.0) and decreased (< 1.5) with HP diet during pregnancy (Figure A-3). Plasma Mg concentrations followed a similar pattern to plasma Ca (Figure A-4).

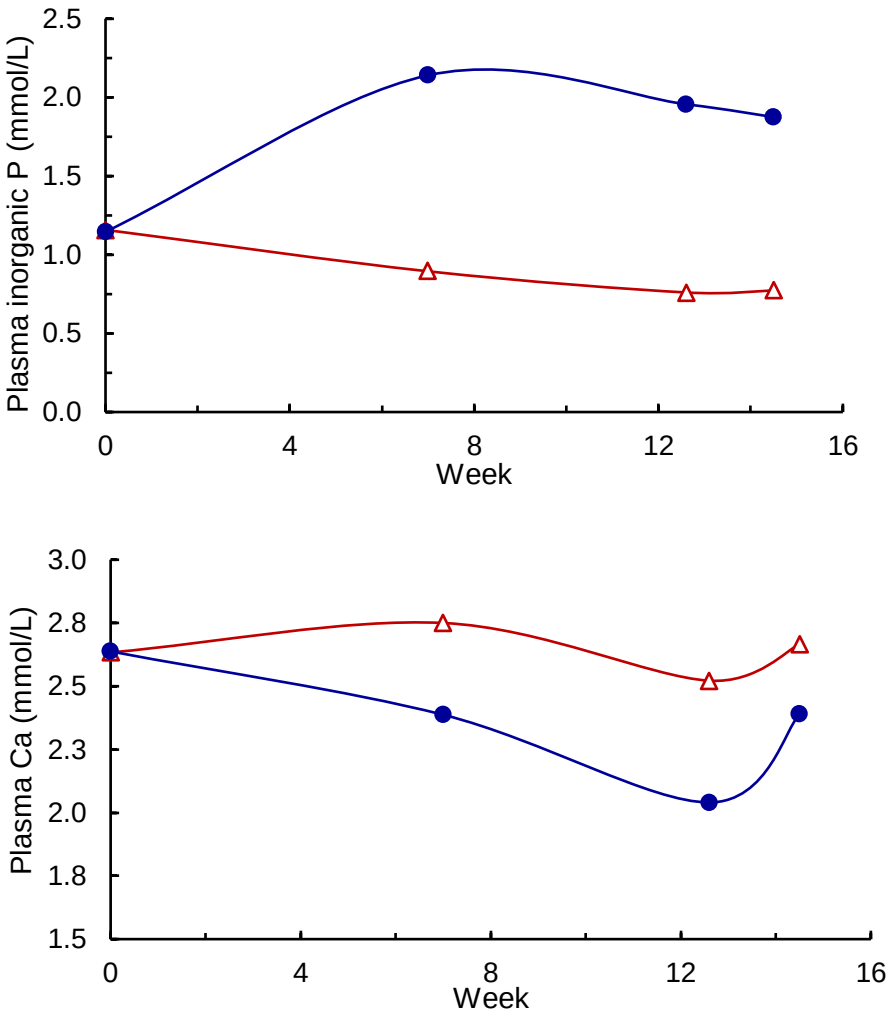


Figure A-3. Pregnancy. Plasma PIP (panel A) and total Ca (panel B) concentrations in FCC during late pregnancy with either low diet P (LP) (Δ) or high diet P (HP) (●)

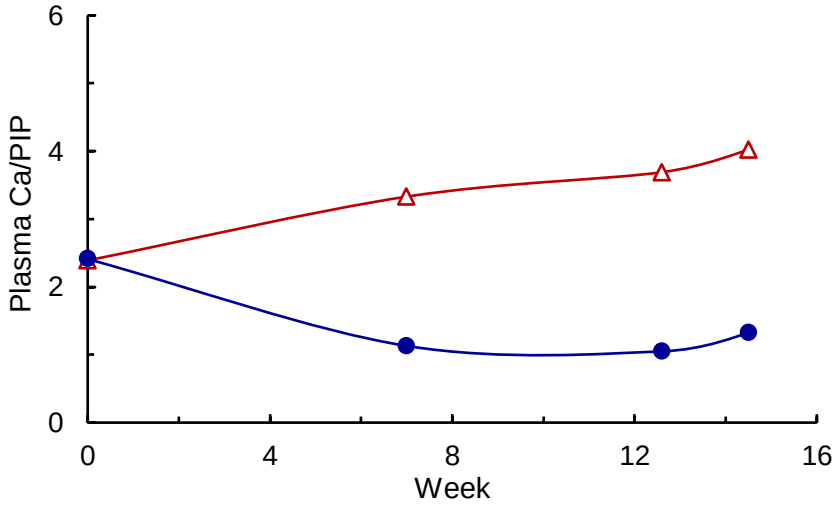


Figure A-3. Pregnancy. Plasma Ca/PIP ratios in FCC during late pregnancy with either low diet P (LP) (Δ) or high diet P (HP) (●).

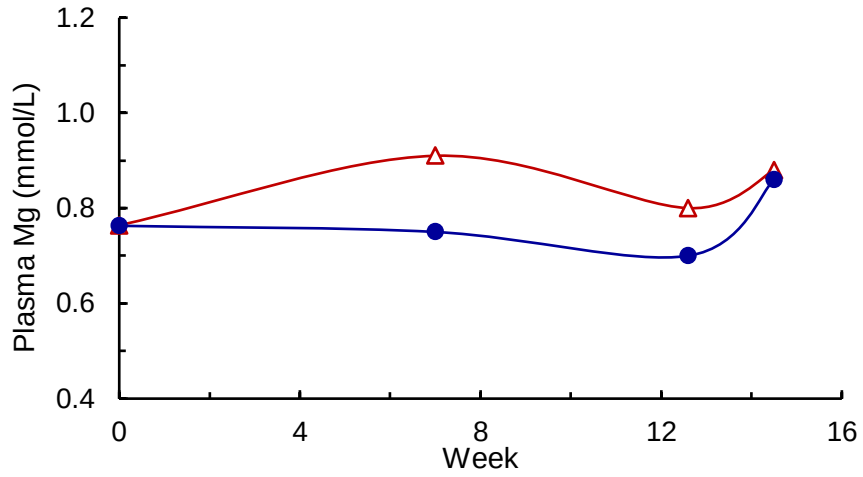


Figure A-4. Plasma Mg concentrations in FCC during late pregnancy with either low diet P (LP) (Δ) or high diet P (HP) (●).

During pregnancy the plasma PTH concentrations exhibited a significant (P<0.01) diet effect, being higher in cows on the HP diet compared to a marked suppression of PTH in cows on the LP diet (Figure A-5). Such divergent PTH responses appear to be related to diet effects on plasma Ca concentrations with a significant relationship between plasma PTH and total Ca concentrations during pregnancy some two months prior to calving as follows: (log_{10} PTH = -2.7 x total Ca + 8.76; R^2 = 0.66, slope p<0.0001, n=40). This is a normal physiological response where low plasma Ca concentration stimulates PTH secretion. There were poorer relationships between PTH levels and plasma inorganic P (R^2 = 0.18) and the Ca:P ratio (R^2 = 0.27) in accord with the hypothesis that PTH is primarily influenced by plasma Ca concentrations rather than by PIP concentrations. Therefore on the HP diet, adequate P status was achieved, albeit the diet was associated with lower blood Ca concentrations and consequent stimulation of PTH to maintain Ca homeostasis. In contrast, the LP diet was associated with higher Ca concentrations and correspondingly PTH was inhibited.

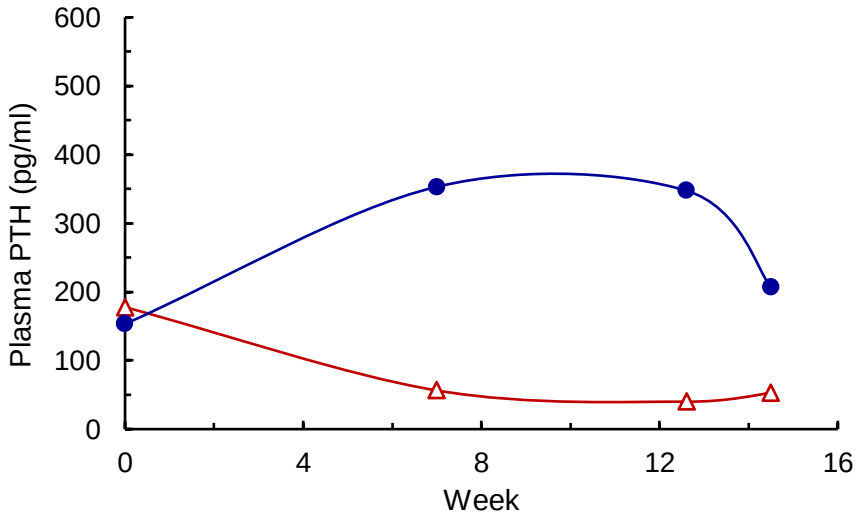


Figure A-5. Pregnancy. Plasma PTH concentrations in FCC during late pregnancy with either low diet P (LP) (Δ) or high diet P (HP) (●).

Plasma 1,25 diOH Vit D concentrations were 160 ± 10 pg/mL at the start of the experiment and after seve weeks on diets were significantly (P<0.01) different, being increased on LP diets (265 pg/mL) whilst being slightly reduced on HP diets (141 pg/mL). However in very late pregnancy there was a marked increase in plasma 1,25 diOH Vit D concentrations, which reduced the difference between the low and high P diets (Figure A-6).

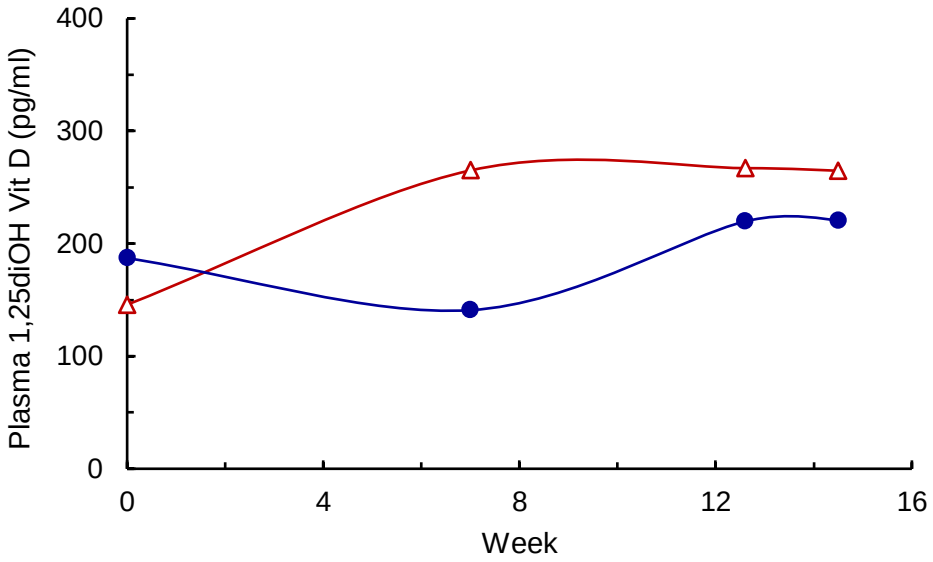


Figure A-6. Pregnancy. Plasma active vit D concentrations in FCC during late pregnancy with either low diet P (LP) (Δ) or high diet P (HP) (●).

Plasma CTX-1 concentrations were high at the start of the experiment (2.8 ± 0.1 ng/ml) and remained at this concentration over the next two months in FCC given the low P diet (Figure A-7). In contrast plasma CTX-1 concentrations of FCC fed the HP diet were markedly decreased during pregnancy. In the last weeks of pregnancy plasma CTX-1 concentrations increased in both the LP and HP diets, but continued to be significantly (P<0.01) different between the diet groups up to calving (Figure A-7).

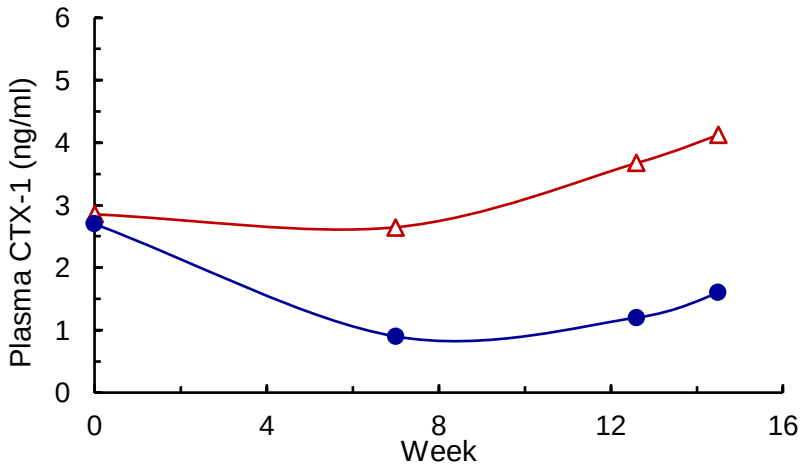


Figure A-7. Pregnancy. Plasma CTX-1 concentrations in FCC during late pregnancy with either low diet P (LP) (Δ) or high diet P (HP) (●).

During pregnancy plasma OCN concentrations were generally high (> 50 ng/ml) in all cows, but OCN concentrations declined in the last month of pregnancy (Figure A-8). A small, but significant (P<0.01) diet effect was observed with HP diets having increased OCN concentrations during pregnancy, although no difference between diets was observed at calving when OCN concentrations reached a nadir (Figure A-8). Plasma BAP concentrations during pregnancy exhibited an inverse response to OCN. Concentrations at the start of the experiment were generally low (>30 U/L) and declined in cows on the HP diet, but increased in cows on the LP diet (Figure A-8). BAP concentrations increased in cows on both diets in late pregnancy.

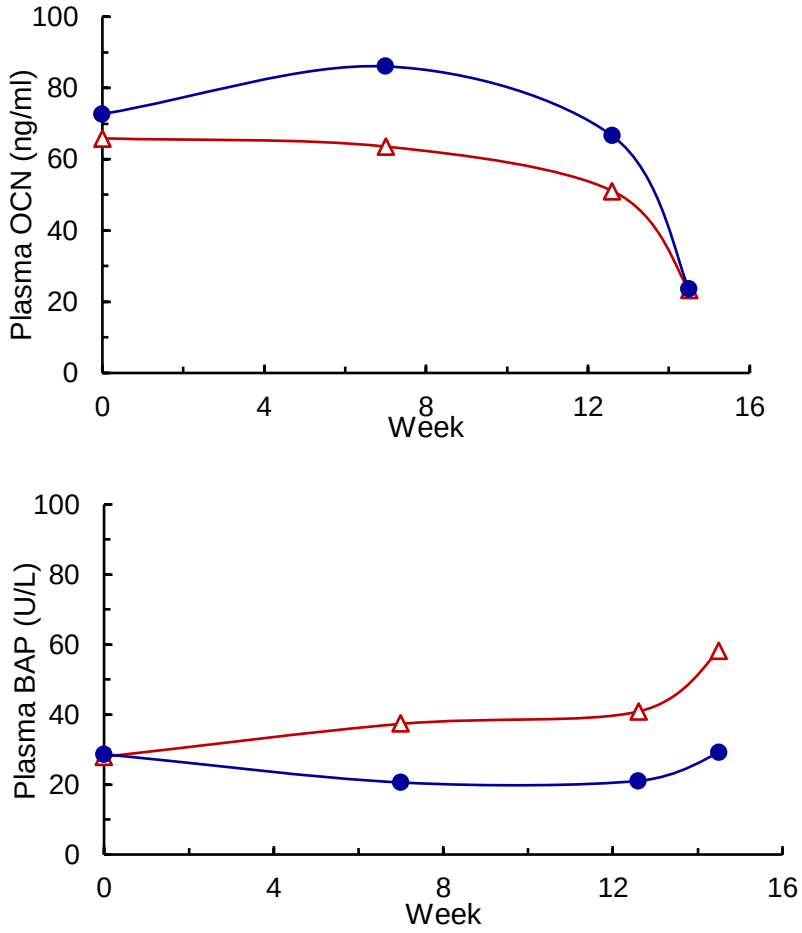


Figure A-8. Pregnancy. Plasma osteocalcin (OCN, panel A) and bone alkaline phosphatase (BAP, panel B) concentrations in FCC during late pregnancy with either low diet P (LP) (Δ) or high diet P (HP) (●).

LACTATION AND REPLENISHMENT. Plasma P, Ca, Mg, endocrine and bone markers

Following calving the PIP concentrations of the LP-LP cows further decreased to 0.5 – 0.6 mmol/L, while the PIP concentrations of the HP-HP cows increased slightly to 2.1 mmol/L (Figure A-9). In cows fed the two other diets (LP-HP and HP-LP) the PIP concentrations changed promptly with changes in diet P. During lactation, the PIP concentrations of both high and low P diets indicated P adequacy and P deficiency respectively. Following weaning, and at the end of the 4 week P replenishment phase, PIP concentrations were in the normal range for each of the treatment groups (HP-HP 2.27, HP-LP 1.89, LP-HP 2.10 mmol/L) except the LP-LP treatment cows which were significantly (P<0.01) lower at 1.44 mmol/L (Figure A-9).

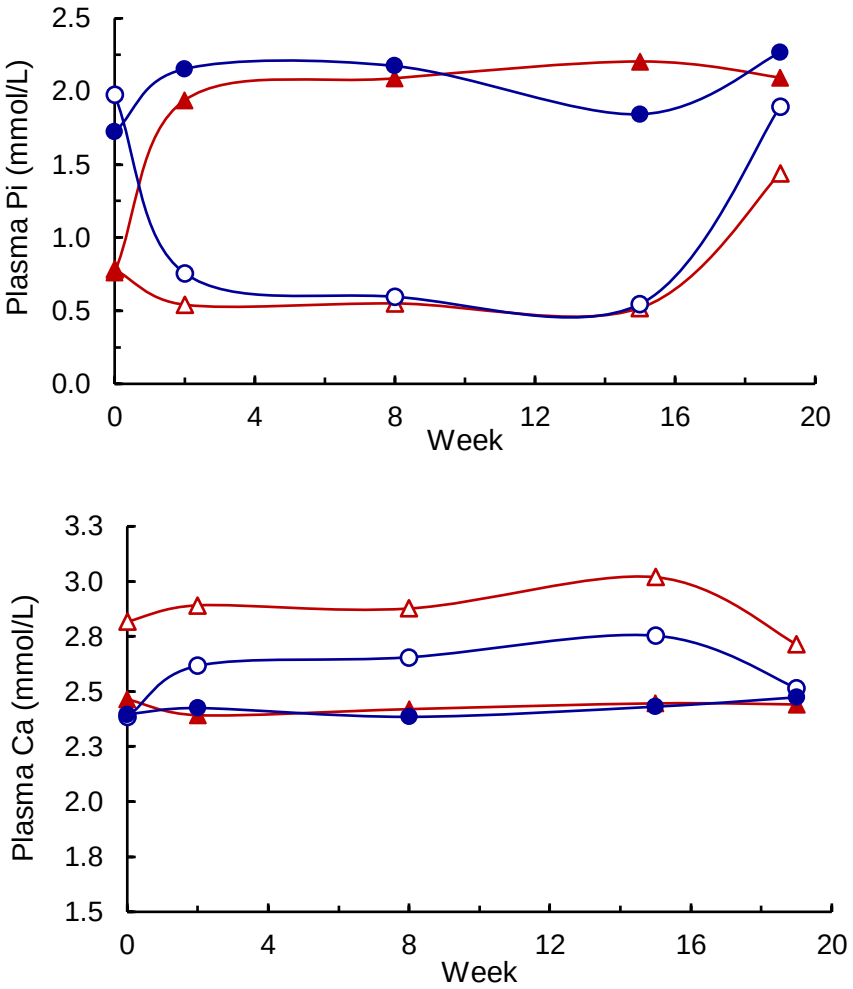


Figure A-9. Lactation and replenishment. Plasma PIP (panel A) and total Ca (panel B) concentrations in FCC during weeks 1-12 lactation and post-weaning during replenishment (weeks 13-18). Cows were fed (LP-LP) (Δ), HP-LP (○), LP-HP (▲) or HP-HP (●) diet in pregnancy and lactation.

During lactation there was a main effect ($P<0.01$) of diet P, and also a diet by time interaction effect ($P<0.01$) on plasma Ca; plasma Ca was higher in animals fed the low P diets during lactation (HP-LP and LP-LP) and increased more in the LP-LP diet than the HP-LP diet (Figure A-9). Ca concentrations in LP-LP diets were at times above normal, i.e. hypercalcemia was observed in some LP-LP cows during lactation. There was a similar effect of diet P during lactation on plasma Mg (Figure A-10). After weaning and P replenishment for six weeks, plasma Ca and Mg in cows given LP during lactation (HP-LP) was similar to cows given HP (LP-HP and HP-HP) during lactation (figures A-9 and A-10). However in LP-LP cows plasma Ca and Mg concentrations remained significantly ($P<0.05$) higher after six weeks of P replenishment.

In lactation cow fed HP diets (HP-HP and LP-HP) had plasma Ca/P ratios below 1.5, whilst in cows fed LP diets (LP-LP and HP-LP) the Ca/P ratio increased markedly, being 5.3 and 6.2 respectively at 15 weeks (Figure A-11). After weaning P replenishment reduced the Ca/P ratio in HP-LP cows to 1.4 and was not different to HP-HP or HP-LP cows (1.1 and 1.2 respectively). But the Ca/P ratio in LP-LP cows remained significantly ($P<0.05$) increased at 2.0. (Figure A-11).

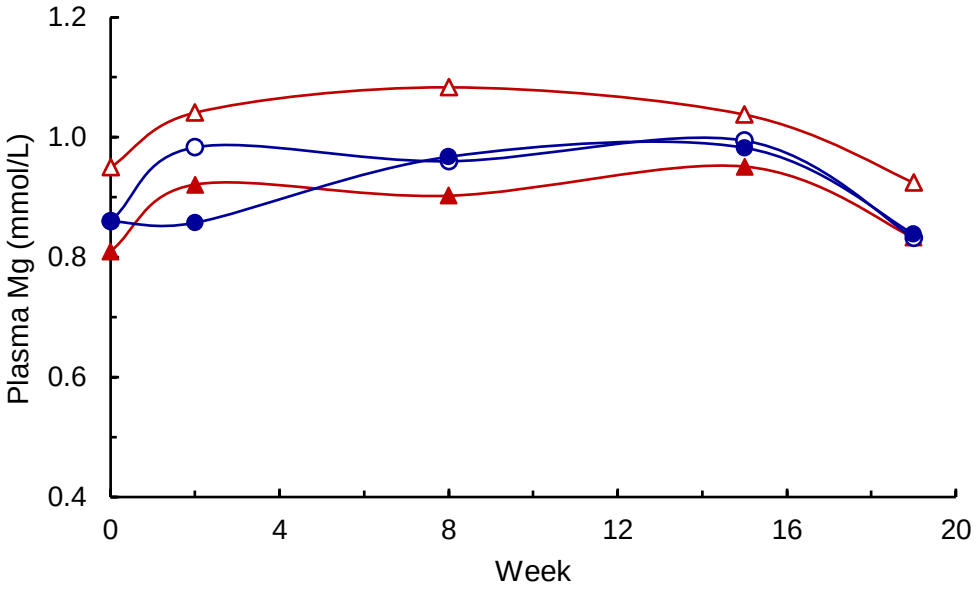


Figure A-10. Lactation and replenishment. Plasma Mg concentrations in FCC during weeks 1-12 lactation and post-weaning during replenishment (weeks 13-18). Cows were fed (LP-LP) (Δ), HP-LP (○), LP-HP (▲) or HP-HP (●) diet in pregnancy and lactation.

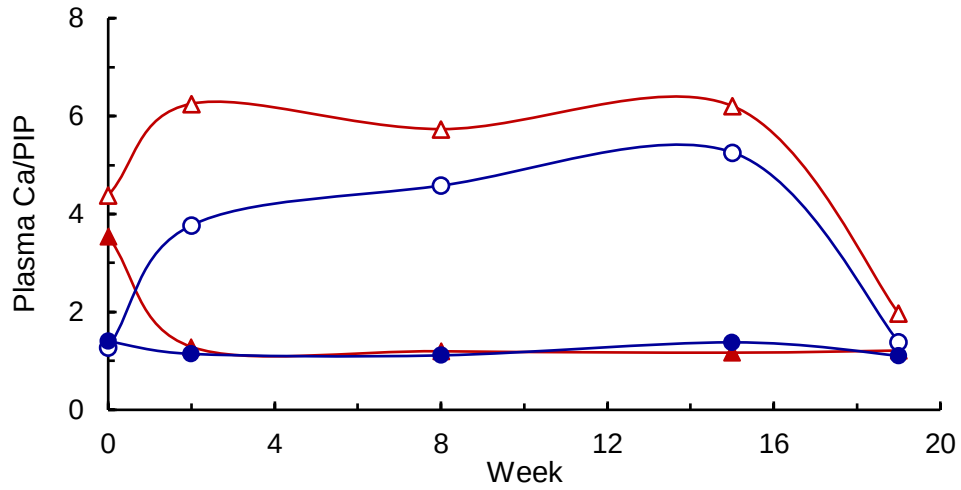


Figure A-11. Lactation and replenishment. Plasma Ca/PIP ratios in FCC during weeks 1-12 lactation and post-weaning during replenishment (weeks 13-18). Cows were fed (LP-LP) (Δ), HP-LP (○), LP-HP (▲) or HP-HP (●) diet in pregnancy and lactation.

Plasma PTH concentrations during lactation were higher in cows fed HP diets than LP diets (Figure A-12). In early lactation 1,25-diOH Vit D concentrations increased in all diets (week 2, Figure A-13) and were highest in cows that received LP diets during pregnancy (LP-HP and LP-LP). At eight weeks after calving 1,25-diOH Vit D concentrations remained significantly ($P<0.05$) higher in these groups, and was also higher in cows fed the LP diet during lactation (HP-LP group) compared to the continued HP (HP-HP) diet. At 15 weeks of lactation 1,25-diOH Vit D concentrations had decreased in all diet groups (Figure A-13), although a small but significant ($P<.0.05$) difference remained between cows on LP diets during lactation (LP-LP and HP-LP, 141 ± 12 and 142 ± 13 pg/mL respectively) and HP

diets during lactation (LP-HP and HP-HP, 91 ± 10 and 93 ± 14 pg/mL respectively). After weaning and four weeks of P replenishment, plasma 1,25-diOH Vit D concentrations did not change in LP-HP and HP-HP cows, but were increased further in both HP-LP (202 ± 22 pg/mL) and LP-LP (309 ± 38 pg/mL) cows (Figure A-13).

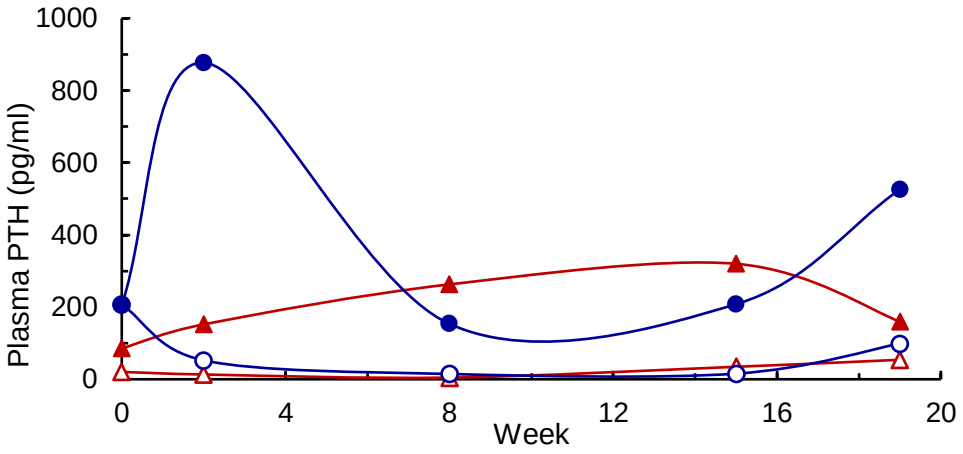


Figure A-12. Lactation and replenishment. Plasma intact parathyroid hormone (PTH) concentrations in FCC during weeks 1-12 lactation and post-weaning during replenishment (weeks 13-18). Cows were fed (LP-LP) (Δ), HP-LP (○), LP-HP (▲) or HP-HP (●) diet in pregnancy and lactation.

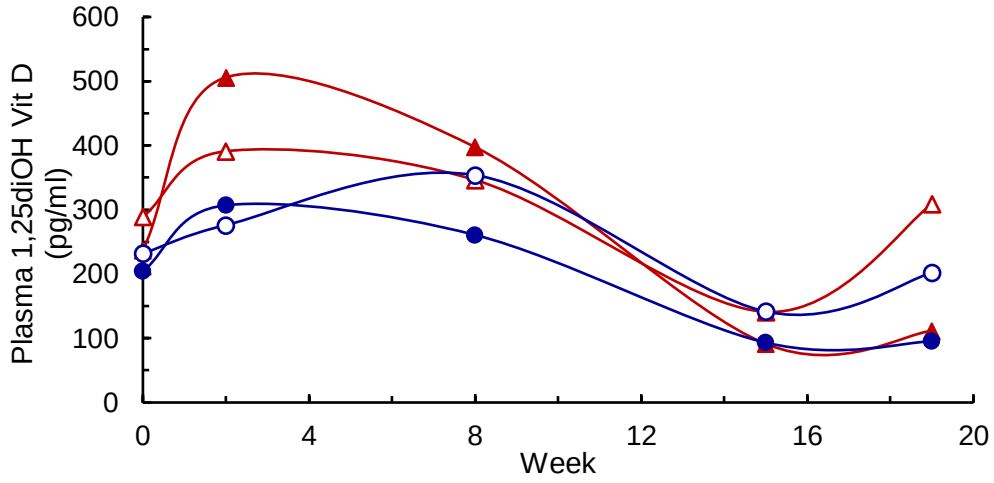


Figure A-13. Lactation and replenishment. Plasma 1,25-diOH Vit D concentrations in FCC during weeks 1-12 lactation and post-weaning during replenishment (weeks 13-18). Cows were fed (LP-LP) (Δ), HP-LP (○), LP-HP (▲) or HP-HP (●) diet in pregnancy and lactation.

In lactation the continuation of the low P diet from pregnancy (LP-LP cows) resulted in a sustained increase in plasma CTX-1 concentrations (Figure A-14). In cows fed a high P diet in pregnancy and change to a low P diet in lactation (HP-LP cows) increased plasma CTX-1 concentrations were observed, albeit these only gradually increased in early lactation to be similar to LP-LP by mid-lactation (Figure A-14). High P diets throughout pregnancy and lactation (HP-HP cows) or a transition from low P diet in pregnancy to high P diet in lactation (LP-HP cows) resulted overall in lower plasma CTX-1 concentrations during lactation. Interestingly as lactation progressed LP-HP cows had significantly ($P<0.05$) lower CTX-1 concentrations than HP-HP cows at 15 weeks lactation (Figure A-

14). Following weaning and P replenishment, HP-HP and LP-HP cows had the lowest CTX-1 concentrations (1.4 ± 0.2 and 1.1 ± 0.2 ng/ml respectively), there was a marked decrease in CTX-1 in HP-LP cows (1.9 ± 0.2 ng/ml), whilst CTX-1 concentrations in LP-LP cows remained very high (3.4 ± 0.5 ng/ml). Indeed plasma CTX-1 concentrations in LP-LP cows remained high throughout pregnancy (2.5 -4.0 ng/mL), lactation (4-5 ng/mL), and even P repletion.

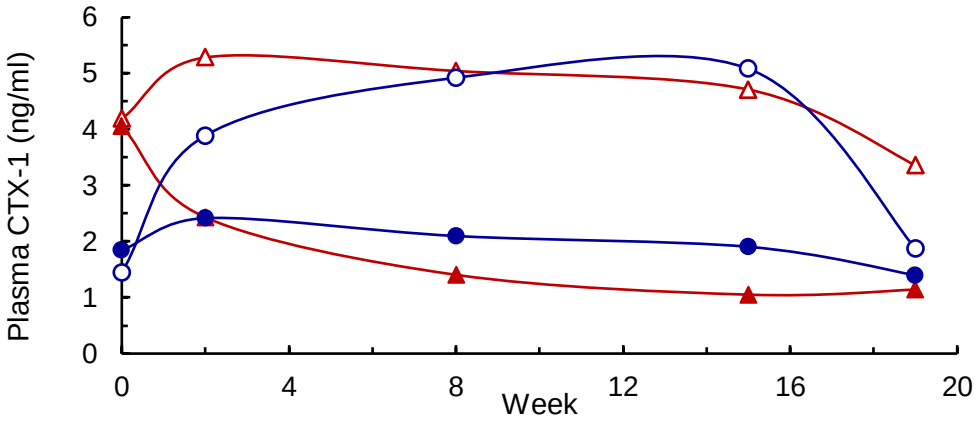


Figure A-14. Lactation and replenishment. Plasma carboxy-terminal telopeptides of Type I collagen (CTX-1) concentrations in FCC during weeks 1-12 lactation and post-weaning during replenishment (weeks 13-18). Cows were fed (LP-LP) (Δ), HP-LP (○), LP-HP (▲) or HP-HP (●) diet in pregnancy and lactation.

In early lactation plasma OCN concentrations increased in all diet groups (Figure A-15). However a more pronounced and significant ($P<0.05$) increase in OCN concentrations occurred in cows given high P during lactation following low P diet during pregnancy (LP-HP group), compared to all other diets at two weeks of lactation. By 15 weeks of lactation OCN concentrations were not different between diet groups (Figure A-15) and returned to concentrations similar to those in pregnancy at the start of the experiment (Figure A-8, panel A). Further increases in plasma OCN concentrations occurred after weaning and during P replenishment.

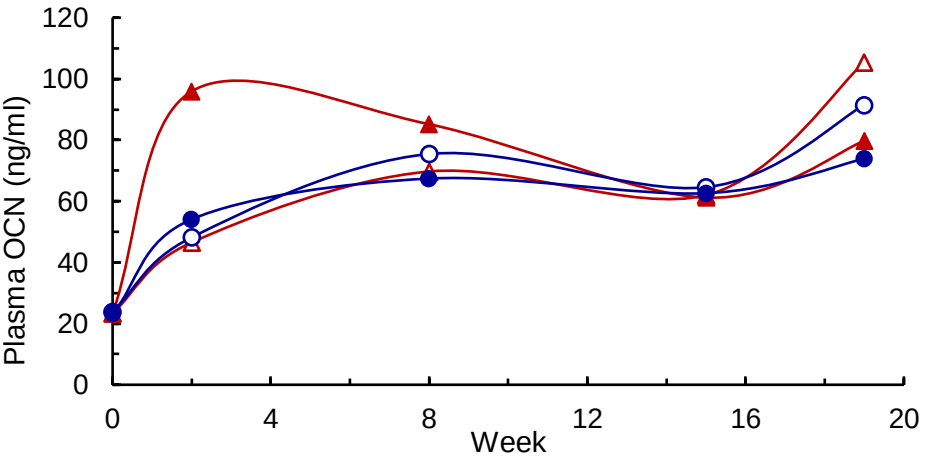


Figure A-15. Lactation and replenishment. Plasma osteocalcin (OCN) concentrations in FCC during weeks 1-12 lactation and post-weaning during replenishment (weeks 13-18). Cows were fed (LP-LP) (Δ), HP-LP (○), LP-HP (▲) or HP-HP (●) diet in pregnancy and lactation.

In early lactation plasma BAP concentrations were low in all in diets following the increase observed in late pregnancy and peak at calving, especially in the LP diet during pregnancy (Figure A-8). As lactation progressed there was a decrease in BAP concentrations in cows given the LP-HP treatment (i.e. high P diets during lactation following low P diet during pregnancy) so that concentrations were similar to those in HP-HP treatment cows (high P diet throughout the experiment). After 3 months of lactation BAP concentrations in LP-LP cows remained significantly (P<.0.01) higher than all other diets (Figure A-16). Interestingly in lactation there was no significant difference in BAP concentrations between HP-HP and HP-LP cows. With P repletion after weaning, BAP concentrations increased in all diets, but remained significantly (P<0.01) higher in LP-LP cows than all other diets.

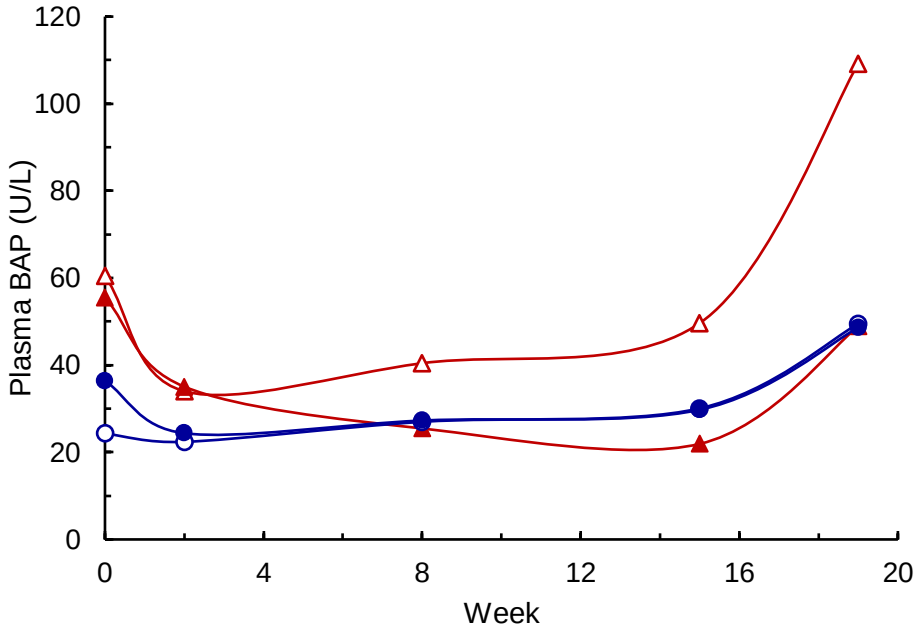


Figure A-16. Lactation and replenishment. Plasma BAP concentrations in FCC during weeks 1-12 lactation and post-weaning during replenishment (weeks 13-18). Cows were fed (LP-LP) (Δ), HP-LP (○), LP-HP (▲) or HP-HP (●) diet in pregnancy and lactation.

Bone growth and histology

Pregnancy

The cortical bone thickness (CBT) and the PSACB of 12th rib bone increased during the last 14 weeks of pregnancy in heifers fed both LowP and HighP diets, by 21% and 40% respectively (Table A-3). This indicated that in these pregnant heifers bone growth (or at least growth of rib bone) continued even when diet P intake was deficient. The mean CBT in the 12th rib of heifers at the start of the experiment was 3.60 (±0.55) mm. At calving the CBT tended (P=0.058) to be greater, and the PSACB was significantly greater (P=0.002), in the HP heifers. Thus there was a large effect of diet P concentration during pregnancy on growth of rib bone.

Lactation interval

At weaning the 11th rib bone was affected by the diet treatments imposed during both pregnancy and lactation (Table A-9). There were no discernible effects of diet during lactation on the CBT or P concentration, but the FCC fed the LP diet during lactation had lower PSACB at weaning (P=0.001)

and lost more PSACB between calving and weaning (means -0.182 and -0.094 mg P/mm^2, respectively; P=0.040). There were also an effect of the diet fed during pregnancy; FCC fed LP during late pregnancy had a lower PSACB at weaning than the FCC fed HP during late pregnancy (means 0.366 and 0.482 mg P/mm^2, respectively; P=0.040), but no diet effect during pregnancy on the decrease in PSACB during lactation.

There were also differences in the 11th rib bone growth during the six week recovery interval as a consequence of the previous diet treatments (Table A-12). The increase in CBT during the recovery interval was greater in FCC which had been fed HP rather than LP during pregnancy (P=0.017), and PSACB also tended (P=0.056) to be greater.

Table A-12. RECOVERY (Post-lactation). Measurements are given of rib bone comprising the cortical bone thickness (CBT), P concentration in cortical bone (P conc) and the index phosphorus per unit surface area of cortical bone (PSACB), and of tuber coxae (hip) bone, of FCC at the end of a six week recovery interval following the imposition of diet treatments where the FCC ingested Low P or high P diets during mid-late pregnancy and/or for 12 weeks during early lactation in a 2x2 factorial design. The diet treatments were LowP-LowP (LP-LP), HighP-LowP (HP-LP), LowP-HighP (LP-HP) and HighP-HighP (HP-HP). All the FCC were fed a high P diet ad libitum during the recovery interval

Measurement	Low P in lactation		High P in lactation		s.e.m.			Prob.		
	LowP-LowP	HighP-LowP	LowP-HighP	HighP-HighP	Preg	Lact	PxL	Preg	Lact	PxL
n	9	9	8	9	--	--	--	--	--	--
Rib bone										
Weaning CBT (mm)	2.51	3.45	3.29	3.69	0.158	0.154	0.224	0.030	0.233	0.251
End R CBT (mm)	3.03	3.49	3.80	3.57	0.165	0.165	0.234	0.620	0.084	0.154
Change in CBT	+0.46	-0.07	+0.51	-0.12	0.161	0.161	0.227	0.017	0.983	0.836
Weaning Pconc (mg/cc)	128	129	127	146	3.80	3.80	5.38	0.143	0.106	0.128
End R Pconc (mg/cc)	117	127	127	136	4.3	4.3	6.0	0.122	0.124	0.989
Change in Pconc (mg/cc)	-0.81	-0.30	-0.10	-10.1	5.5	5.5	7.8	0.886	0.904	0.262
Weaning PSACB (mg/mm^2)	0.313	0.440	0.419	0.524	0.0205	0.0205	0.0290	<0.001	0.001	0.929
End R PSACB (mg/mm^2)	0.352	0.452	0.486	0.488	0.027	0.027	0.038	0.195	0.035	0.214
Change in PSACB (mg/mm^2)	+0.037	+0.008	+0.067	-0.053	0.026	0.026	0.037	0.056	0.675	0.230
Tuber coxae										
Weaning_Tb_Th (µm)	94.9b	106.9ab	114.3a	116.6a	3.4	3.6	5.0	0.146	0.006	0.335
End R Tb_Th (µm)	106.0b	128.3a	122.6ab	136.5a	4.3	4.4	6.3	0.005	0.051	0.494
Change in Tb_Th (µm)	11.1	21.4	8.3	19.9						
Weaning Osteoid	26.9a	21.5b	10.6a	8.5b	2.4	2.5	3.5	0.262	<0.001	0.629
End R Osteoid	29.1	22.1	17.1	17.2	3.3	3.3	4.8	0.435	0.080	0.451
Change in Osteoid	+2.2	+0.6	+6.5	+8.7						
Weaning BV-TV (%)	8.9b	10.5b	10.4b	13.4a	0.8	0.8	1.1	0.024	0.041	0.182
End R BV-TV (%)	8.6b	13.2a	11.6ab	12.9a	0.8	0.9	1.2	0.018	0.273	0.166
Change in BV-TV	-0.3	+2.7	+1.2	-0.5						

Lower case superscript letters indicate differences between means.

Recovery interval

Since the *tuber coxae* can only be biopsied twice (once on each side) in the present experiment these samples were obtained at weaning, when the greatest difference between treatment groups was expected, and at the end of the replenishment period after weaning to evaluate the cow's ability to start depositing bone again.

There was a significant effect of diet P on trabecular bone volume (BV/TV) at weaning with both main effects (provision of HP in pregnancy or lactation) significant (P=0.024 and P=0.041, respectively) but there was no interaction effect (Table A-5). The animals fed HighP during both pregnancy and lactation (HP-HP) had greater BV/TV (13.4%) than the other three groups. Cows fed LowP during pregnancy and lactation (LP-LP) had almost 35% less trabecular bone volume (8.9%) than cows fed HighP during pregnancy and lactation (HP-HP). Cows fed HighP only during pregnancy (HP-LP) or lactation (LP-HP) had similar BV/TV (10.4% and 10.5%) that were about 25% less than cows fed the HP-HP diet. Thus, changes in bone volume at weaning were a combined effect of diet P during pregnancy and lactation. There was also a significant effect of diet P during lactation on average trabecular thickness (Tb.Th) at weaning (P=0.006) and on osteoid volume (P<0.001). Animals fed LowP during pregnancy and lactation (LP-LP) had less (94.9 µm) than cows fed HighP during lactation (HP-HP and LP-HP) with trabeculae in the LP-LP group 23% thinner than in cows fed the HP-HP diet (116.6 µm). Changes in Tb.Th were affected most by diet P during lactation (P=0.006) with the cows fed HighP only during lactation (114.3 µm) having significantly greater Tb.Th than lows fed LP-LP diets while cows fed HighP only during pregnancy (HP-LP) did not (106.9 µm). Similarly there was a significant effect of diet P during lactation on the relative volumes of osteoid (un-mineralised new bone) and mineralized bone (p=0.001). Heifers fed the LowP diet during lactation (HP-LP and LP-LP) had very thick layers of un-mineralised osteoid over large areas of trabecular bone with 21% (HP-LP) and 29% (LP-LP) of bone being un-mineralised at the end of lactation. This was likely to represent true osteomalacia due to P deficiency and demonstrated that although there was an attempt to deposit new bone during this period, LowP diet during lactation prevented bone mineralisation leading to poorer quality bone formation. There was a high degree of variability in bone morphology in LP-LP treatment bone samples. Some had very high osteoid volume indicating clear osteomalacia but bone that was still attempting to form new bone. Others had very thin trabeculae with minimal osteoid suggesting minimal attempt to form new bone and severe loss of bone from mobilisation. The total bone volume (BV/TV) was similar between cows fed HP-LP and LP-HP diets. The higher volume of osteoid in cows that consumed HighP only during pregnancy (HP-LP) means that the actual volume of mineralised bone in these cows was less than in cows fed HighP only during lactation.

Following six weeks of HighP replenishment post-weaning there were still significant diet P effects on BV/TV (P=0.038) and Tb.Th (P=0.01) but not on osteoid. Cows that had previously been on HP-HP treatment (i.e. HighP through both pregnancy and lactation) showed an increase in Tb.Th but minimal change in overall bone volume. This suggested that these heifers had been able to maintain maximal possible bone growth and volume throughout pregnancy and lactation. Heifers fed the LowP diet throughout late pregnancy and lactation (LP-LP) showed some increase in trabecular thickness but this was primarily un-mineralised bone. There was almost no overall increase in trabecular bone volume in these animals. Severe P deficiency associated with some degree of osteomalacia appears to reduce the capacity of the animal to rapidly start forming bone when the animals return to a P adequate diet. Heifers that had previously been fed (HP-LP and LP-HP) diets showed increases of 10-20% in bone volume with associated increases in trabecular thickness during this six week period.

Assessment of osteoid volume following the six weeks of P replenishment post-weaning demonstrated that while cows in the LP-LP and HP-LP treatment groups had maintained high osteoid volumes, animals in HP-HP and LP-HP groups had also increased the proportion of osteoid as part of the increase in bone volume. The thickened osteoid seams during this replenishment stage represented rapid bone formation rather than pathological failure of mineralisation. Results from bone biopsy analysis show dramatic mobilisation of both cortical and trabecular bone in response to P deficiency (Figures A-17 and A-18). These results agree with the results of plasma markers of bone mobilisation including CTX-1, calcium and PIP. Even cows fed HighP during both pregnancy and lactation (HP-HP) mobilised rib cortical and possibly trabecular bone. Some bone mobilisation is the normal physiological response to high mineral demands, particularly during lactation. However these first-calf cows consuming LowP diets during late pregnancy or lactation suffered so much bone mobilisation and poor bone mineralisation that they were left with osteomalacia, a pathological bone disease characterised by thick seams of un-mineralized bone (osteoid) as well as osteoporosis (reduced bone volume). There was evidence of a strong carry-over effect of a HighP diet during pregnancy. Although cows in good P status at the end of pregnancy but fed a LowP diet during lactation developed some degree of osteomalacia they had improved bone volume and Tb.Th compared to animals that had been fed LowP in pregnancy and lactation. They had similar bone responses to cows fed LowP during pregnancy but HighP during lactation. Cows fed HighP either during pregnancy only or during lactation only were also able to much more rapidly initiate bone formation (deposition) when placed on a replenishment diet after weaning compared to cows fed the LP-LP diet. This suggests that rapid increases in bone formation was possible when P supplementation in pregnancy or lactation had prevented heifers losing large amounts of trabecular bone.

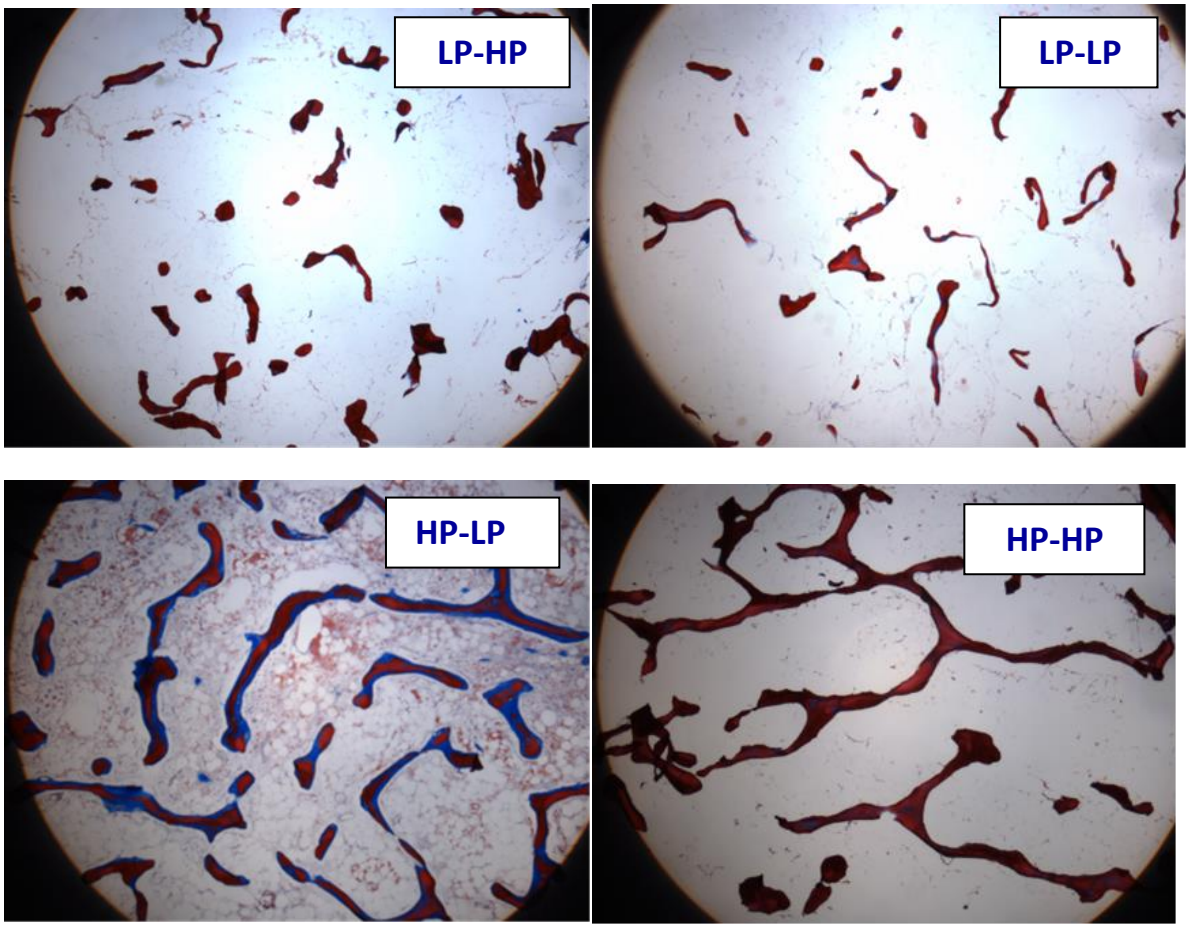
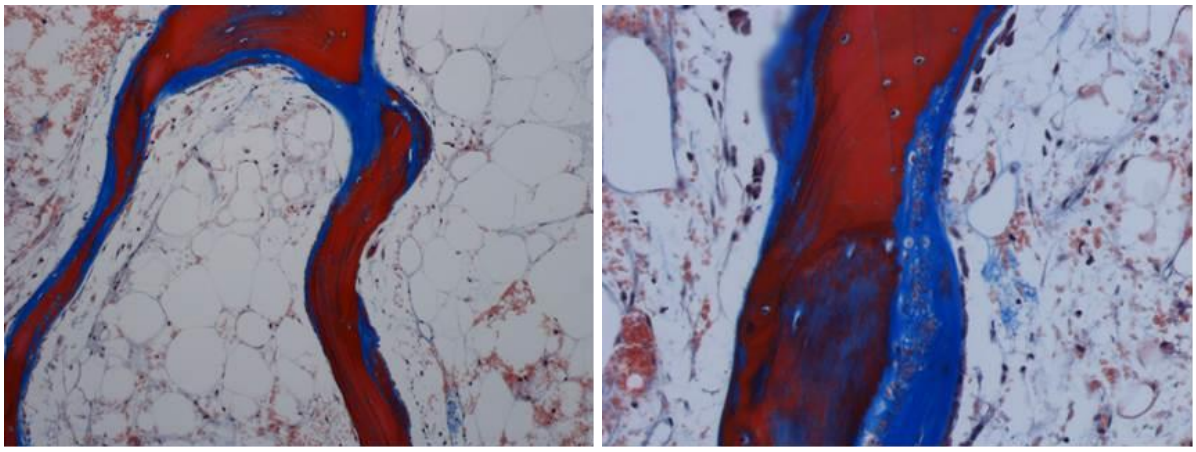


Figure A-17. Pregnancy and lactation. Typical images of bone histology sampled following 3 months of lactation for the diet treatment groups HP-LP, LP-HP, LP-LP and HP-HP, respectively. Masson's stain shows osteoid, unmineralised bone as a blue colour.



Figures A-18. Pregnancy and lactation. Typical close-up images demonstrating the Masson's stain shows osteoid, un-mineralized bone as a blue colour in the LP-LP treatment heifers. Samples were obtained after heifers had been fed severely P deficient diets for four months during late pregnancy followed by three months of lactation.

4.1.5 Discussion

Pregnancy

It was clear that there were major benefits of providing an adequate P rather than a P-deficient diet to late-pregnant heifers. Voluntary intake was reduced by the P deficient diet. Lower ME intake was associated with lower total LW and conceptus-free LW shortly before and immediately after parturition. The minor loss in CF-LW in late pregnancy (about 10 kg) was comparable with that often observed with breeders grazing dry season pasture in endowed environments or intermediate environments during good seasonal conditions. The alleviation of the P deficiency increased voluntary intake and was associated with substantial CF.LW gain rather than loss during the second half of pregnancy. Thus benefit would be expected with P supplementation of P deficient young breeders during the late dry season under comparable pasture conditions.

There was some carryover benefit of high P status (from a high P diet) during late pregnancy on voluntary intake and milk production in early lactation, especially in those FCC changed to the LP diet at calving. DM intake in these animals decreased from 7.56 to 6.13 and then 6.07 kg DM/day, which was on average 28% higher than for the LP-LP FCC (Table A-5) during the three total collection intervals at one, seven and 12 weeks of lactation. Nevertheless the DM intake and milk production of these FCC to the high P diet during lactation were much greater than the responses observed as a consequence of the carryover effects from pregnancy. Thus the recommendation to the cattle industry should not be changed; i.e. that it is important to feed P supplements during the wet season if it possible to do so. The best strategy is to supplement young cows with P through lactation. However, if this strategy is not possible, young cows should benefit from improved P nutrition in late pregnancy even if they are subsequently underfed for P in early lactation.

The young cows commenced the experiment in marginal P status, as indicated by relatively initial low PIP concentrations. Thus it was not unexpected that plasma CTX-1 levels, as indicator of bone mobilisation, were reasonably high (>2.0 mmol/L) at the start. P supplementation during pregnancy resulted in a marked decrease in CTX-1, indicative of lesser bone mobilisation in these cows. Such CTX-1 concentrations of about 1.0 ng/ml in the P supplemented cows are similar to those reported in multiparous dairy cows in late pregnancy (Ekelund *et al.* 2006; Puggaard *et al.* 2014). Immediately prior to calving, there was a small increase in CTX-1 indicating a degree of mobilisation of bone Ca and P reserves in P adequate cows to meet demands of foetal skeletal growth. As expected CTX-1 concentrations in these FCC consuming the low P diet substantially increased throughout late pregnancy through to calving. By calving, CTX-1 concentrations were >4 mmol/L, indicative of major mobilisation of bone Ca and P reserves in late pregnancy with low P diet. Such CTX-1 levels observed here are similar to those previously reported in dairy cows in early lactation (Ekelund *et al.* 2006; Puggaard *et al.* 2014), when major bone mobilisation is known to occur.

As a biomarker of bone formation, we utilised intact osteocalcin (OCN), a bone specific protein secreted by osteoblasts that are responsible for bone formation. At the start of the experiment, there was good rate of bone formation in the heifers with OCN levels being >50 ng/ml. During most of late pregnancy OCN concentrations remained high, but decreased in late pregnancy to calving. The moderate OCN concentrations indicate continued bone formation with growth as expected in pregnancy in heifers and also likely in young cows. Notably there was some indication that the high P

diet promoted more bone deposition than low P diet in late pregnancy, with OCN levels being higher in the two months before calving.

Overall, considering bone turnover (rate of bone mobilisation versus bone formation) the high / adequate P diet considerably decreased turnover, that is, favoured bone deposition above mobilisation. A clear increase in OCN (formation) together with lower CTX-1 (mobilisation) shows that bone turnover in the high P diet was markedly reduced by P supplementation in late pregnancy. In contrast, bone turnover was increased under the low P diet. This was reflected in bone biopsy measurements with heifers fed P during pregnancy having greater increases in rib bone cortical thickness. Bone mobilisation led to marked losses in cortical and trabecular bone by the time of weaning and this was partly explained by diet P during pregnancy. Such changes in bone turnover are reflected in maternal weight loss in low P diet (12 kg loss) versus high P diet (37 kg gain) from mid-pregnancy to calving.

Lactation

After calving changes in dietary P treatments (LP to HP, HP to LP) resulted in rapid changes in plasma P and Ca concentrations. As controls, cows remaining on the low P diet remained hypophosphatemic, whilst cows on the high P diet exhibited P concentrations within the normal homeostatic range, despite the increase in mineral demands due to lactation. Therefore the PIP results support the concept that PIP concentrations reflect current diet P intake, rather than being a more general measure of P status that reflects bone mineral stores. Interestingly for plasma Ca, similar to late pregnancy, there was a clear effect with lactating young cows on the high P diet exhibiting Ca concentrations at the lower end of the normal range (towards hypocalcemic), whilst young cows on low P diets were at the high end of normal range (towards hypercalcemic). Such divergent Ca and PIP results suggest that a composite index of the two measures, such as Ca to P ratio, may be a useful marker of P deficiency in cattle.

The dynamic changes in plasma CTX-1, associated with evidence from the changes in rib bone of marked loss in bone mass during lactation in the current study, supports the concept that CTX-1 is a good marker of current bone mobilisation in young cows. It appears that in early lactation mobilisation of bone mineral reserves is mandatory to meet the Ca and P demands for milk production, and this occurs even if diet Ca and P intakes are in excess of requirements. In accord with this hypothesis the FCC fed the high P diets (HP-HP) in the present experiment had slightly increased plasma CTX-1 concentrations from early lactation through to weaning at 3 months. The CTX-1 concentrations observed in these HP-HP beef genotype FCC in the present study are quite low compared to multiparous dairy cows, and presumably reflect the differences in mineral demand and genetic differences for high milk production. For young cows kept on the low P diet (LP-LP) during pregnancy and lactation, CTX-1 concentrations increased after calving and remained very high throughout lactation, which is indicative of marked bone mobilisation. This was supported by bone biopsy results indicating that by weaning these cows had almost 35% less trabecular bone and 30% less CBT than cows fed the HP diet throughout both late pregnancy and lactation. Provision of additional diet P during lactation following a P deficient state in pregnancy (LP-HP group) resulted in a marked decrease in the need for bone mobilisation, as indicated by reduced CTX-1 concentrations

in these cows. Supplementation of P in pregnancy reduced the amount of bone mobilisation, as indicated by lower CTX-1 concentrations, in early lactation.

In general, bone deposition as indicated by OCN levels was low at calving and increased during lactation in all treatments. This OCN pattern is typical to that reported in dairy cows (Ekelund *et al.* 2006), and reflects a slow increase in bone deposition during lactation. However a discernible diet treatment effect was observed in cows given additional P during lactation (LP-HP group), with increased bone deposition being apparent in the first months of lactation.

Overall bone turnover for all dietary treatments was higher in lactation than in pregnancy. In early lactation bone turnover (mobilisation) was greatest, but with ongoing lactation in the P adequate young cows bone mobilisation decreased and bone deposition increased. In contrast with low P diets in lactation there was little reduction in bone mobilisation. Interestingly, providing additional diet P after calving (LP-HP cows) reduced bone turnover, even when compared to continual provision of adequate P in both late pregnancy and lactation (HP-HP cows). Bone biopsy results indicated that feeding adequate P during pregnancy or lactation led to less cortical and trabecular bone loss than in the P deficient diets. While both LP-HP and HP-LP diets led to similar protection from severe bone loss, there was evidence that the FCC fed P during lactation had greater levels of mineralization. These results are in accord with the strategy to supplement young cows with P during lactation in the wet season. However there was also reduced bone turnover during early lactation in young cows given P supplementation in late pregnancy (HP-LP). These FCC appeared to require less bone mobilisation in early lactation to meet the demands for milk production. Nevertheless during later lactation they appeared to have substantial bone turnover. Therefore a secondary nutritional strategy would be to provide P supplement in late pregnancy.

P replenishment after weaning.
After weaning, P supplementation for six weeks resulted in normal P and Ca levels in each of the treatment groups except for the LH-LH cows which had been given the low P diets during both late pregnancy and lactation. These cows had not reached a state of mineral homeostasis after six weeks of being fed a HP diet at the end of the recovery interval, as indicated by lower PIP, higher total Ca, and higher Mg than the other treatments. Bone markers in these cows indicated ongoing higher bone turnover with increased bone mobilisation (higher CTX-1), and reduced bone formation (low OCN). In all other diets plasma mineral concentrations were normal, CTX-1 was decreased, and OCN increased during the replenishment period. Bone biopsy results confirmed that cows fed low P during pregnancy and lactation had failed to increase their bone volume during replenishment while the other treatment groups all showed improvement in bone deposition over this period. Altogether these results support the concept that a P replenishment period of six weeks after weaning in first-calf cows may be sufficient to correct P deficiency in either pregnancy (LP-HP) or lactation (HP-LP), but not a P deficiency in both pregnancy and lactation.

Apart from the ability of the breeder to deposit and later mobilise P, the 54 kg higher LW of breeders at parturition due to adequate P nutrition in late pregnancy can be expected to have major benefits for breeder herd performance.

Experiment Section 3. Pens_B experiment.

Pens_B. Replenishment of phosphorus body reserves by mature *Bos indicus* cross breeder cows post-weaning

4.1.6 Summary of experiment

An experiment examined the responses of mature pregnant recently-weaned breeder cows (n=40) housed in individual pens and fed moderate or high ME diets and low (LP) or high P (HP) diets for 14 weeks in a 2x2 factorial experimental design. PIP concentrations indicated that animals fed LP diets were severely deficient and those fed HP diets adequate in P (means 0.6 and 1.8 mmol/L). HP increased ($P<0.001$) voluntary intake of DM and ME and liveweight (LW) gain, particularly in the high ME diets. The HP diet increased ($P<0.05$) rib cortical bone thickness (CBT), trabecular bone volume and PSACB. The HP diet improved mineral homeostasis with lower concentrations of Ca and a lower Ca/P ratio in plasma. Lower concentrations of CTX-1 and active 1,25-diOH Vitamin D3 in plasma indicated lower bone resorption in the HP diets. Furthermore independent of ME intake the HP diet increased circulating IGF-1 which was indicative of better metabolic status. In conclusion, mature pregnant breeder cows fed P-deficient diets post-weaning responded to additional diet P with substantial increases in intakes of DM and ME, and increased deposition of P into rib and pelvic bones. The effects of the additional diet P on intake and LW gain were much greater in cows given a high ME content rather than a moderate ME content diet. The experiment supports the hypothesis that providing P supplements to mid-pregnant breeders during the late wet and early dry seasons and post-weaning will improve the LW and bone P reserves of mature breeders.

4.1.7 Background

If breeder cows mobilize body P reserves in late pregnancy and/or lactation then in cows calving annually it is necessary to replenish body P before the next lactation to maintain a replete P status of the animals. In cows calving annually it is important to know whether such replenishment can occur during the late wet season or dry season if P supplements are fed when usually pasture quality is declining or later only senesced pastures are available.

Experiment Pens_B was designed to examine the effects of diet P deficiency or adequacy on voluntary intake of DM and metabolisable energy (ME), liveweight change and changes in body reserves (soft tissues and bone P) of mature *Bos indicus* cross cows fed moderate or high ME content diets for three months commencing post-weaning. The breeders used had all reared a calf while grazing pastures expected from the land system to be deficient to marginal in P.Breeders were expected to be in low P at the commencement of the experiment. A moderate ME content (ModE) diet was intended to represent the ME intake in the early dry season senesced pasture as is often available when breeders are weaned early in the dry season, and where recently weaned cows would be expected to be in about LW maintenance. The high ME (HighE) diet was intended to represent, in metabolisable energy intake and N content, late wet season pasture in northern Australian rangelands of higher quality and where substantial LW gain could be expected. The HighE diets were nominally the same as those fed during lactation in the Pens_A experiment, and also in the subsequent Pens_C and Pens_E experiments of the project to facilitate comparisons between experiments and to understand the potential benefits of being able to provide high quality pasture

to cows post-weaning.

4.1.8 Materials and Methods

Mature breeder cows (n=40) *ca.* 5/8 *Bos indicus* x 3/8 *Bos taurus* crossbred Droughtmaster (>F2) and 6 - 11 years of age and were selected from the commercial herd on Spyglass Cattle Research Facility (100 km W of Townsville, Queensland, Australia) in the seasonally dry tropics. This herd had grazed northern Speargrass native pastures in open Eucalyptus woodland of low to moderate soil P. The breeders had been mated first as two year old heifers and then annually, most recently from January 2014. The herd was blood sampled (5 May 2014) for plasma inorganic phosphorus (PIP). The herd was pregnancy tested by rectal palpation (June 2014) and cows selected on the criteria of lactating through the 2013-14 wet season, 2–3 months pregnant, in body condition score (BCS) 2.0–3.3 (on a 5-point scale; CSIRO 2007) and with a temperament suitable for intensive pen experiments. The calves were weaned immediately before this pregnancy test. One month before selection of the cows PIP was (mean ± sd) 1.13 ± 0.38 mmol/L indicating that animals were in low to moderate P status. The cows were relocated to the Brian Pastures Research Facility, Gayndah.

Housing of animals and diet treatments
After arrival at Brian Pastures (20 June 2014) the animals were allowed to recover and fed barley straw, and blood sampled on the 23 June 2014. PIP concentration was (mean ± sd, range) 1.78 ± 0.41, 1.02 - 2.73 mmol/L indicating that animals were in moderate P status. The cows were all fed a low P mixed diet from the 23 - 26 June 2014. Cows were allocated to the four experimental diets (26 June 2014), which were fed for 14.5 weeks (101 days). The cows were (mean ± sd) 430 ± 45 kg total liveweight, BCS 2.7 ± 0.35, 2.0-3.3. Foetal age was 12 ± 1.2, 9-14 weeks and thus the cows were in mid-pregnancy during the experiment. The cows were again blood sampled on the 30 June 2014. Cows were housed and fed in individual pens (*ca.* 33 m^2) with a concrete floor in the half of the pen nearest to the feed bunk and with the remainder soil. The part of each pen with concrete flooring and the feed bunk were roofed, and additional shade was provided with canvas sails.

The main ingredients of the total mixed ration diets were coarsely chopped wheat straw, wheat flour and sugar and the constituents and the composition of each of the four diets are given in Table B-1. The diet treatments were: Diet 1, Moderate ME, Low P (ModE-LP) Diet 2, Moderate ME, High P (ModE-HP), Diet 3, High ME, Low P (HE-LP), and Diet 4, High ME, High P (HE-HP). The high ME content diet was nominally the same as that fed during lactation during the Pens_A experiment. For the moderate ME content diet the proportion of wheat straw was increased to 0.67, and the proportions of flour and sugar decreased to 0.19 and 0.09, respectively. The HP diets contained calcium phosphate (Kynophos, KK Animal Nutrition Pty Ltd, Umbogintwini, South Africa) to meet the expected needs of the animals for phosphorus. Designated diets were offered at *ca.* 10% in excess of the actual intakes by individual animals to achieve *ad libitum* intakes. Feed refusals were collected weekly. Cows were introduced to the treatment diets over an interval of four days during which time additional straw was added to the diet to minimize the risk of acidosis. Water was freely available from troughs in each pen.

Table B-1. Pens_B. The ingredients (g as-fed/kg) and the composition (g as-fed/kg for dry matter concentration and g/kg DM for the other constituents) of the mixed diets containing low or high

concentrations of P and fed to cows in mid-pregnancy. Mean and SD (in parenthesis) of the constituents (n = 5)

Ingredient (as fed, g/kg)	Treatment diets			
	ModE-LP	ModE-HP	HE-LP	HE-HP
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 sulphate	3.1	3.1	4.8	4.7
Sodium chloride	3.1	3.1	4.8	4.7
Rumigro premix	0.50	0.50	0.77	0.76
Elanco rumensin 100	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)
Ca	3.02 (0.335)	4.30 (0.868)	2.96 (0.332)	4.74 (0.530)
P	0.68 (0.071)	1.58 (0.356)	0.76 (0.057)	2.11 (0.282)
Mg	0.91 (0.05)	0.95 (0.06)	0.72 (0.06)	0.83 (0.01)
S	2.0 (0.36)	1.8 (0.32)	2.3 (0.33)	2.0 (0.16)
Ca/P	4.5 (0.50)	2.9 (0.20)	3.9 (0.23)	2.2 (0.13)

[A,] Kynophos.

The LW of each cow and calf was measured, and cow BCS (1-5 scale) estimated, each week. Voluntary intake of feed of each cow-calf pair was measured on a weekly basis from the amount of feed offered and refused. Samples of feed offered were obtained on each occasion a batch of diet was mixed and samples of feed refusals were obtained weekly. Total collections (TC) of all faeces excreted were made over fiveday intervals during weeks 3, 8 and 14 (TC1, TC2 and TC3, respectively) while the animals were constrained to the front section of the pen with concrete flooring (*ca.* 14 m^2) and by frequent collection of the faeces from the floors. The feed offered and refused during the total collection intervals were sampled and bulked. The faeces excreted each day were collected, mixed and a 10% subsample was stored frozen, and at the end of each collection interval these samples were mixed and subsampled. Feed offered and refused during other intervals of the experiment were bulked on a monthly basis. Feed and faeces samples were dried at 60°C and were

then ground through a 1 mm screen (Christie and Norris Mill, Chelmsford, UK) and retained for analysis.

Blood samples were obtained at fortnightly intervals by jugular puncture using vacutainers (BD, Diagnostics, Plymouth, UK) containing lithium-heparin and were centrifuged (3,000 g x 15 min) to separate plasma. Urine samples *(ca.* 30-60 ml) obtained by stimulation of the vulva during the weeks before and after the TC2 and TC3 total collections of faeces, were acidified (pH < 3) by addition of 4 M sulphuric acid, and were stored for subsequent analysis of purine derivatives and P. Samples of plasma and urine were stored frozen.

Biopsies of external cortical bone from the 12th rib and of *tuber coxae* bone were obtained at the commencement and at the end of the experiment using a modification of the procedures described by Little (1972), Kidd *et al*. (2014) and as described in Appendix 3. The depth of fat at the P8 and rib sites, and eye muscle area (EMA) were measured by ultrasound (Esaote Pie Medical Aquila with a 3.5 Mhz linear array transducer (Pie Medical Imaging, Maastricht, The Netherlands) by an experienced operator at the beginning and end of the experiment.

Laboratory procedures

Organic matter were determined by incineration (550^{o}C for 8 h). Concentrations of P, Ca, Mg and S in feed offered throughout the experiment, in feed offered, feed refused and faeces during the total collection intervals were analysed using an inductively coupled plasma spectrometer (Optima 7300 DV, Perkin Elmer, Waltham, MA, USA) after nitric-perchloric acid mixture digestion. Samples of feed offered were also analysed for total N, NDF, ADF, lignin and crude fat by a commercial laboratory (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). Plasma samples were analysed colormetrically for plasma inorganic P (PIP), Ca and Mg in the DAF Coopers Plains laboratory (Beckman Coulter Au680 analyser with OSR6122, OSR 6189 and OSR6189 assay kits respectively). The concentration of P in urine obtained before and after TC3 was analysed colormetrically (Goodwin 1970). Rib bone biopsy samples were carefully scraped to remove trabecular bone before measuring CBT using vernier callipers and specific gravity (SG) by gravimetric procedures. Plasma concentrations of hormones and bone metabolism markers were determined using commercial assay kits and in accordance with the manufacturer's instructions (a full list of hormones and markers is given in Appendix 2). These constituents were parathyroid hormone (PTH; A11930, Beckman Coutler), 25OH Vit D and 1,25-diOH Vit D (AA-35F1 and AA-54F2, Immunodiagnostic Systems), CTX-1 (Crosslaps AC-02F1, Immunodiagnostic Systems), bone alkaline phosphatase (BAP; MicroVue 8012, Quidel), and osteocalcin (OCN; MicroVue 8002, Quidel). ALP and AST were analysed with kits (Beckman Coulter) which are based on recommendations of the International Federation for Clinical Chemistry. Bone samples for histology were fixed in 10% neutral buffered formalin for at least eight weeks. Samples were decalcified in 14% EDTA for at least 12 weeks and then embedded in parrafin. Sections (5 µm) were stained with Masson's trichrome and were photographed on a microscope at 2, 10 and 20 x magnification. Measurments of cortical thickness, trabecular volume, trabecular thickness, osteoid % and trabecular separation were performed using the image analysis program ImageJ with the pluggin BoneJ (Doube *et al.* 2010).

Calculations and statistical analyses

The estimated weight of the conceptus was calculated as described by O'Rourke *et al.* (1991) and the conceptus-free LW (CF-LW) was calculated by subtraction of the estimated conceptus weight from the total LW (T-LW). The LW change of the cows was calculated using two approaches; (i) from the linear regression of LW with time during the 14.5 weeks (LWG(R)), and difference between the LW measured on day 4 and the mean of the LW on day 101 (LWG(D)). Apparent digestibility of DM and organic matter (OM) were calculated by conventional procedures. The ME/DM content (M/D) of the mixed diets fed in the pens during was calculated from the organic matter digestibility (OMD) measured during the three total collection intervals as: M/D = 0.169 (%OMD) – 1.986 where M/D was MJ ME/kg DM (CSIRO 2007). Fermentable metabolisable energy (FME) content was calculated from total ME content by subtraction of the lipid content of the diet and assuming that the latter contained 35 ME MJ ME/kgDM (AFRC 1991). The volume of urine excreted during week 14 was calculated from the creatinine concentration and assuming a daily creatinine of 0.91 mmol/kg $W^{0.75}$ (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 difference between intake and faecal excretion (I-F) of P and Ca during the total collection intervals was calculated from the respective measurements. P balance was calculated from P intake minus the excretion of P in faeces and urine (measured from samples obtained shortly before and after the week 14 total collection). The concentration of P in cortical rib bone was calculated as: Pconc (mgP/cc) = 228.8 SG – 261 (Dixon *et al.* 2018). An index of rib bone P, the P in cortical bone per unit surface area of cortical bone (PSACB, mgP/mm^2), was calculated as the product of P concentration in external cortical bone and CBT (Dixon et al. unpublished). The requirements for P and Ca were calculated using the CSIRO spreadsheet Ca/P Requirements.

Statistical analysis of the results was conducted by analysis of variance in a 2x2 factorial design using Genstat (release 16.1, VSN International Ltd, Hemel Hemstead, UK). Measurements of the animals at the commencement of the experiment (estimated foetal age, cow age, initial cow total liveweight, cow concept-free liveweight, cow body condition score, rump fat, rib fat, eye muscle area, cortical bone thickness, specific gravity and P concentration) were examined as potential covariates and were included in the statistical model when significant (P<0.05). This is indicated in the respective tables. For analyses of blood mineral, hormone and bone metabolism markers a repeated measures ANOVA with main effects of diet and time was used, and subesquently effects at individual time points were compared using a factorial ANOVA with posthoc pair-wise protected LSD tests.

4.1.9 Results

Voluntary intake, diet digestibility, LW change and rumen microbial synthesis.
The cows were in good health throughout the experiment. The average concentrations of P in the diets were 0.68 and 0.76 g/kg DM for the ModE-LP and HE-LP diets, and 1.58 and 2.11 g/kg DM for the ModE-HP and HE-HP diets (Table B-1).

The voluntary intake (Figure B-1 and Table B-2) declined through the 14.5 week feeding interval and in a repeated measures ANOVA model there was an effect of time (P<0.001). With the exception of the HE-LP diet during week 1 there was no evidence of interaction between diet and time on voluntary intake. Intake of the HE-HP treatment diet (21.5 g DM/kg T-LW) was consistently higher (P<0.05) than that for the ModE-HP and HE-LP diets (14.9 and 15.8 g DM/kg T-LW), which were

higher (P<0.05) than for the ModE-LP diet (13.4 g DM/kg T-LW) (Table B-2). Mean organic matter (OM) digestibility, and hence estimated ME content, was affected by both the type of diet (HE versus LE) and by P content, and there was an interaction where the digestibility of the HE-LP and HE-HP diets were similar and higher than the digestibility of the ModE-LP diet, which was was higher than for the ModE-HP diet (Table B-1). Voluntary intakes of metabolizable energy calculated from average DM intake and OM digestibility were 112 and 115 kJ ME/kgLW.day for ModE-LP and ModE-HP treatments, increasing (P<0.05) to 148 and 204 kJ ME/kgLW.day for the HE-LP and HE-HP diets, respectively (Table B-2). Microbial crude protein (MCP) synthesis was similar in the ModE-LP, ModE-HP and HE-LP diets (155-182 g MCP/day but higher (310 g MCP/day) in the HE-HP diet (Table B-2). This was associated with an increase in the efficiency of microbial synthesis from 3.26 – 3.71 to 5.30 g MCP/MJ FME in the HE-HP diet.

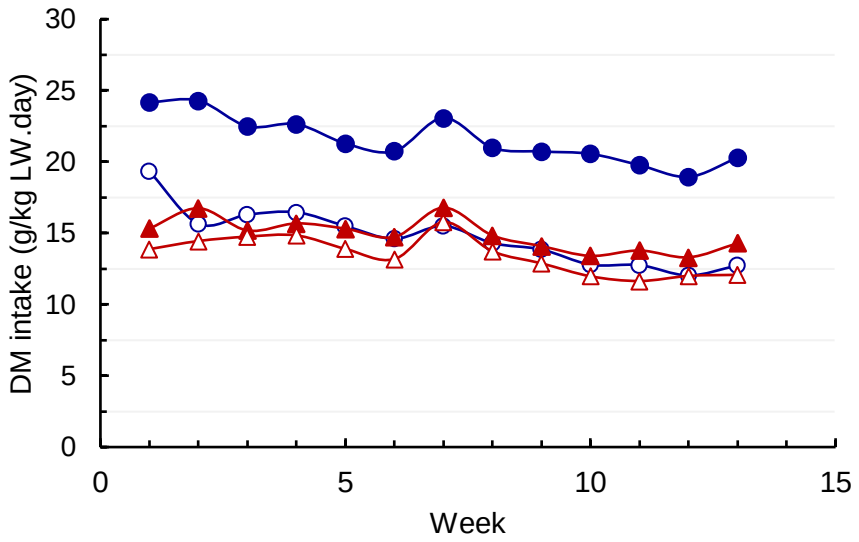


Figure B-1. Pens_B. Voluntary dry matter intake (VI) (g DM/kg LW.day) of cows fed the four diet treatments comprising HighE-LP(○), HighE-HP (●), ModE-LP (Δ) and ModE-HP (▲).

Table B-2. Experiment Pens_B. The voluntary intakes of DM, diet in vivo OM digestibility (DMD) at three total collection intervals (TC1, TC2 and TC3), estimated ME content of the diet and estimated ME intake, intake and excretion of P and Ca, and microbial protein synthesis in mature cows fed moderate ME (ModE) or high ME (HE) content diets containing low (LP) or high (HP) concentrations of phosphorus (P) for 14 weeks during mid-pregnancy (n=10)

Measurement	Diet treatments				s.e.m.					Probability			
	ModE-LP	ModE-HP	HE-LP	HE-HP	E	P	ExP	T	Cov	E	P	ExP	T
n	10	10	10	10	-	-	-	-	-	-	-	-	-
Calculated P requirement (g/day)	7.3	10.2	10.2	19.3	-	-	-	-	-	-	-	-	-
Actual P intake (g P/day)	3.7	10.3	5.2	21.4	-	-	-	-	-	-	-	-	-
Intake (Proportion of requirement)	0.51	1.01	0.51	1.11	-	-	-	-	-	-	-	-	-
DM intake (kg/day)	5.47^{c}	6.54^{b}	6.83^{b}	10.14^{a}	0.15	0.15	0.21		A	<0.001	<0.001	<0.001	<0.001
DM intake (g/kg T-LW.day)	13.4^{c}	14.9^{b}	15.8^{b}	21.5^{a}	0.30	0.30	0.41	--	A	<0.001	<0.001	<0.001	<0.001
DM intake (g/kg CF-W.day)	13.7	15.2	16.4	21.9	1.46	1.46	2.06	--	A	<0.001	<0.001	<0.001	<0.001
OMD (g/kg) TC1	618^{b}	516^{c}	676^{a}	622^{ab}	13	13	19	--	--	<0.001	<0.001	0.213	--
OMD (g/kg) TC2	629^{b}	622^{b}	695^{a}	725^{a}	9	9	13	--	--	<0.001	0.371	0.168	--
OMD (g/kg) TC3	610^{c}	579^{d}	645^{b}	690^{a}	7	7	11	--	--	<0.001	0.540	<0.001	--
OMD(g/kg) Mean	619^{b}	572^{c}	672^{a}	680^{a}	7	7	11	8	--	<0.001	0.055	0.009	<0.001
ME content (MJ/kg)	8.47^{b}	7.68^{c}	9.37^{a}	9.46^{a}	0.12	0.12	0.16	--	--	<0.001	0.41	0.011	--
MEI (MJ/day)	45.8^{c}	50.7^{c}	64.2^{b}	96.0^{a}	1.42	1.38	1.96		A	<0.001	<0.001	<0.001	--
MEI (kJME/kg T-LW.day)	112^{c}	115^{c}	148^{b}	204^{a}	2.7	2.6	3.7		B	<0.001	<0.001	<0.001	--
Microbial CP (g/day)	354^{b}	358^{b}	387^{b}	573^{a}	17.4	17.4	24.6			<0.001	<0.001	<0.001	--
Microbial CP (g/MJ FME.day)	7.77^{b}	8.30^{b}	6.78^{b}	6.34^{a}	0.40	0.40	0.56		A	0.015	0.944	0.398	--

E, main effect of energy content of the diet; P, main effect of phosphorus content of the diet; T, time effect in repeated measures model.

Means with different superscripts are different (at P<0.05) calculated from the lsd for the E x P interaction.

Covariates: A, cow age and initial cow LW. B, cow age; C, Initial cow T-LW and Day of pregnancy.

Across the diets the intakes of P during the various TC intervals ranged from 2.2 – 4.3 g P/day for the LP diets, and 9.1 – 22.9 gP/day for the HP diet, the variation associated with the changes in both P concentration in the diet and voluntary intakes. Mean faecal excretion ranged from 7.0 – 11.7 g P/day across the diets (Table B-3). P excretion in urine was ≤0.2 g P/day. P retention was negative in the two low P diets (-4.5 and -5.8 g P/day in the ModE-LP and HE-LP diets, respectively) indicating net mobilization from body tissues of these amounts of P in the low P diets. The ModE-HP diet cows were in approximate P balance (+0.2 g P/day) and the HE-HP cows retained substantial P (+6.5 g P/day). The intake and excretion of Ca followed a similar pattern (Table B-4) with the ModE-LP and ModE-HP diet cows at approximate Ca balance, the HE-LP diet cows in a small negative balance (-2.4 g Ca/day) and the HE-HP diet cows retained substantial Ca (11.9 g Ca/day).

The LW gain in each of the treatments was consistent through the 14.5 weeks of the experiment (Figure B-2). There was a small LW loss in the ModE-LP cows (-0.07 kg/day) and inclusion of P (ModE-HP diet) improved (P<0.05) this to a LW gain (+0.32 kg/day) (Table B-5). The addition of P to the high ME content diet increased (P<0.05) LW gain from 0.29 to 1.05 kg/day. Thus the effect of additional diet P to increase LW gain was much greater in cows given the high ME content diet than the moderate ME content diet. An unexpected result was that the LW gain of the ModE-HP and HE-LP diets were similar (0.32 and 0.29 kg/day even though the ME intake was 29% higher (P<0.05) in the HE-LP diet (Table B-2). There were only small changes during the experiment in BCS, fat depth at the rump and rib sites and EMA in the ModE-LP, ModE-HP and HE-LP diets, but all of these values were higher (P<0.05) in the HE-HP diet treatment cows than the other diets at the end of the experiment (Table B-5). This was in accord with the higher LW gain in the HE-HP diet.

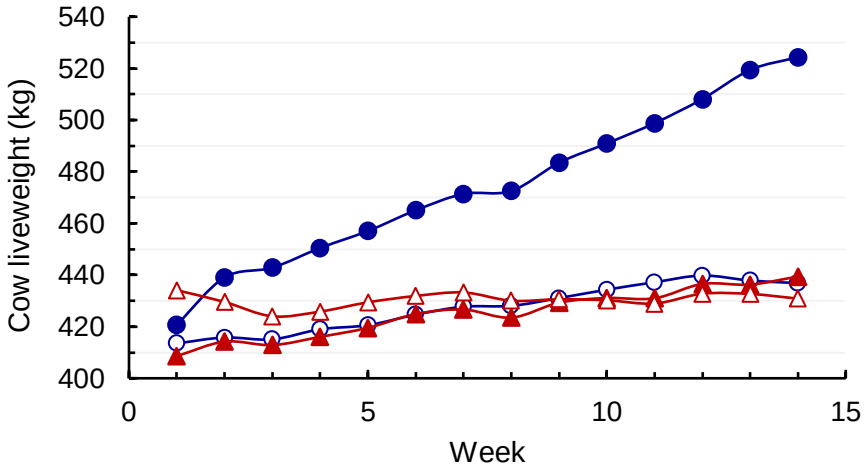


Figure B-2. Pens_B. Total liveweight of cows fed the four diet treatments comprising HighE-LP(○), HighE-HP (●), ModE-LP (Δ) and ModE-HP (▲).

Table B-3. Pens_B. The intakes of P, faecal excretion of P, difference between intake and faecal excretion (I-F) of P, and urinary excretion of P, during three total collection intervals (TC1, TC2 and TC3) and the mean in mature cows fed moderate ME (ModE) or high ME (HE) content diets containing low (LP) or high (HP) concentrations of phosphorus (P) for 14 weeks during mid-pregnancy (n=10)

Measurement	Diet treatments				s.e.m.				CoV	Probability			
	ModE-LP	ModE-HP	HE-LP	HE-HP	E	P	ExP	Time		E	P	ExP	T
P intake_TC1 (g/day)	3.1^{c}	9.1^{b}	4.2^{c}	17.4^{a}	0.36	0.36	0.51	--	No	<0.001	<0.001	<0.001	--
P intake_TC2 (g/day)	3.4^{c}	9.5^{b}	3.2^{c}	21.3^{a}	0.51	0.51	0.72	--	No	<0.001	<0.001	<0.001	--
P intake_TC3 (g/day)	2.2^{d}	10.7^{b}	4.3^{c}	22.9^{a}	0.32	0.32	0.45	--	No	<0.001	<0.001	<0.001	--
P intake_mean (g/day)	2.9^{c}	9.8^{b}	3.9^{c}	20.5^{a}	0.35	0.35	0.50	0.18	No	<0.001	<0.001	<0.001	<0.001
P faeces_TC1 (g/day)	5.8^{b}	7.4^{b}	7.0^{b}	10.3^{a}	0.60	0.60	0.85	--	No	0.026	0.008	0.317	--
P faeces_TC2 (g/day)	7.3	8.7	10.4	11.4	1.25	1.25	1.77	--	No	0.114	0.507	0.899	--
P faeces_TC3 (g/day)	7.7	10.0	8.5	13.4	1.30	1.30	1.83	--	No	0.258	0.062	0.480	--
P faeces_mean (g/day)	7.0	8.7	8.6	11.7	0.83	0.83	1.17	0.61	No	0.056	0.050	0.580	0.029
P (I-F)_TC1 (g/day)	-2.8^{c}	+1.7^{b}	-2.8^{c}	+7.2^{a}	0.60	0.60	0.85	--	No	0.003	<0.001	0.004	--
P (I-F)_TC2 (g/day)	-3.9^{bc}	+0.8^{b}	-7.2^{c}	+9.9^{a}	1.32	1.32	1.86	--	No	0.131	<0.001	0.003	--
P (I-F)_TC3 (g/day)	-5.1^{c}	+0.7^{b}	-4.2^{bc}	+9.5^{a}	1.30	1.30	1.84	--	No	0.011	<0.001	0.052	--
P (I-F)_mean (g/day)	-4.1^{c}	+1.1^{b}	-4.7^{c}	+8.8^{a}	0.85	0.85	1.20	0.61	No	0.007	<0.001	0.002	0.491
Urine volume_TC3 (L/day)	24.1^{bc}	29.0^{ab}	33.8^{a}	17.3^{ac}	3.07	3.07	4.34	-		0.826	0.196	0.021	-
P in urine (g/day)	0.2	0.2	0.2	0.0	0.07	0.07	0.10	--		0.396	0.475	0.274	--
P retention (g/day)	-4.6^{c}	+0.3^{b}	-6.3^{c}	+6.6^{a}	0.87	0.87	1.23	--	No	0.072	<0.001	0.003	--
P retention (mg/kg T-LW.day)	-10.5^{c}	+0.6^{b}	-15.0^{c}	13.9^{a}	2.00	2.00	2.83	--	No	0.133	<0.001	0.004	--

E, main effect of energy content of the diet; P, main effect of phosphorus content of the diet; T, time effect in repeated measures model.
Means with different superscripts are different (at P<0.05) calculated from the lsd for the E x P interaction.

Table B-4. Pens_B. The intakes of Ca, faecal excretion of Ca, difference between intake and faecal excretion (I-F) of Ca, and urinary excretion of Ca, during three total collection intervals (TC1, TC2 and TC3) and the mean in mature cows fed moderate ME (ModE) or high ME (HE) content diets containing low (LP) or high (HP) concentrations of phosphorus (P) for 14 weeks during mid-pregnancy (n=10)

Measurement	Diet treatments				s.e.m.				CoV	Probability			
	ModE-LP	ModE-HP	HE-LP	HE-HP	E	P	ExP	Time		E	P	ExP	T
Ca intake_TC1 (g/day)	19.9	24.2	20.7	42.0	1.04	1.04	1.47	--	No	<0.001	<0.001	<0.001	--
Ca intake_TC2 (g/day)	23.4	26.6	23.9	44.1	1.22	1.22	1.73	--	No	<0.001	<0.001	<0.001	--
Ca intake_TC3 (g/day)	14.7	30.3	21.0	48.3	0.90	0.90	1.28	--	No	<0.001	<0.001	<0.001	--
Ca intake_mean (g/day)	19.3	27.0	21.9	44.8	0.92	0.92	1.30	0.46	No	<0.001	<0.001	<0.001	<0.001
Ca faeces_TC1 (g/day)	17.2	21.5	21.3	29.2	1.55	1.55	2.19	--	No	0.012	0.010	0.432	--
Ca faeces_TC2 (g/day)	19.4	23.3	27.7	29.8	2.70	2.70	3.82	--	No	0.063	0.442	0.831	--
Ca faeces_TC3 (g/day)	17.7	23.3	20.2	29.0	2.25	2.25	3.19	--	No	0.215	0.033	0.614	--
Ca faeces_mean (g/day)	18.1	22.7	23.1	29.5	1.67	1.67	2.37	1.19	No	0.020	0.028	0.701	0.185
Ca (I-F)_TC1 (g/day)	+2.7	+2.7	-0.7	+12.8	1.45	1.45	2.05	--	No	0.107	0.003	0.003	--
Ca (I-F)_TC2 (g/day)	+3.9	+3.3	-3.8	+14.0	2.50	2.50	3.53	--	No	0.674	0.022	0.015	--
Ca (I-F)_TC3 (g/day)	-3.0	+7.0	+0.9	+19.3	2.11	2.11	2.98	--	No	0.011	<0.001	0.167	--
Ca (I-F)_mean (g/day)	+1.2	+4.3	-1.2	+15.3	1.48	1.48	2.09	1.19	No	0.049	<0.001	0.003	0.469
Ca in urine (g/day)	1.6	4.2	1.9	5.1	0.48	0.48	0.68	--	No	0.369	<0.001	0.723	--
Ca retention (g/day)	-0.46[b]	-1.2[b]	-3.1[b]	+9.9[a]	1.68	1.68	2.37	--	No	0.092	0.017	0.008	--
Ca retention (mg/kg T-LW.day)	+0.8[a]	+0.9[b]	-5.6[b]	+24.9[a]	3.66	3.66	5.18		No	0.102	0.007	0.007	--

E, main effect of energy content of the diet; P, main effect of phosphorus content of the diet; T, time effect in repeated measures model.
Means with different superscripts are different (at P<0.05) calculated from the lsd for the E x P interaction.

Table B-5. Pens_B. The liveweight (LW), conceptus-free LW (CF-LW) and body condition score (BCS), and changes in these variables in mature cows fed moderate ME (ModE) or high ME (HE) content diets containing low (LP) or high (HP) concentrations of phosphorus (P) for 14 weeks during mid-pregnancy (n=10). In addition measurements of the fat depth measured at the rump and rib sites and eye muscle area, and the changes in these measurements during the experiment are given

Measurement	Diet treatments				s.e.m.				Probability		
	ModE-LP	ModE-HP	HE-LP	HE-HP	E	P	ExP	Cov	E	P	ExP
Initial T-LW (kg)	434	409	414	421	8.9	8.9	12.6	--	0.739	0.475	0.207
Initial CF-LW (kg)	430	405	410	417	8.9	8.9	12.6	--	0.740	0.477	0.218
T-LW change(D) (kg)	+6	+29	+26	+97	3.4	3.4	4.8	--	<0.001	<0.001	<0.001
T-LW change(D) (kg/day)	0.07^{c}	0.32^{b}	0.29^{b}	1.05^{a}	0.037	0.037	0.052	--	<0.001	<0.001	<0.001
CF-LW change(D) (kg)	-7^{a}	$+17^{b}$	$+14^{b}$	$+84^{c}$	3.4	3.4	4.9	--	<0.001	<0.001	<0.001
CF-LW change(D) (kg/day)	-0.08^{a}	$+0.19^{b}$	$+0.16^{b}$	$+0.91^{c}$	0.037	0.037	0.053	--	<0.001	<0.001	<0.001
Initial BCS	2.6	2.7	2.7	2.7	0.07	0.07	0.10	--	0.551	0.619	1.00
BCS change	-0.10	0.25	0.27	1.25	0.08	0.08	0.11	--	<0.001	<0.001	0.008
Initial rump fat (mm)	1.5	2.3	2.0	1.7	0.25	0.25	0.35	--	0.888	0.484	0.130
Change in rump fat	0.5	0.5	2.3	8.7	0.32	0.30	0.43	A	<0.001	<0.001	<0.001
Initial rib fat (mm)	1.4	1.5	1.6	1.3	0.15	0.15	0.21	--	1.000	0.632	0.341
Change in rib fat	-0.2	0.2	0.9	4.4	0.23	0.23	0.32	--	<0.001	<0.001	<0.001
Initial EMA	50.5	53.5	53.5	56.4	1.83	1.83	2.59	--	0.265	0.265	0.985
Change in EMA	-3.2	0.9	0.7	14.3	1.01	1.01	1.42	B	<0.001	<0.001	0.002

E, main effect of energy content of the diet; P, main effect of phosphorus content of the diet; T, time effect in repeated measures model.
Means with different superscripts are different (at P<0.05) calculated from the lsd for the E x P interaction.
Covariates, A, cow age; B, Initial EMA and Day of pregnancy.

Table B-6. Pens_B. Plasma bone metabolite measurements of mature cows fed high ME (HE) or moderate ME (ModE) content diets containing low (LP) or high (HP) concentrations of phosphorus (P) at 14 weeks during mid-pregnancy (n=10)

Measurement	Diet treatments				s.e.m.					Probability			
	ModE-LP	ModE-HP	HE-LP	HE-HP	E	P	ExP		Cov	E	P	ExP	
Plasma P (mmol/L)	0.67^{a}	2.17^{b}	0.51^{a}	2.11^{b}	0.104	0.104	0.146		-	n.s.	***	n.s.	
Plasma Ca (mmol/L)	2.53^{b}	2.17^{a}	2.50^{b}	2.17^{a}	0.051	0.051	0.072		-	n.s.	***	n.s.	
Plasma Mg (mmol/L)	0.87^{b}	0.77^{a}	0.88^{b}	0.80^{a}	0.031	0.031	0.043		-	n.s.	**	n.s.	

E, main effect of energy content of the diet; P, main effect of phosphorus content of the diet; T, time effect in repeated measures model.
Means with different superscripts are different (at P<0.05, n.s., not significant P>0.05) calculated from the lsd for the E x P interaction.

Plasma P, Ca and Mg

Three days after commencement of the diet treatments there were significant (P<0.01) differences in PIP between the low P diets (ModE-LP 0.92 and HE-LP 0.73 mmol/L) and the high P diets (ModE-HP 1.36 and HE-HP 1.43 mmol/L) (Figure B-3A). In the high P diets PIP concentrations gradually increased during the experiment in the HE-HP and ModE-HP diet cows and indicated that these were adequate in P throughout the experiment, whilst cows on low P diets (HE-LP and ModE-LP) were severely P deficient (PIP < 1mmol/L); The PIP was declined sharply within three days of introduction of the treatment diets and PIP continued to decline through the following 14 week experimental interval (Figure B-3A). The plasma concentrations of Ca and Mg showed opposite responses to the PIP concentrations, increasing on low P diets and remaining low on high P diets (Figure B-3B, Table B-6). Plasma Ca/P ratio was also consistently high in the LP diets (Figure B-4). No effect (P>0.10) of diet ME content was observed on any of the plasma mineral concentrations.

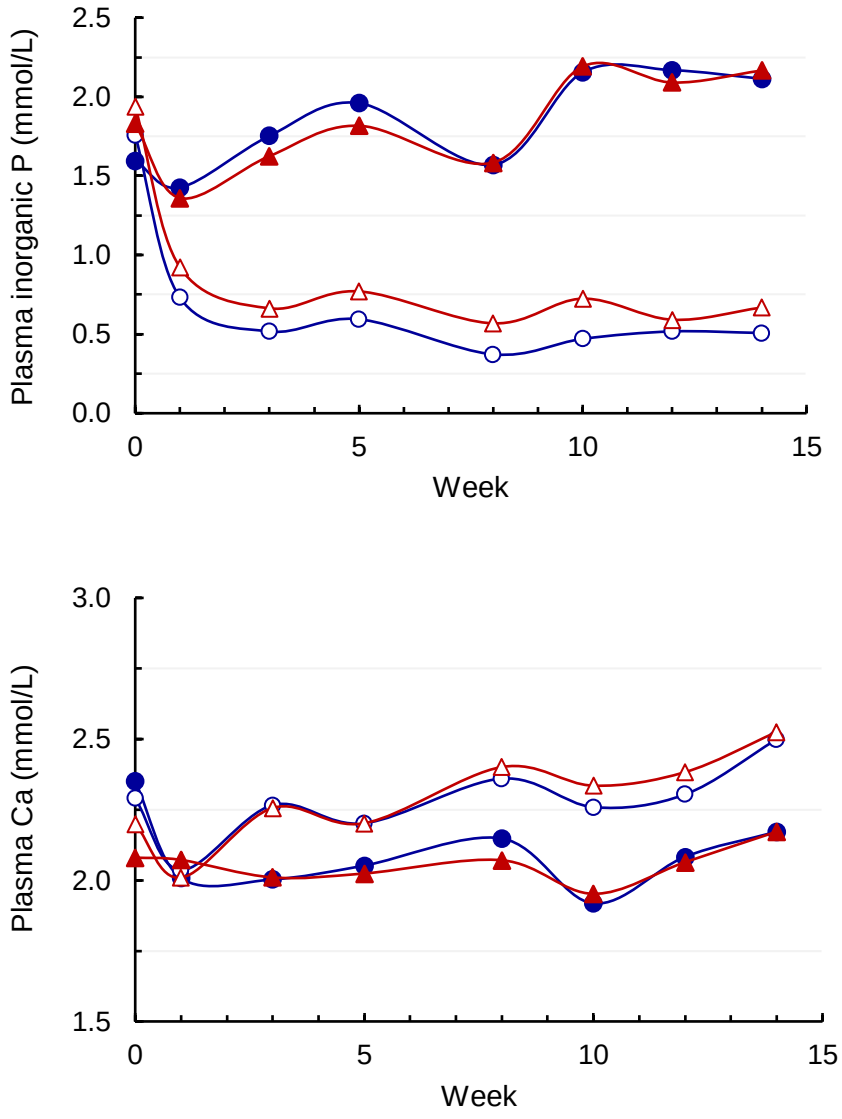


Figure B-3A and 3B. Pens_B. Plasma inorganic P (PIP) (mmol/L) and plasma total Ca (mmol/L) in cows fed the four diet treatments comprising HighE-LP(○), HighE-HP (●), ModE-LP (Δ) and ModE-HP (▲). Plasma concentrations were affected by diet (P<0.01) but not energy (P>0.10), and there was a diet x time interaction (P<0.0001).

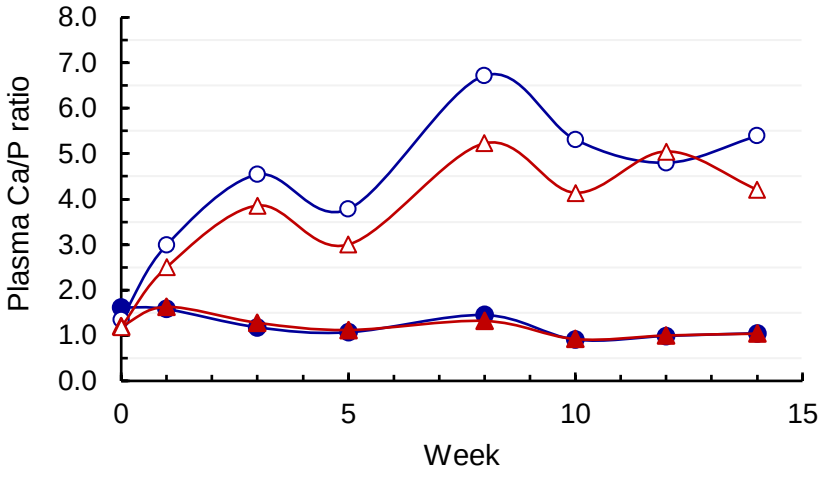


Figure B-4. Pens_B. The ratio of plasma plasma total Ca (mmol/L) to plasma inorganic P (PIP) (mmol/L) to in cows fed the four diet treatments comprising HighE-LP(○), HighE-HP (●), ModE-LP (Δ) and ModE-HP (▲).

Endocrine markers

Plasma PTH concentrations were variable at 2 and 4 weeks (Figure B-5). As the diet treatment interval progressed a significant ($P<0.05$) effect of diet P became evident, and by 14 weeks PTH concentrations were lower in low P diets (ModE-LP and HE-LP) than in adequate P diets (ModE-HP and HE-HP; Table B-6). PTH concentrations were correlated with plasma Ca concentrations as follows: ($\log_{10}$ PTH = -2.2 x total Ca + 7.097; $r^2 = 0.49$, slope $p<0.0001$, n=32).

Active Vitamin D (1,25-diOH VitD3) concentrations, but not precursor Vitamin D (25-OH Vit D3) concentrations, were affected by diet (Table B-6) with the former increased ($P<0.05$) 2-fold in the low P diets (ModE-LP and HE-LP) (Figure B-6). Plasma concentrations of the precursor 25-OH Vit D3 did not change over time (Table B-6). The ratio of active to precursor Vitamin D was higher in the low P diets and was highest in HE-LP cows.at 14 weeks.

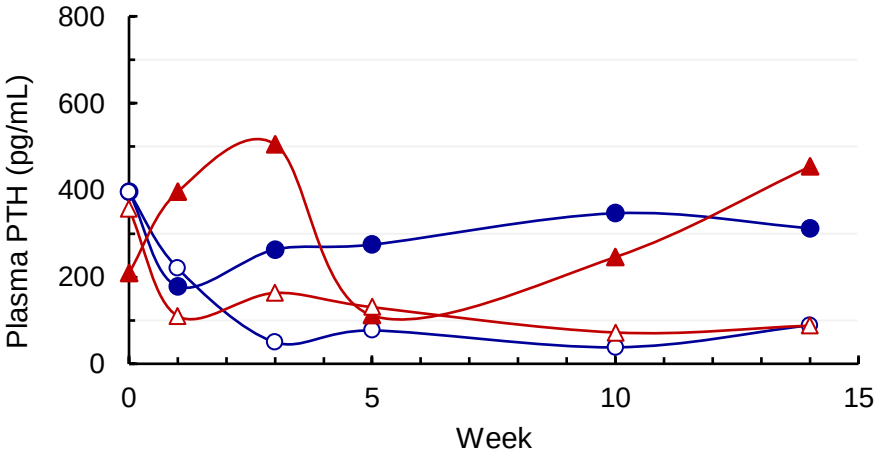


Figure B-5. Pens_B. Plasma Parathyroid Hormone (PTH) concentrations in cows fed the four diet treatments comprising HighE-LP(○), HighE-HP (●), ModE-LP (Δ) and ModE-HP (▲).

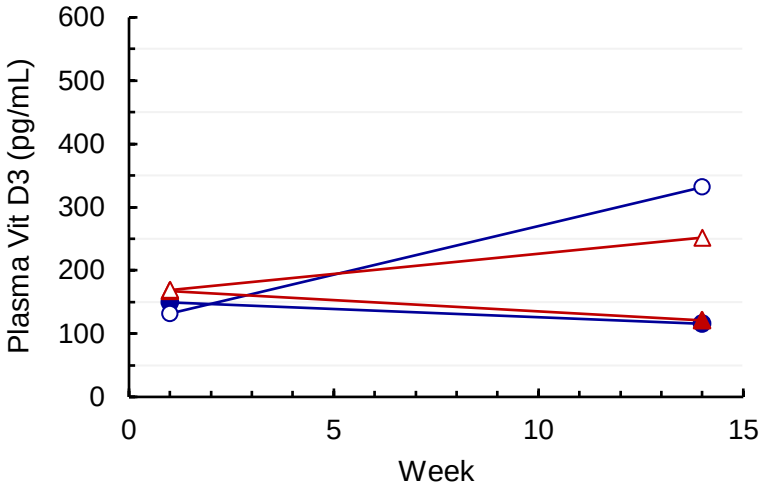


Figure B-6. Pens_B. Plasma 1, 25 dihydroxy Vitamin D concentrations in cows fed the four diet treatments comprising HighE-LP(○), HighE-HP (●), ModE-LP (Δ) and ModE-HP (▲).

In contrast there were interactions between diet P and ME by time for the plasma IGF-1 concentrations (P<0.001). IGF-1 concentrations were markedly increased in the HE-HP diet within 2 weeks (from initial 85 to 188 ng/ml at 2 weeks) and IGF-1 concentrations remained significantly (P<0.01) higher than in all the other diets throughout the experiment (Figure B-7). The HE-LP and ModE-HP diets had similar IGF-1 concentrations throughout the experiment, and IGF-1 concentrations were significantly (P<0.05) higher in both of these diets than in the ModE-LP diet (Table B-6).

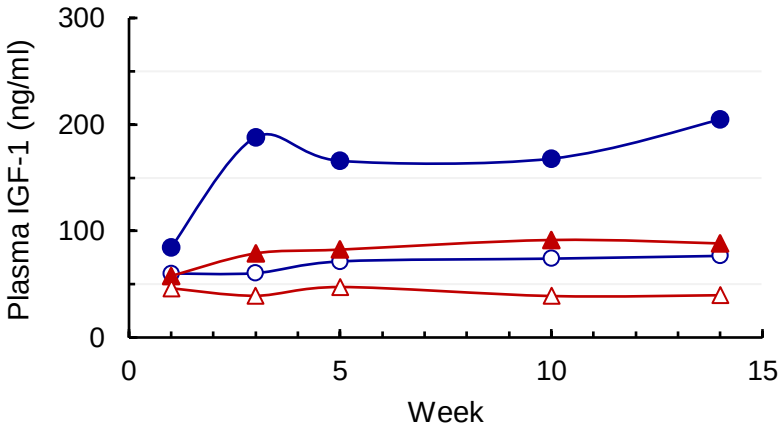


Figure B-7. Pens_B. Plasma IGF-1 concentrations in cows fed the four diet treatments comprising HighE-LP(○), HighE-HP (●), ModE-LP (Δ) and ModE-HP (▲).

Bone markers

CTX-1 concentrations were low in the cows initially when they were all fed a low P diet (0.57 ± 0.05 ng/mL), and increased (P<0.01) to 0.93 ± 0.07 ng/mL (average across in all treatments) by 3 days after introduction of the treatment diets. During the 14.5 weeks when diet treatments were fed plasma CTX-1 concentrations markedly increased in P deficient diets (ModE-LP and HE-LP), but decreased more in the high P diets (Table B-6) (Figure B-8). There was a significant (P<0.05) main effect of diet ME on CTX-1, with higher CTX-1 concentrations on moderate ME diets (Table B-6). CTX-

1 concentrations were negatively correlated with PIP (r = -0.72, P<0.001) but positively correlated with total plasma Ca concentrations (r = +0.66, P<0.001).

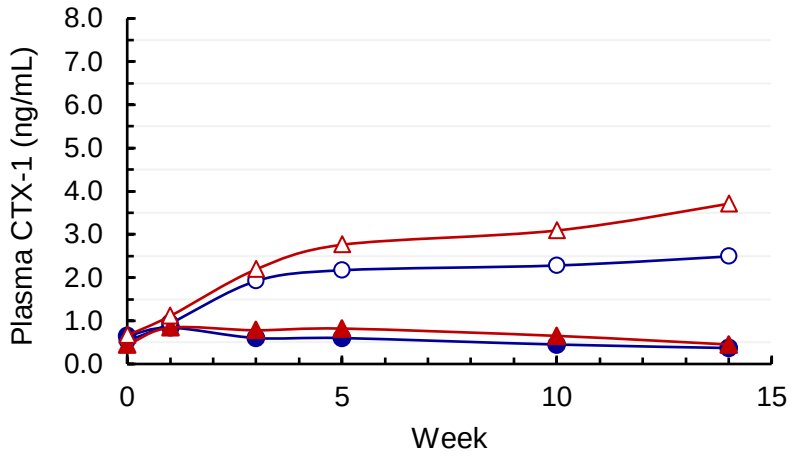


Figure B-8. Pens_B. Plasma CTX-1 concentrations in cows fed the four diet treatments comprising HighE-LP(○), HighE-HP (●), ModE-LP (Δ) and ModE-HP (▲).

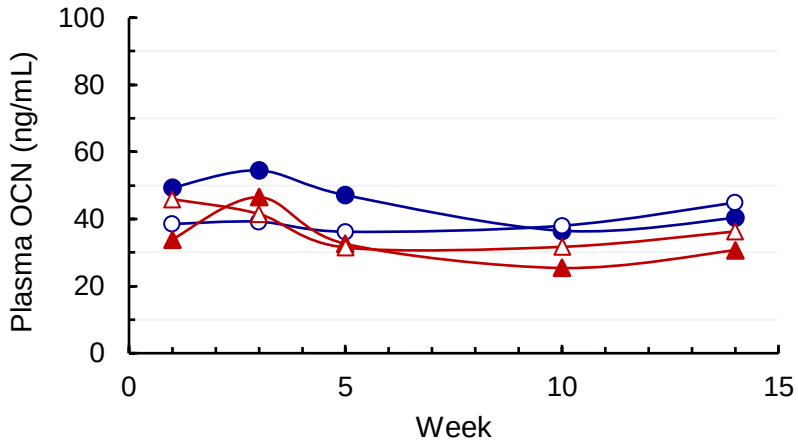


Figure B-9. Pens_B. Plasma osteocalcin (OCN) concentrations in cows fed the four diet treatments comprising HighE-LP(○), HighE-HP (●), ModE-LP (Δ) and ModE-HP (▲).

Plasma OCN concentrations decreased (P<0.01) during the 14.5 weeks of the experiment (Figure B-9) and there was no effect (P>0.05) of diet P on OCN. However there was a significant main effect (P<0.05) of diet ME content with higher OCN concentrations in the high ME diets, but this effect was small and not readily apparent in pair-wise comparisons (Table B-6). The CTX-1/OCN ratios with time are given in Figure 10 and show similar trends between diets. In contrast, there were marked main effects of diet P and ME on plasma BAP concentrations, and also interactions (P<0.01) of both diet P and ME with time (Figure B-11, Table B-6). Overall, low P diets (HE-LP and ModE-LP) increased BAP concentrations, whilst high P diets (ModE-HP and HE-HP) decreased BAP concentrations. In addition, high E diets increased BAP compared to moderate E diets. At 14.5 weeks BAP concentrations were different (P<0.05) among all the diets (Table B-6).

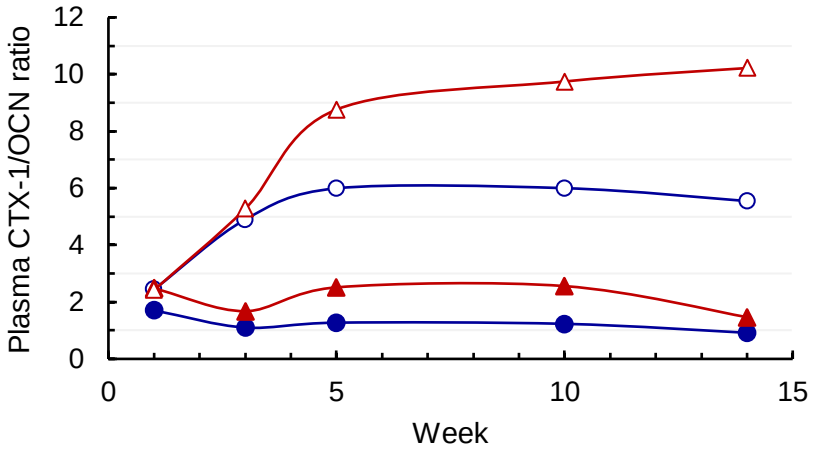


Figure B-10. Pens_B. Plasma CTX-1/OCN ratio in plasma of cows fed the four diet treatments comprising HighE-LP(○), HighE-HP (●), ModE-LP (Δ) and ModE-HP (▲). CTX-1 concentrations were affected by diet P (P<0.05) and diet E (P<0.05), and there was a diet P x time interaction (P<0.05).

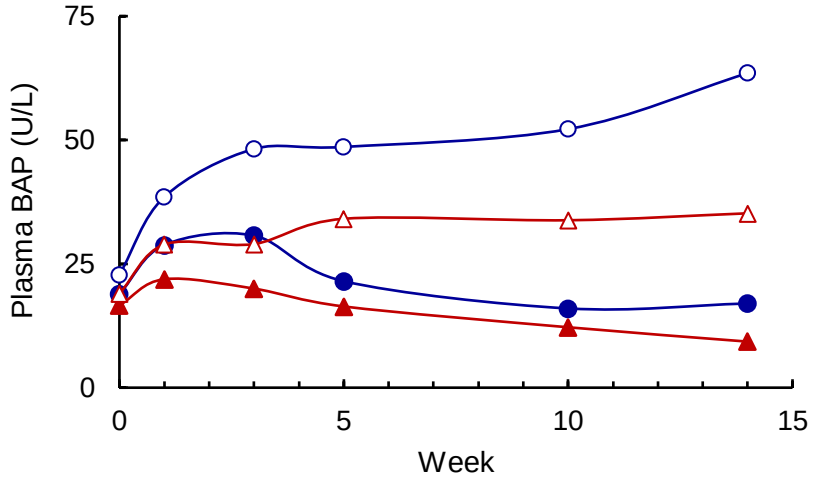


Figure B-11. Pens_B. Plasma concentrations of bone alkaline phosphatase (BAP) (U/L) in cows fed the four diet treatments comprising HighE-LP(○), HighE-HP (●), ModE-LP (Δ) and ModE-HP (▲). BAP concentrations were affected by diet P (P<0.05) and diet E (P<0.05), and there was a diet P x time interaction (P<0.05).

Total alkaline phosphatase (ALP) concentrations in plasma were affected (P<0.01) by the main effect of diet P and there was also an interaction of diet P by time; however there was no main effect of diet E. There was large variability in ALP concentrations among animals and reduced differences between diets at 14.5 weeks (Table B-6, Figure B-12). Plasma AST concentrations showed that all the cows, with the exception of one animal at one time point, had normal liver function during the experimental interval with AST < 170 U/L. Therefore AST did not aid in diagnosis of the persistent high ALP concentrations (ALP > 200 U/L) seen in six animals. BAP and ALP concentrations were poorly correlated (r = +0.14, P=0.41).

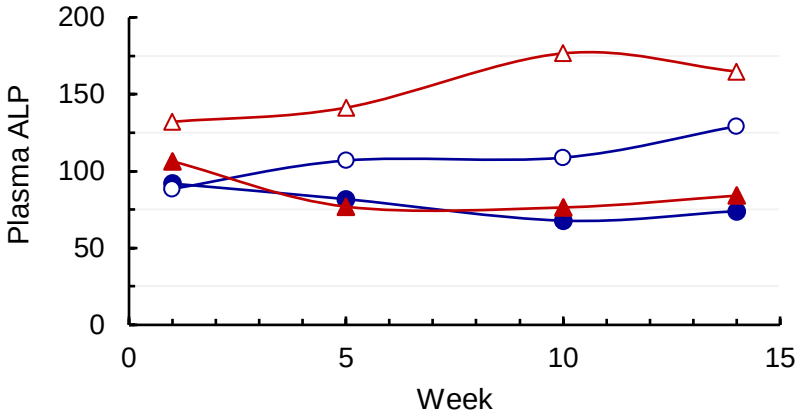


Figure B-12. Pens_B. Plasma concentrations of total alkaline phosphatase (ALP) (U/L) in cows fed the four diet treatments comprising HighE-LP(○), HighE-HP (●), ModE-LP (Δ) and ModE-HP (▲).

Changes in rib bone and tuber coxae trabecular bone

There was little change in cortical bone thickness (CBT) during the experiment in the two high P diets, but CBT in the two low P diets decreased ($P<0.05$) by on average 10.2% (Table B-7), indicating substantial net mobilization of rib bone P in the P deficient cows. There were similar changes in PSACB (Table B-7). There was no main effect of diet P on the P concentration of the cortical bone ($P>0.05$), but this P concentration was reduced ($P<0.01$) on average by 6% in cows fed the high ME content diets (Table B-7). PSACB decreased on average by 13.1% in the cows fed the two LowP diets but did not change in the cows fed the HighP diets.

Trabecular thickness, bone and osteoid volume, and the proportions of mineralised bone and osteoid was similar among treatment groups at the start of the experiment (Figure B-13). Cows given high P diets (HE-HP and ModE-HP) increased their trabecular bone volume by about 29% and trabecular thickness by about 20% during the 14.5 weeks. When initial BCS or cow age were used as covariates in statistical analysis, there was a significant ($P<0.05$) diet P effect with cows on high P diets having greater bone volume and trabecular thickness than cows on low P diets. Cows on low P diets (HE-LP and ModE-LP) exhibited almost no change in trabecular bone volume or trabecular thickness during the experiment (Table B-7). Thus the effect of diet P on trabecular bone appeared to be an improvement independent of diet ME content. In cows fed moderate ME diets (ModE-HP and ModE-LP), a high P content improved bone gain in association with some increase in liveweight. However cows fed high P diets (HE-HP and ModE-HP) had similar gains in bone volume despite large differences in intake and liveweight gain between these groups. Bone restoration therefore may not occur as quickly as the rapid gains in liveweight seen in cows fed the HE-HP diet.

The different responses seen in the rib cortical bone than in the tuber coxae trabecular bone in this experiment may have a number of explanations. These findings are consistent with site-specific differences in post-weaning bone restoration as seen in mice and humans where trabecular bone was deposited more rapidly than cortical bone in some studies (Liu *et al.* 2012). This may be due simply to the larger surface area over which trabecular bone can be formed. In cows fed low P diets, there was an increased proportion of un-mineralised osteoid in the trabecular bone (Table B-8; Figure B-13). Hence although the absolute volume of bone did not appear to change in cows fed low P diets, the volume of mineralised bone and thus of P reserves were reduced. This effect is more readily seen in trabecular bone where there is a larger surface area on which osteoid can form.

Table B-7. Measurements in rib bone biopsy samples of cortical bone thickness (CBT), P concentration (mg P/cc), PSACB index, porosity of cortical bone and the changes in these measurements during the experiment

Measurement	Diet treatments				s.e.m.	Cov	Probability		
	ModE-LP	ModE-HP	HE-LP	HE-HP	E or P		E	P	ExP
n	10	10	9	8	-	-	-	-	-
CBT, initial (mm)	3.76	3.98	3.54	3.66	0.146	--	0.211	0.419	0.811
CBT, final (mm)	3.33	3.61	3.28	3.83	0.135	A	0.601	0.036	0.472
CBT change (mm)	-0.41	-0.13	-0.46	+0.10	0.135	A	0.602	0.036	0.472
CBT change (%)	-8.9	-2.1	-11.5	+4.0	3.63	A	0.676	0.036	0.394
Pconc, initial (mg/cc)	150	148	149	148	2.4	--	0.866	0.573	0.895
Pconc, final (mg/cc)	149	152	137	142	2.3		0.003	0.240	0.701
Pconc change (g/cc)	0	3	-12	-7	2.3	C	0.004	0.202	0.720
Pconc change (%)	0.2	2.3	-7.9	-4.0	1.57	C	0.003	0.193	0.693
PSACB, initial (mgP/mm^2)	0.565	0.591	0.527	0.533	-	-	-	-	-
PSACB, final (mgP/mm^2)	0.502	0.572	0.436	0.549	0.0216		0.493	0.014	0.403
PSACB change (mgP/mm^2)	-0.063	-0.019	-0.091	+0.015	0.0200	D	0.545	0.018	0.502
PSACB change (%)	-9.6	-1.5	-16.5	+3.5	3.71	D	0.413	0.016	0.447
n	9	8	8	6	-	-	-	-	-
CB porosity, initial V-451	2.75	3.70	2.73	5.08	-	-	-	-	-
CB porosity, final V-452	3.59	1.87	6.19	3.38	0.574		0.020	0.012	0.514
CB porosity, change V-453	+0.84	-1.83	+3.46	-1.70	0.555		0.097	<0.001	0.129
CB porosity, change (%) V-454	+28	-40	+164	-42	24.5		0.071	<0.001	0.061

E, main effect of energy content of the diet; P, main effect of phosphorus content of the diet; T, time effect in repeated measures model.
Covariates, A, initial CBT; B, Initial SG; C, Initial Pconc; D, Initial PSACB; E, initial BCS; F, Cow age.

Table B-8. Pens_B. Measurements of tuber coxae of bone volume (BV/TV), trabecular thickness (Tb.Th), trabecular thickness (Tb.Th, max), osteod as % total bone, mineralized bone as % of total bone and the changes in these measurements during the experiment

Measurement	Diet treatments				s.e.m.			Cov	Probability		
	ModE-LP	ModE-HP	HE-LP	HE-HP	E	P	ExP		E	P	ExP
n	10	9	10	8	-	-	-	-	-	-	-
BV/TV, initial (%)	8.36	8.34	8.90	7.93	-	-	-	-	-	-	-
BV/TV, final (%)	8.40	11.36	10.17	11.23	0.731	0.731	1.033		0.437	0.065	0.365
BV/TV, change (%)	0.04	2.93	-0.22	2.85	1.014	1.014	1.433		0.905	0.050	0.951
BV/TV, change (% change)	11.1	40.1	7.6	39.4	12.33	12.33	17.43		0.908	0.095	0.937
Osteoid, initial (%)	16.4	13.5	21.2	20.2	-	-	-	-	-	-	-
Osteoid, final (%)	23^{b}	19^{b}	32^{c}	9^{a}	2.4	2.4	3.4		0.905	<0.001	0.008
Osteoid, change (%)	+6	+5	+9	-13	3.8	3.8	5.3		0.166	0.043	0.072
Osteoid, change (% change)	334	41	157	-55	83	83	117		0.257	0.043	0.737
Mineralized, initial (%)	7.1	7.3	7.2	6.5	-	-	-	-	-	-	-
Mineralized, final (%)	6.4	9.1	6.8	10.3	0.54	0.54	0.76		0.314	<0.001	0.614
Mineralized, change (%)	-0.73	+1.83	-1.09	+3.75	0.82	0.82	1.154		0.504	0.004	0.335
Mineralized, change (% change)	+1	+33	-4	+63	11.0	11.0	15.5		0.427	0.004	0.266
Tb.Th, initial (µm)	113	103	105	107	-	-	-	-	-	-	-
Tb.Th, final (µm)	108	127	127	135	4.7	4.7	6.6		0.055	0.057	0.454
Tb.Th, change (µm)	-4.5	+23.4	+17.7	+26.2	5.5	5.5	7.8		0.122	0.029	0.227
Tb.Th, change (%)	-2.2	+22.5	+17.0	24.7	5.1	5.1	7.2		0.153	0.036	0.253
Tb.Th-max, initial (µm)	253	238	262	293	-	-	-	-	-	-	-
Tb.Th-max, final (µm)	254	316	306	303	14.2	14.2	20.0		0.346	0.154	0.122
Tb.Th-max, change (µm)	1	76	54	14	21.5	21.5	30.4		0.869	0.565	0.070
Tb.Th-max, change (%)	4.2	34.6	25.9	11.6	8.2	8.2	11.6		0.956	0.496	0.067

E, main effect of energy content of the diet; P, main effect of phosphorus content of the diet; T, time effect in repeated measures model.

Means with different superscripts are different (at P<0.05) calculated from the lsd for the E x P interaction.

Covariates, A, initial CBT; B, Initial SG; C, Initial Pconc; D, Initial PSACB; E, initial BCS; F, Cow age

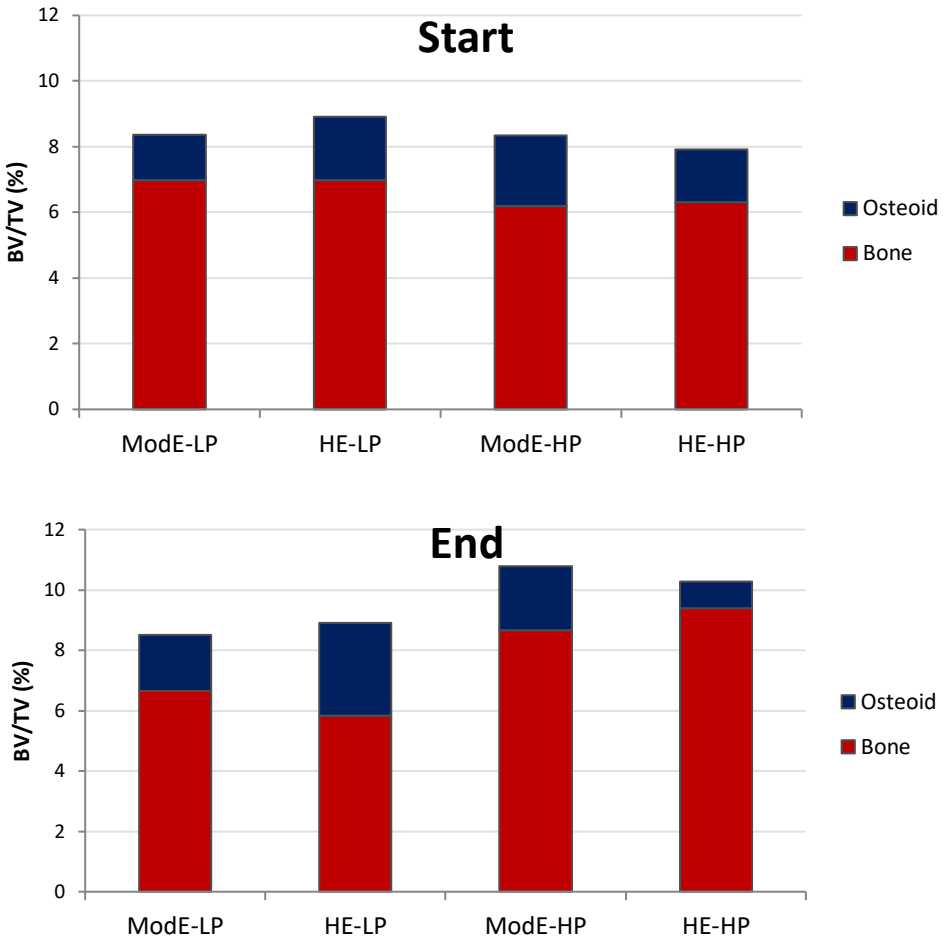


Figure B-13. Trabecular bone volume (BV/TV, %) showing the proportion of the total that is mineralised bone (red) or unmineralised osteoid (blue) at the start and end of cows fed the four diet treatments comprising HighE-LP(○), HighE-HP (●), ModE-LP (Δ) and ModE-HP (▲).

4.1.10 Discussion

If breeder cows mobilize their body reserves of energy and P to maintain lactation, it is essential that the cow calving annually recover body condition and body P reserves in preparation for the following late dry season and wet season when lactating. This is widely recognized in the context of body energy reserves and the importance to retain body condition on breeders, but should be equally valid for body P reserves. It is important to understand the circumstances under which breeders can replenish their body P reserves as both soft tissue and bone. There may be important relationships between P body reserves and LW and/or BCS. Higher LW and BCS will have the obvious benefits of greater amounts of P in soft tissue but may also be associated with the amount and availability of bone P reserves. Under established and usual management routines in northern Australia breeders will be weaned in the late wet season or early dry season. Thus the pasture available for cows post-weaning is usually senesced early dry season pasture expected to provide sufficient ME for slow live weight gain, and later with advance of the dry season LW maintenance or slow LW loss.

The present experiment indicated that if the pasture available is of sufficient ME and N content to allow substantial LW gain then there will be a large response to P supplementation as increased

pasture intake, LW gain, and improved P reserves. If the quality of the pasture available is suitable only for LW maintenance or slow LW gain then P supplementation of P deficient pasture during mid-pregnancy is not likely to increase pasture intake, but there is likely to be benefit as increased animal LW and some repletion of skeletal P reserves. This leads to a recommendation that cattle will respond to a low level of P supplementation during the early to mid dry season.

In practice provision of a high quality pasture diet post-weaning is only likely to be achieved routinely on commercial properties by early weaning (e.g. in March) and grazing breeders on late wet season pasture and/or by using higher quality pastures during this part of the annual cycle for breeders. The additional cost of managing low LW weaners may be justified to provide opportunity to obtain large responses to P supplements and replenish body P reserves in breeders grazing P-deficient rangelands, or to reduce the amounts of P supplements fed annually. Early weaning is clearly a well-established and widely used practice. It is important to be able to evaluate the importance of the advantages of good seasonal conditions when late wet season grass-legume pastures may retain their high nutritive value and present an opportunity for managers to provide high nutrient intakes for breeders in the early dry season.

It was unexpected that the P supplementation of the moderate ME content diet was associated with an increased LW change even though there was no change measured in ME intake per cow per day, or per kg LW; however the increase in T-LW and in CF-LW due to this additional P was statistically significant ($P<0.05$). This contrasts with the paradigm that the benefits of providing P to P deficient cattle is mediated entirely through increased voluntary intake of DM and ME. This result in the present experiment suggests that in at least some circumstances the provision of P is associated with an increase in the efficiency of utilization of ME for LW maintenance and/or LW gain. In contrast when a high ME, high N content, but P deficient diet was fed there were large increases in voluntary DM intake and estimated ME intake, and there was also a large increase in LW gain. The very low plasma PIP in the HE-LP animals (0.51 mmol/L) indicated that these animals were in extreme P deficiency. Thus, if it is possible to wean cows onto high quality pasture of sufficient ME and protein content to allow rapid growth, then large responses can be expected to P supplementation of pasture deficient in P.

The present study considered non-lactating breeder cows in mid-pregnancy when the demand of the foetus for P, and for other nutrients, is low. The responses as feed intake and LW change of the cows given the moderate ME content diet were comparable with those of the heifers during pregnancy in the Pens_A experiment which were fed the same nominal diet. The importance of the present study for the northern cattle industry is that there is opportunity to replenish body soft tissue reserves, and to some extent skeletal P reserves by providing P supplements for P deficient pastures. This opportunity will occur when breeders are grazing lower quality, lower ME content pastures. If breeders can be weaned onto better quality pastures then the benefits of P supplementation as LW gain and body P reserves will be considerably enhanced.

Taken in conjunction with the results of the Pens_C experiment it is likely that a viable strategy for producers who for practical reasons are not able to supplement with P during the early to mid wet season, is to instead rely on cows mobilizing body reserves during the early-mid wet season and during lactation, and to replenish these P reserves by P supplementation as early as possible during the mid to late wet season and the early dry season. Such a strategy will increase the P requirements

of breeders in the late wet season and the early dry season for replenishment of body P reserves as well as for the LW change at the time.

4.2 Experimental Section 4. Pens_C experiment

Title. Pens_C. Mobilization of phosphorus from body reserves by mature *Bos indicus* cross breeder cows during early lactation

Undertaken for the task 2 and 3 objectives

4.2.1 Summary of experiment

This experiment investigated the responses of mature breeders fed a P-adequate diet or several severely P-deficient diets in early lactation. Thirty-two *Bos indicus* cross cows initially in adequate P status (6-11 years, (initial mean ± sd) liveweight (LW) 474 ± 57 kg; body condition score 3.5 ± 0.55) were housed in individual pens from calving. For 14 weeks the cows were fed *ad libitum* a diet high or low in P (HP or LP) or calcium (Ca) (HCa or LCa) as follows: HP-HCa, LP-HCa and LP-LCa. A fourth diet comprised LP-LCa but with inclusion of ammonium chloride to achieve a negative DCAD diet (LP-LCa-D). These diets contained 9.4-9.7 MJ ME/kg (calculated from in vivo OMD) and 113-126 g CP/kg DM. The Ca:P ratios of these diets were 2:1, 5:1, 3:1 and 3:1, respectively. Liveweight, intake and milk production were measured, and blood was sampled weekly or fortnightly. Voluntary intake (VI) was higher in the cows fed the HP-HCa diet (21.5 g DM/kg LW) than any of the LP diets (P<0.001). The HP-HCa cows gained substantial LW (0.45 kg/day) while the cows fed the LP-HCa and LP-LCa diets lost substantial LW (P<0.01; -0.19 and -0.24 kg/day). Cows fed the LP-LCa-D diet had higher (P<0.05) VI 18.5 g DM/day) and LW gain (+0.19 kg/day) than the LP-HCa diet cows. Neither milk production nor calf growth were affected by diet (P>0.10). Rib bone biopsies after 14 weeks indicated that cortical bone thickness (CBT) and PSACB were higher in the HP diet cows than the LP diet cows, and PSACB in the LP-LCa-D diet was lower (P<0.05) than in the other two LP diets. Plasma inorganic P (PIP) concentrations indicated that the cows fed the three low P diets were severely P-deficient. Plasma PTH concentrations decreased and plasma Ca increased during lactation. CTX-1 concentrations, an marker of bone resorption, increased markedly in all low P diets and was higher (P<0.01) in cows fed the LP-LCa-D diet. Similarly low P diets increased (P<0.01) BAP and 1,25 di-OH Vit D concentrations after one month of lactation. It is concluded that mature *Bos indicus* cross cows calving in adequate P status can maintain milk production and calf growth when fed severely P deficient diets but there was substantial mobilization of body tissues. The negative DCAD diet increased bone P mobilization and reduced the LW loss in these lactating cows. Further research is needed to confirm these responses, to investigate the optimal diet DCAD and to examine possible adverse effects of excessive bone mobilization especially where subsequent body P replenishment is delayed.

4.2.2 Background

The objective of this experiment was to examine the effects of diet P deficiency on performance of mature cows during early to mid-lactation when cows calved in high P status. Three severely P deficient diets and a P adequate diet and were fed for the first 14 weeks of lactation to examine effects on intake, liveweight, lactational performance, and changes in P body reserves in this class of cow. Measurements of metabolites were included to elucidate the importance of various physiological mechanisms to the animal performance. In addition the effects of two diet variations were examined: (i) low calcium: phosphorus(Ca:P) ratio, and (ii) of a negative DCAD (diet cation-

anion difference). It has been shown in a number of studies with growing ruminants that a high Ca:P ratio in the diet reduces the rate of mobilization of body P reserves. Also extensive experimentation in the dairy industry has shown that negative DCAD diets around the time of parturition increase bone mobilization for Ca and reduce the risk of milk fever. Continued use of a negative DCAD diet in lactation might enhance bone P mobilization and increase milk production, but with the potential risk of excessive bone mobilization.

4.2.3 Materials and methods

General

Mature *Bos indicus x Bos taurus (ca. 5/8-3/4 B. indicus)* Droughtmaster breeder cows (6-11 years, n = 32) from the commercial herd of the DAF Spyglass Research Facility (Charters Towers) herd were used in the experiment. These breeders had been relocated to Brian Pastures Research Facility (Gayndah) in June 2014 and most (n= 30) had been used in the Pens_B experiment described above where diets low or high P, and low or high metabolisable energy (ME) content, were fed for 14 weeks during mid-pregnancy. Two additional cows from the same relocated group which had been grazing pastures growing on high P soils were also used in the present experiment. From the 29 September 2014 (Day of year from the 1 January 2014 (DOY) 272) at the end of the Pens_B experiment the cows were held in a 4.3 ha paddock with negligible pasture and fed as a group until parturition when cows with their calves entered the present experiment. At the end of the Pens_B experiment these cows were in either low or high P status and ranged in body condition score (BCS) (1-5 scale; CSIRO 2007) depending on their previous diet treatments. Between the experiments the cows were fed *ad libitum* as a group on baled oat hay in long form offered in hay feeders, and were also offered in separate troughs twice weekly the daily equivalent (per cow per day, as-fed) of 2 kg whole cottonseed, 0.50 kg cottonseed meal, 0.05 kg calcium phosphate and 0.05 kg limestone; the minerals were mixed with the cottonseed meal and this mixture was fed on top of the whole cottonseed in the trough. At 2-3 week intervals during this preparatory phase the cows were weighed without fasting, BCS estimated, and blood sampled for mineral measurements. The cows which were used in the present experiment calved from the 16 December 2014 (DOY 350) through to 18 February 2015 (DOY 414) (i.e. over 64 days) and thus the diet during the interval preceding the present experiment was fed to individual cows for between 11 and 20 weeks during late pregnancy. When in late pregnancy (December 2014) the cows were (mean ± sd) 508 ± 56 kg liveweight (LW) and 3.3 ± 0.65) BCS. On the day of parturition or the following day (n = 22 and 10, respectively) cows with their calves were weighted and moved to individual pens where they remained throughout the experiment. The LW and BCS of the cows immediately after parturition (Day 1) was (mean ± sd) 474 ± 57 kg LW and 3.5 ± 0.55 BCS. At calving the cows were allocated at random to one of the four diet treatments. Groups of four cows calving over several days were considered as a block and were allocated to adjacent pens in the open-sided animal shed; thus these blocks encompassed variation associated with both date of calving and position of pens within the animal house. Cows given the high and low phosphorus diets in the previous Pens_B experiment were similarly represented in each diet of the current experiment.

Housing of animals and diet treatments

Cows-calf pairs were housed and fed from parturition in individual pens (33 m^2) with a concrete floor in the half the pen nearest to the feed bunk and with the remainder as soil. The part of each pen with concrete flooring and the feed bunk were roofed, and additional shade was provided with

canvas sails. Water was freely available from troughs in each pen. Designated diets were offered at *ca.* 10% in excess of the actual intakes by individual animals to achieve *ad libitum* intakes. Feed refusals were collected weekly. The main ingredients of the total mixed ration diets were coarsely chopped wheat straw, wheat flour and sugar and the constituents and the composition of each of the 4 diets are given in Table C-1. Two of the diets (Diets 1 and 2) were nominally the same high P and low P diets as fed during lactation in the previous Pens_A experiment. Diet 3 was similar to Diet 2 but modified with removal of the limestone to provide a diet with lower Ca:P ratio. Diet 4 was based on Diet 3 but included ammonium chloride (5 g/kg as fed) to provide a more negative diet anion-cation difference (DCAD) diet. Diets low or adequate in P were prepared by the exclusion or incorporation, respectively, of calcium phosphate. The diets were:
Diet 1. HP-HCa. P and Ca adequate. High P and High Ca.
Diet 2. LP-HCa. P deficient and Ca adequate. Low P and High Ca.
Diet 3. LP-LCa. P and Ca deficient. Low P and Low Ca.
Diet 4. LP-LCa-D. P and Ca deficient. Low P and Low Ca.
Cows were introduced to the treatment diets over an interval of four days during which additional straw was added to the diet to allow adaptation and to minimize risk of acidosis.

Measurement and sampling procedures

The LW of each cow and calf was measured, and cow BCS was estimated, each week. Voluntary intakes of feed of each cow-calf pair was measured on a weekly basis from the amount of feed offered and refused. Samples of feed offered were obtained on each occasion a batch of diet was mixed and samples of feed refusals were obtained weekly for each pen. Samples of feed offered and refused during three 5-day total collection intervals during weeks 4, 8 and 14 of lactation (TC1, TC2 and TC3, respectively) were collected and sub-sampled. The total faeces excreted by the cows during these intervals were collected while the animals were constrained to the front section of the pen with concrete flooring (*ca.* 14 m^2). The faeces excreted each day were collected, mixed and a 10% subsample was stored frozen. At the end of each collection interval the daily samples of faeces were mixed and subsampled. Feed offered and refused during other intervals of the experiment were bulked on a monthly basis. Feed and faeces samples were dried at 60°C and were then ground through a 1 mm screen in a laboratory mill (Christie and Norris Mill, Chelmsford, UK) and retained for analysis.

Jugular blood samples were obtained at fortnightly intervals using vacutainers (BD Diagnostics, Plymouth, UK) containing lithium heparin as an anticoagulant, and were centrifuged (3,000 g x 10 min) to separate plasma. Milk production was measured fortnightly by mechanical milking (DeLaval Model DVP 170/340) following i/v injection of 10 IU oxytocin (Ilium, Syntocin) for milk letdown; each cow was milked twice during the day with a four hour interval. Daily milk production was calculated from the weight of milk secreted during this interval and subsamples of milk were stored frozen for subsequent analyses of composition. Urine samples were successfully obtained by stimulation of the vulva from the majority of the cows three days before and two days after the total collection interval in week 14. These samples were acidified to pH < 3 by addition of 4 M sulphuric acid and were stored frozen for subsequent analysis of purine derivatives and P. Biopsies of the external cortical bone of 11th rib were obtained following the procedures of Little (1972) 1-2 weeks and 14-15 weeks after parturition. Realtime ultrasound scanning (Esaote Pie Medical Aquila with a 3.5 Mhz linear array

Table C-1. Pens_C. The ingredients (g as-fed/kg) and the composition (g as-fed/kg for dry matter concentration and g/kg DM for the other constituents) of the mixed diets containing low or high concentrations of P and fed to the lactating cows. Mean and sd (in parenthesis) of the constituents sampled weekly are given

Attribute	Treatment diets			
Ingredient (as fed, g/kg)	HP-HCa	LP-HCa	LP-LCa	LP-LCa-D
Wheat straw	509	514	517	516
Wheat flour	280	283	285	284
Sugar	140	141	142	142
Canola oil	22.1	22.3	22.4	22.4
Urea	22.1	22.3	20.4	17.3
Calcium phosphate (Kynophos)	9.4	0.0	0.0	0.0
Limestone	4.7	4.7	0.0	0.0
Ammonium sulphate	4.7	4.7	4.8	4.74
Ammonium chloride	0.0	0.0	0.0	5.04
Sodium chloride	4.7	4.7	4.7	4.72
Magnesium oxide	2.33	2.35	2.36	2.36
Rumigro premix	0.762	0.769	0.767	0.773
Elanco rumensin 100	0.265	0.268	0.267	0.269
Composition				
Dry matter (g/kg as fed)	948	946	947	944
Organic matter (g/kg DM)	915 (3.3)	921 (5.5)	923 (2.8)	925 (3.3)
Crude protein	126 (8.7)	122 (15.4)	120 (14.3)	113 (2.1)
NDF	388 (34)	396 (28)	415 (25)	397 (5)
ADF	294 (15)	286 (27)	283 (20)	286 (11)
Lignin	38 (5)	35 (9)	31 (4)	27 (8)
Crude fat	36 (3)	35 (2)	34 (2)	35 (4)
Ca	4.63 (0.81)	2.79 (0.37)	1.72 (0.73)	1.53 (0.16)
P	2.16 (0.51)	0.52 (0.07)	0.57 (0.23)	0.53 (0.05)
Mg	1.99 (0.37)	1.69 (0.34)	1.89 (0.32)	1.77 (0.37)
S	2.31 (0.19)	2.10 (0.36)	3.00 (0.36)	2.18 (0.22)
Ca:P	2.24 (0.63)	5.49 (0.96)	3.02 (0.55)	2.89 (0.30)
DCAD	-3 (3.0)	-1 (4.1)	-1 (3.1)	-10 (3.5)

Organic constituents sampled (means of 4 samples)
Mineral constituents sampled weekly (means of 20 samples)

transducer (Pie Medical Imaging, Maastricht, The Netherlands) was used to measure fat depth at the rib and P8 rump sites, and of eye muscle area (EMA) on two occasions early and late in lactation. Also ultrasonography (Honda HS200V with a 7.5MHz linear array transducer (Honda Electronics Co. Ltd, Toyohashi, Japan) was used to examine ovarian activity in the cows in early March and late July 2015 for the presence of follicles, corpus luteum and corpus albicans (Johnston *et al.* 2014).

Laboratory procedures

Organic matter were determined by incineration (550^{o}C for 8 h). Samples of feed offered throughout the experiment, in feed offered, feed refused and faeces during the total collection intervals and milk, were digested in a nitric-perchloric acid mixture, and the concentrations of P, Ca, Mg and S analysed using an inductively coupled plasma spectrometer (Optima 7300 DV, Perkin Elmer, Waltham, MA, USA). Samples of feed offered were also analysed for total N, NDF, ADF, lignin and crude fat by a commercial laboratory (Dairy One, Ithaca, USA). The concentrations of fat, protein and lactose in milk was measured by mid-infrared analyses (Australian Herd Recording Services, Tasmania). Rib bone biopsy samples were carefully scraped to remove trabecular bone before measuring cortical bone thickness (CBT) using Vernier callipers and specific gravity (SG) by gravimetric procedures.

Plasma samples were analysed calorimetrically for plasma inorganic P (PIP), Ca and Mg in the DAF Coopers Plains laboratory (Beckman Coulter Au680 analyser with OSR6122, OSR 6189 and OSR6189 assay kits respectively). Also the concentration of P in urine obtained shortly before and after the TC3 in week 14 was measured colorimetrically (Goodwin 1970). Plasma concentrations of hormones and bone metabolism markers were determined using commercial assay kits in accordance with the manufacturer's instructions. Assays were parathyroid hormone (PTH; A11930, Beckman Coutler), 25OH Vit D and 1,25-diOH Vit D (AA-35F1 and AA-54F2, Immunodiagnostic Systems), carboxy-terminal telepeptides of type 1 collagen (CTX-1) (Crosslaps AC-02F1, Immunodiagnostic Systems), bone alkaline phosphatase (BAP; MicroVue 8012, Quidel), and osteocalcin (OCN; MicroVue 8002, Quidel).

Calculations and statistical analyses

The LW change of the cows during lactation was calculated using two approaches; (i) from the linear regression of LW with time from day 7 to day 98 (LWG(R)), and from the LW measured on day 7 and the mean of the LW on days 91 and 98 (LWG(D)). The LW gain of the calves were calculated by linear regression of LW measured at birth and weekly during the experiment. Apparent digestibility of DM and organic matter (OM) were calculated by conventional procedures. The ME content (M/D) of the mixed diets fed in the pens was calculated from the organic matter digestibility (OMD) measured during the three total collection intervals as: M/D = 0.169 (%OMD) – 1.986 where M/D was MJ ME/kg DM (CSIRO 2007, p. 8). Fermentable metabolisable energy (FME) content was calculated from total ME content by subtraction of the lipid content of the diet and assuming that the latter contained 35 ME ME/kgDM (AFRC 1993). The volume of urine excreted during week 14 was calculated from the creatinine concentration and assuming a daily creatinine of 0.91 mmol/kg $W^{0.75}$ (Chen *et al*. 1995). The amounts of P and Ca secreted in milk were calculated from the measured amounts of milk and their concentrations in milk. The energy content of milk (MJ/kg) was calculated from the concentrations of fat, protein and lactose (CSIRO 2007 p 46). The difference between intake and faecal excretion (I-F) of P and Ca during the total collection intervals was calculated from these measurements. P retention was calculated from P intake minus the excretion of P in faeces, urine (measured from samples obtained shortly before and after the week 14 total collection) and in milk. The concentration of P in cortical rib bone was calculated as: Pconc (mgP/cc) = 228.8 SG) – 261 (Dixon *et al.* 2017). An index of rib bone P, the P in cortical bone per unit surface area of cortical bone (PSACB, mgP/mm^2), was calculated as the product of P concentration in external cortical bone and CBT (Dixon *et al.* 2017). Calculation of the requirements for P and Ca using the CSIRO spreadsheet Ca/P Requirements.

Statistical analyses was done by ANOVA using Genstat (release 16.1, VSN International Ltd, Hemel Hemstead, UK) to compare among the treatments. The effects of animal blocks at allocation (based on calving date confounded with pen position within the animal house) and of a number of potential covariates (initial cow LW and BCS, bone attributes, fat depth, EMA and calving date) were examined and included when appropriate and significant (P<0.05). Where measurements were made at intervals (LW, intake, digestibility, bone biopsies, concentrations of blood minerals and hormones) the effects of diet and time were examined in a repeated measures ANOVA with main effects of diet and time. Pair-wise comparisons between means were made using the protected LSD procedure at the P<0.05 level of significance.

4.2.4 Results

Animal health

All the cows gained LW during late pregnancy when group-fed the common high P diet from the 29 September 2014 (DOY 271) until parturition. Individual animals calved between the 16 December 2014 and 18 February 2015 (DOY 350-414) and thus the cows were fed the common diet for 79-143 days. By the 3 December 2014 (DOY 337) the difference in LW between animals in the highest and lowest treatment groups in the Pens_B experiment had been reduced from 83 kg to 62 kg LW due to compensatory growth. On the 3 December 2014 plasma PIP and Ca were (mean ± sd) 2.00 ± 0.48 and 2.09 ± 0.20 mmol/L, respectively, and did not differ (P>0.10) between the previous treatment groups fed the low P or high P diets in mid-pregnancy. During the lactation phase two calves died for causes unrelated to the experiment and these cows were removed from the experiment. Also one cow-calf pair was removed from the experiment one week earlier than planned due to the poor BCS of the cow; the results for this cow-calf pair was included in the data set. The cows and calves were otherwise in good health throughout the experiment.

Composition of the diets

The mean concentrations of P in the three LP diets (Table C-1) ranged from 0.52-0.57 (g P/kg DM) and was 2.16 g P/kg DM in the HP-HCa diet. Ca/P ratios were 2.2, 5.5 and 3.0:1 in the HP-HCa, LP-HCa and LP-LCa diets, respectively.

Liveweight and LW change of the cows and calves

The LW of the cows immediately after calving was (mean ± sd) 473 ± 57 kg. On average across all treatments the cows lost 24 (sd 16) kg from day 1 to day 7 (Figure C-1A). Between days 7 and 14 the LP-LCa and LP-HCa cows lost an average of 9 (sd 18) kg and the LP-LCa-D treatment cows 27 (sd 19) kg, respectively. Because of these initial substantial LW losses by the cows in each of the diet treatments, the LW changes during early lactation were calculated from the LW measured from day 7 to 98 rather than LW starting on day 1. During the 13 weeks from day 7 to 98 of lactation the cows given the HP-HCa diet gained 44 kg LW (0.50 kg/day) when calculated by difference, or 0.45 kg/day (equivalent to 41 kg over 13 weeks) when calculated by regression, and gained 0.05 BCS units (Table C-2). During the same interval the LW change of the cows fed the LP-HCa and the LP-LCa diets were lower (P<0.0001) than for the HP-HCa diet (Figure C-1A,Table C-2), and these two LP diets lost similar amounts of LW (17 and 21 kg when calculated by difference, and 22 and 17 kg (-0.24 and -0.19 kg/day) when calculated by regression. The cows fed the LP-LCa-D diet were calculated by difference to have gained 17 kg (0.19 kg/day), or if calculated by regression to have gained 42 kg (0.47 kg/day) from day 7 to day 98. However in the cows fed this LP-LCa-D diet there was substantial LW loss from

day 7 to 14 and the regression relationships between LW and time for individual animals were often poor. It was also observed that the decrease in BCS of 0.9 units, and in eye muscle area (EMA) measured by ultrasound, in the LP-LCa-D diet cows was similar the the decreases in BCS and EMA measured in the LP-HCa and LP-LCa diets (Table C-2). Taken together these measurements indicated that the LW gain in the LP-LCa-D diet cows was lower than indicated by the measurements of LW and the actual LW change was intermediate between the LW gain for the HP-HCa diet and the LW losses for the other two low P diets.

Birth weight of the calves averaged 30.0 (± 4.9) kg and was not affected by the experimental treatments during the previous Pens_B experiment (Table C-2). The increase in individual calf LW during the experiment was always well described by the linear regression of calf LW with time (R^2 ≥0.97). Calf LW gain was not affected (P>0.05) by the treatment diet fed to the cow-calf pairs and ranged from 0.58 to 0.69 kg/day. None of the LP diets decreased (P>0.05) calf growth during early lactation (Table C-2).

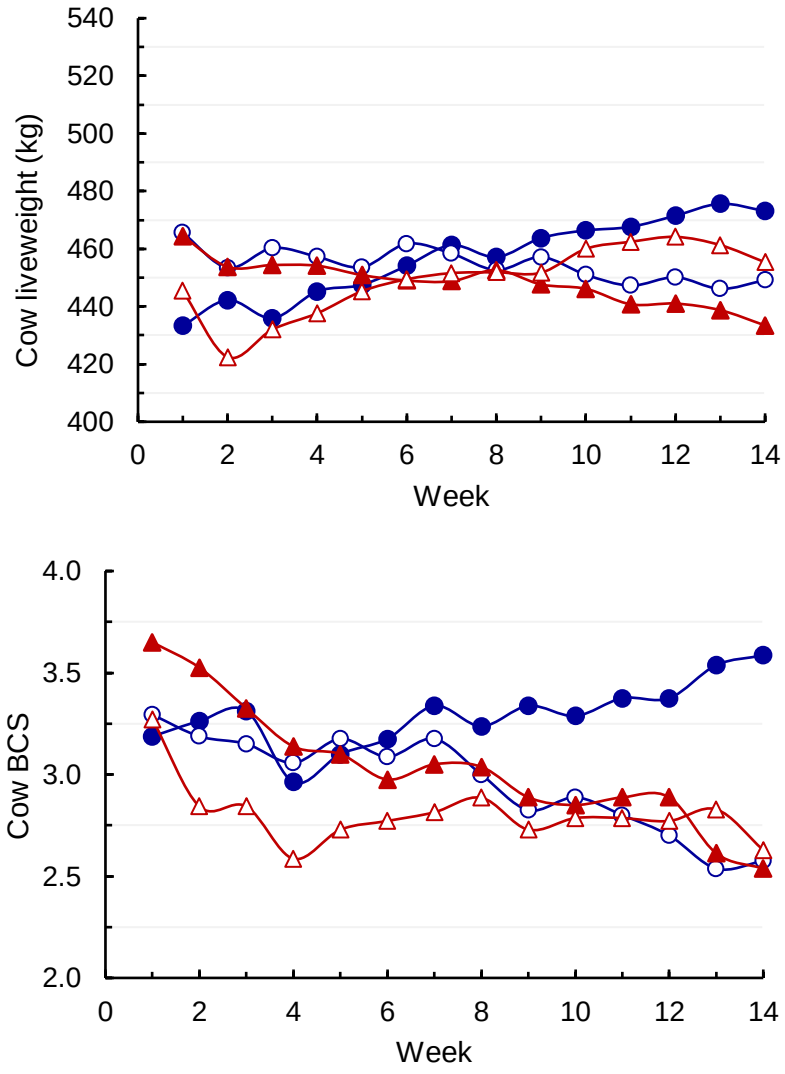


Figure C-1A and 1B. Pens_C. Liveweight and body condition score (BCS) of cows fed the four diets comprising HP-HCa (●), LP-HCa (○), LP-LCa (▲) and the LP-LCa-D diets (Δ).

Table C-2. Pens_C. The LW and BCS in cows, growth of calves, microbial protein synthesis, rib bone biopsy measurements and fat cover and eye muscle area (EMA), and the changes in these measurements during the 14 weeks of early lactation, in cows fed 4 diets (n=7-8). The cows were fed ad libitum on diets of high P (HP-HCa) or low in P (HP –LCa, LP-LCa, LP-LCa-D (see text)

Measurement	HPHCa	LPHCa	LPLCa	LPLCa-D	Cov	s.e.m.	P
n	8	8	7	7	-	-	-
Cow Initial LW (kg)	433	460	465	443		-	-
Cow LW change (kg)(Diff)[AB]	44 a	-17 bc	-21 c	17 ab	1, 4	10.1	<0.01
Cow LW change (kg/day)(Diff)[A]	0.50 a	-0.19 b	-0.24 c	0.19 a	1, 4	0.115	<0.01
Cow LW change (kg/day)(Regn)[P]	+0.451a	-0.242b	-0.190b	+0.467a	-	0.102	<0.001
Cow Initial BCS	3.4	3.4	3.8	3.4		-	-
Cow BCS change[C]	+0.05 a	-1.2 b	-1.0 b	-0.9 b	6	0.22	<0.01
Calf birth weight (kg)	28.3	29.6	30.9	31.3	7	1.64	0.004
Calf LW at 98 days (kg)	96.4	88.1	96.3	93.5	-	5.21	ns
Calf LW gain (kg/day)(Regn)[D]	0.69	0.58	0.64	0.64	-	0.048	ns
Urine volume (L/day)	11.1	9.3	8.3	8.4	-	1.70	0.62
Initial CBT (mm)	3.79	3.99	3.69	3.39	-	0.206	n.s.
Initial P conc (mg/cc)	155	155	146	153	-	4.5	n.s.
Initial PSACB	0.587	0.624	0.541	0.517	-	0.0409	n.s.
Weaning CBT (mm)	4.40 a	4.01 ab	3.70 b	3.76 b	22	0.167	0.033
Weaning P conc (mg/cc)	153a	134c	145ab	139bc	4, 5	3.55	0.008
Weaning PSACB	0.671a	0.566b	0.528ab	0.498c	25	0.0229	<0.001
CBT (change%)	29.3 a	9.5 b	-4.4 c	5.8 bc	18,2	3.8	<0.001
P conc (change%)	-3 a	-9 ab	-3 a	-16 b	1,	3.1	0.040
PSACB (change%)	+29 a	+7 b	-3 c	-9 c	21,2	2.5	<0.001
Initial rump fat depth (mm)	3.9	3.5	4.3	3.1	-	-	-
Rump fat change (mm)[E]	-1.6	-1.6	-0.7	-2.1	-	0.484	0.25
Initial rib fat depth (mm)	2.1	1.9	2.0	2.0	-	-	-
Rib fat change (mm)[F]	-0.3	-0.3	-0.1	-0.4	-	0.146	0.58
Initial EMA (mm^2)	48.8	51.5	41.3	52.9	-	-	-
EMA change (mm^2)[G]	4.9	1.7	-0.8	-1.3	-	1.96	0.146

[abc] Means within rows with different superscripts are different at P<0.05.

A, LW change calculated as the difference between days 7 and 98.

B, covariates 1, carry-over P effect; 4, age of cow; 6, initial cow BCS.; 7, initial cow LW; 18, cow CBT change in pregnancy; 19, cow SG change in pregnancy; 20, cow Pconc change in pregnancy; 21, cow PSACB change in pregnancy; 22, cow CBT at calving; 25, cow PSACB at calving.

Voluntary intake, diet digestibility, mineral balance and microbial protein synthesis

In each of the diets there was a gradual increase in voluntary intake (VI) during the first 4 weeks from 11-13 g DM/kg LW in week 1 to 17-24 g DM/kg LW by week 4 (Figure C-2). When voluntary DM intake was examined in a repeated measurements ANOVA the VI increased from weeks 1 to 4, but did not change from weeks 5 to 14. On average over the 14 weeks the voluntary intake was higher ($P< 0.05$) in the cows fed the HP-HCa diet (mean 21.5 g DM/kg LW) than the LP-LCa-D diet (18.5 g DM/kgLW), and intake was higher for this latter diet than for the other two low P diets (LP-HCa and LP-LCa; 16.7 and 17.3 g DM/kg LW, respectively) (Table C3).

Table C-3. Pens_C. Measurements of voluntary intake, organic matter digestibility, estimated ME intake and P balance in cows fed high phosphorus (HP) or low P (LP) content diets containing high calcium (HCa) or low Ca (LCa) concentrations, or the low P low Ca diet with addition of ammonium chloride to provide a negative DCAD diet during 3 total collection intervals

Measurement	Diet treatments				s.e.m.			Probability		
	HP-	LP-	LP-LCa	LP-LCa-	Die	Tim	DxT	Diet	Tim	DxT
n	8	8	7	7	-	-	-	-	-	-
DM intake (kg/d)_mean[A]	10.22	7.71	8.19 b	8.55 b	0.4	0.2	0.667	<0.0	0.0	0.3
DM intake (g/kg	21.5 a	16.7 c	17.3 bc	18.5 b	0.5	0.6	1.350	<0.0	<0.	0.1
OM digestibility	699	698	693	727	18	-	-	n.s.	-	-
OM digestibility	705	681	676	640	14	-	-	n.s.	-	-
OM digestibility	671	643	653	660	24	-	-	n.s.	-	-
OM digestibility	692	674	674	676	13.	8.9	13.4	0.74	0.0	0.2
ME content (MJ ME/kg	9.73	9.47	9.40	9.44	0.2	-	-	0.72	-	-
ME intake (MJ	98.8 a	69.9 c	69.5 c	80.6 b	3.3	-	-	<0.0	-	-
ME intake (est kJ ME/kg	210a	158b	161b	175b	6.4	-	-	<0.0	-	-
P intake (g P/day)_TC1	23.6 a	4.6 b	4.2 b	4.4 b	1.1	-	-	<0.0	-	-
P intake (g P/day)_TC2	26.8 a	4.5 b	4.9 b	5.1 b	1.0	-	-	<0.0	-	-
P intake (g P/day)_TC3	24.2 a	3.4 b	4.6 b	5.1 b	1.1	-	-	<0.0	-	-
P intake (g P/day)_mean	24.9 a	4.2 b	4.6 b	4.9 b	0.8	0.4	0.866	<0.0	0.1	0.5
P faeces (g P/day)_TC1	13.2 b	6.3 c	6.0 c	7.5 c	0.6	-	-	<0.0	-	-
P faeces (g P/day)_TC2	14.7 b	6.7 c	6.5 c	7.9 c	1.0	-	-	<0.0	-	-
P faeces (g P/day)_TC3	19.1 a	5.0 c	6.4 c	7.1 c	1.8	-	-	<0.0	-	-
P faeces (g P/day)_mean	15.7 a	6.0 b	6.3 b	7.5 b	0.9	0.5	1.325	<0.0	0.3	0.0
P (I-F) (g P/day)_TC1	+10.5 a	-1.8 c	-1.8 c	-3.1 c	1.0	-	-	<0.0	-	-
P (I-F) (g P/day)_TC2	+12.1 a	-2.1 c	-1.6 c	-2.8 c	1.2	-	-	<0.0	-	-
P (I-F) (g P/day)_TC3	+4.7 b	-1.6 c	-1.8 c	-2.0 c	1.1	-	-	<0.0	-	-
P (I-F) (g P/day)_mean	+9.1 a	-1.9 b	-1.7 b	-2.6 b	0.7	0.6	1.24	<0.0	0.2	0.0
P intake (mg	54.9 a	9.4 b	10.3 b	10.4 b	1.3	-	-	<0.0	-	-
P faeces (mg	34.9 a	13.6	14.1 b	16.6 b	1.9	-	-	<0.0	-	-
P (I-F) (mg	21.3 a	-4.3 b	-3.8 b	-6.3 b	1.4	-	-	<0.0	-	-

A, adjusted for covariates 5 (calving date) and 7 (initial cow LW). B, adjusted for covariate 7 (initial cow LW) (P<0001). C, Calculated as P intake – P in faeces

Table C-4. Pens_C. The intake and faecal excretion of calcium (Ca) in mature cows fed high phosphorus (HP) or low P (LP) content diets containing high calcium (HCa) or low Ca (LCa) concentrations, or the low P low Ca diet with addition of ammonium chloride to provide a negative DCAD diet for 14 weeks during early lactation (n=7 or 8)

Measurement	Diet treatments				s.e.m.			Probability		
	HP-	LP-	LP-LCa	LP-LCa-	Die	Tim	DxT	Diet	Tim	DxT
n	8	8	7	7	-	-	-	-	-	-
Ca intake (g Ca/day)_TC1	47.6	22.9	11.9	11.4	2.8	-	-	<0.0	-	-
Ca intake (g Ca/day)_TC2	53.0	23.4	15.4	13.3	2.6	-	-	<0.0	-	-
Ca intake (g Ca/day)_TC3	49.8	18.2	12.4	14.2	3.2	-	-	<0.0	-	-
Ca intake (g	50.1 a	21.5	13.2 c	13.0 c	2.1	1.2	2.94	<0.0	0.2	0.6

Ca faeces (g Ca/day)_TC1	29.1 b	22.5	13.4	12.8	1.8	-	-	<0.0	-	-
Ca faeces (g Ca/day)_TC2	28.0 b	22.6	13.5	12.3	1.4	-	-	<0.0	-	-
Ca faeces (g Ca/day)_TC3	38.4 a	18.7	13.4	10.9 e	3.6	-	-	<0.0	-	-
Ca faeces (g	31.8 a	21.3	13.4 c	12.0 c	1.9	1.0	2.55	<0.0	0.6	0.02
Ca (I-F) (g Ca/day)_TC1	+18.5	+0.4	-1.5	-1.3	2.2	-	-	<0.0	-	-
Ca (I-F) (g Ca/day)_TC2	+25.0	+0.8	+1.9	+1.0	2.6	-	-	<0.0	-	-
Ca (I-F) (g Ca/day)_TC3	+11.4	-0.5	-1.0	+3.3	2.5	-	-	<0.0	-	-
Ca (I-F) (g Ca/day)_mean	+18.3 a	+0.2 b	-0.2 b	+1.0 b	1.6	1.1	2.48	<0.0	0.0	0.0
Ca intake (mg	110.7	48.4	29.9 c	28.1 c	3.5	-	-	<0.0	-	-
Ca faeces (mg	70.8 a	49.0	30.1 c	26.8 c	3.4	-	-	<0.0	-	-
Ca (I-F) (mg	+43.3 a	+0.28	-0.35 b	+1.05	3.2	-	-	<0.0	-	-

A, adjusted for covariates 5 (calving date) and 7 (initial cow LW).

B, adjusted for covariate 7 (initial cow LW) (P<0001).

C, Calculated as P intake – P excreted in faeces.

Table C-5. Pens_C. The concentrations and production of milk constituents by mature cows fed high phosphorus (HP) or low P (LP) content diets containing high calcium (HCa) or low Ca (LCa) concentrations, or the low P low Ca diet with addition of ammonium chloride to provide a negative DCAD diet for 14 weeks during early lactation (n=7 or 8). sem, standard error of the mean

Measurement	Diet treatments				s.e.m.			Probability		
	HP-HCa	LP-HCa	LP-LCa	LP-LCa-D	Diet	Time	DxT	Diet	T	DxT
n	8	8	7	7	-	-	-	-	-	-
Milk production (kg/day)	5.81	4.57	5.93	5.31	0.462	0.255	0.661	0.186	0.010	0.049
Milk composition										
Fat (g/kg)	41.5 a	32.2 b	40.5 a	36.8 ab	0.215	0.158	0.363	0.026	<0.001	0.401
Protein (g/kg)	26.7	24.0	24.0	26.4	0.11	0.04	0.14	0.190	0.012	0.030
Lactose (g/kg)	51.1	51.3	50.4	51.4	0.45	0.46	0.96	0.465	0.683	0.351
Ca (g/kg)	1.234	1.226	1.224	1.241	0.0237	0.0200	0.0441	0.956	0.101	0.349
P (g/kg)	0.896	0.863	0.869	0.848	0.0199	0.0190	0.0415	0.364	0.052	0.394
Mg (g/kg)	0.0959	0.0946	0.0926	0.0949	0.00336	0.00182	0.00476	0.915	0.004	0.404
Energy (MJ/kg)	3.08 a	2.66 b	2.96 a	2.90 ab	0.096	0.061	0.148	0.040	<0.001	0.402
Milk energy (MJ/day)	18.1	12.6	17.7	15.3	1.43	0.92	1.43	0.051	0.010	0.051
Milk Ca (g/day)	7.23	5.68	7.21	6.60	0.562	0.318	0.814	0.206	0.009	0.046
Milk P (g/day)	5.29	4.00	5.11	4.49	0.393	0.240	0.593	0.116	0.049	0.083

Table C-6. Pens_C. The concentrations, production and estimated retention of P during the three intervals when total collections of faeces were measured in mature cows fed high phosphorus (HP) or low P (LP) content diets containing high calcium (HCa) or low Ca (LCa) concentrations, or the low P low Ca diet with addition of ammonium chloride to provide a negative DCAD diet for 14 weeks during early lactation (n=7 or 8) s.e.m., standard error of the mean of treatment

Measurement	Diet treatments				s.e.m.			Probability		
	HP-HCa	LP-HCa	LP-LCa	LP-LCa-D	Diet	Time	DxT	Diet	Time	DxT
n	8	8	7	7	-	-	-	-	-	-
Urinary P in week 14 (g P/day)	0.21	0.03	0.02	0.06	0.052	-	-	0.058	-	-
Milk P secretion (g P/day)										
Milk P (g P/day)_week 4	5.2 abc	4.2 cd	6.4 a	4.0 cd	0.56	-	-	0.022	-	-
Milk P (g P/day)_week 8	5.7 ab	4.0 cd	5.0 abcd	4.8 bcd	0.44	-	-	0.102	-	-
Milk P (g P/day))_week 14	4.7 bcd	3.5 d	4.1 cd	4.3 bcd	0.49	-	-	0.461	-	-
Milk P (g P/day))_mean	5.2	3.9	5.2	4.4	0.42	0.19	0.52	0.111	0.009	0.027
Estimated P retention (g P/day)										
Estimated P retention (g P/day)_wk 4	+5.1 a	-6.0 c	-8.3 c	-7.1 c	1.23	-	-	<0.001	-	-
Estimated P retention (g P/day)_wk 8	+6.2 a	-6.2 c	-6.6 c	-7.7 c	1.12	-	-	<0.001	-	-
Estimated P retention (g P/day)_wk 14	-0.4 b	-4.9 c	-6.2 c	-6.4 c	1.28	-	-	0.009	-	-
Estimated P retention (g P/day)_mean	3.6	-5.7	-6.0	-7.1	0.81	0.59	1.26	<0.001	0.591	0.009
Milk P (mg P/LW.day)										
Milk P (mg P/LW.day)_week 4	10.4 a	-12.9 c	-18.2 c	-16.9 c	2.41	-	-	<0.001	-	-
Milk P (mg P/LW.day))_week 8	13.7 a	-14.1 c	-14.6 c	-17.4 c	2.50	-	-	<0.001	-	-
Milk P (mg P/LW.day)_week 14	-0.3 b	-11.1 c	-13.6 c	-14.1 c	2.65	-	-	0.003	-	-
Milk P (mg P/LW.day)_mean	7.9	-12.7	-15.4	-16.1	1.58	1.29	2.64	<0.001	0.619	0.014

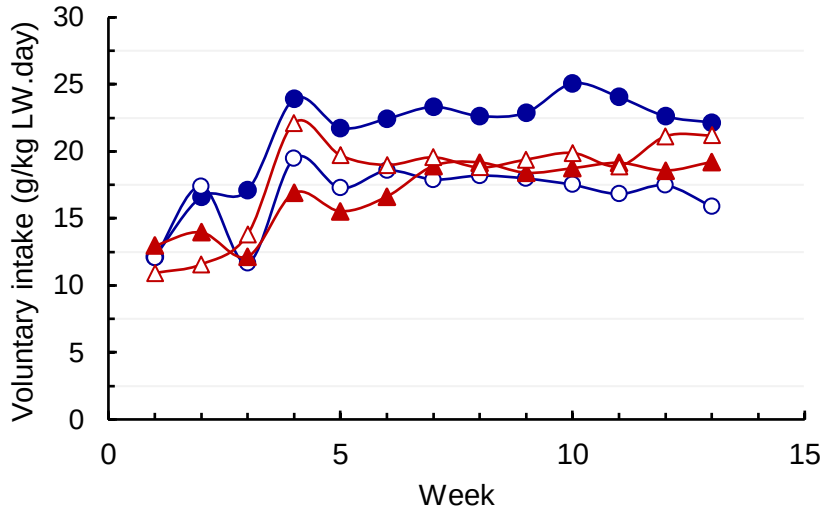


Figure C-2. Pens_C. Voluntary intake (VI) (g DM/kg LW.day) of cows fed the four diets comprising HP-HCa (●), LP-HCa (○), LP-LCa (▲) and the LP-LCa-D diets (Δ). VI was affected by diet (P<0.05) and Time (P<0.05).

In a repeated measurements ANOVA model the OM digestibility (mean of 679 g/kg across treatment diets and the three total collection intervals) did not differ (P>0.10) among the diets, but OM digestibility was higher (P<0.05) in week 4 (704 g/kg) than in weeks 8 and 14 (676 and 657 g/kg respectively; Table C-3). Intake of metabolisable energy (ME) per kg LW was greater (P<0.001) for the HP-HCa diet (210 versus 158-175 kJ ME/kg LW.day). Intakes and faecal excretion of P and Ca were higher for the HP-HCa diet than for each of the three LP diets, as expected from the differences in diet P concentration (Table C-3 and C-4). On average over the three total collection intervals the amount of P ingested but not excreted in faeces (I-F) averaged +9.1 g P/day in the HP-HCa cows but -2.6 to -1.9 g P/day in the three low P diets. There was also a significant diet x time interaction in the repeated measured ANOVA such that the (I-F) during week 14 in cows fed the HP-HCa diet (+4.7 g P/day) was lower (P<0.05) than in weeks 4 and 8 (+10.5 and +12.1, respectively), while this (I-F) amount was negative for each of the three low P diers in each of the three total collection intervals (range -2.8 to -1.6 g P/day). On average over the three total collection intervals the amount of Ca ingested but not excreted in faeces (I-F)) averaged 18.3 g Ca/day in the HP-HCa cows but -0.2 to +1.0 g Ca/day in the three low P diets (Table C-4). There was a tendancy (P= 0.055) for a diet x time interaction such that the (I-F) during week 14 in cows fed the HP-HCa diet (11.4 g Ca/day) tended to be lower than in weeks 4 and 8. The (I-F) amount was lower (P<0.001) for each of the three low P diets in each of the three total collection intervals (range -1.5 to +3.3 g Ca/day). Rumen microbial protein synthesis did not differ among the diet treatments (Table C-2).

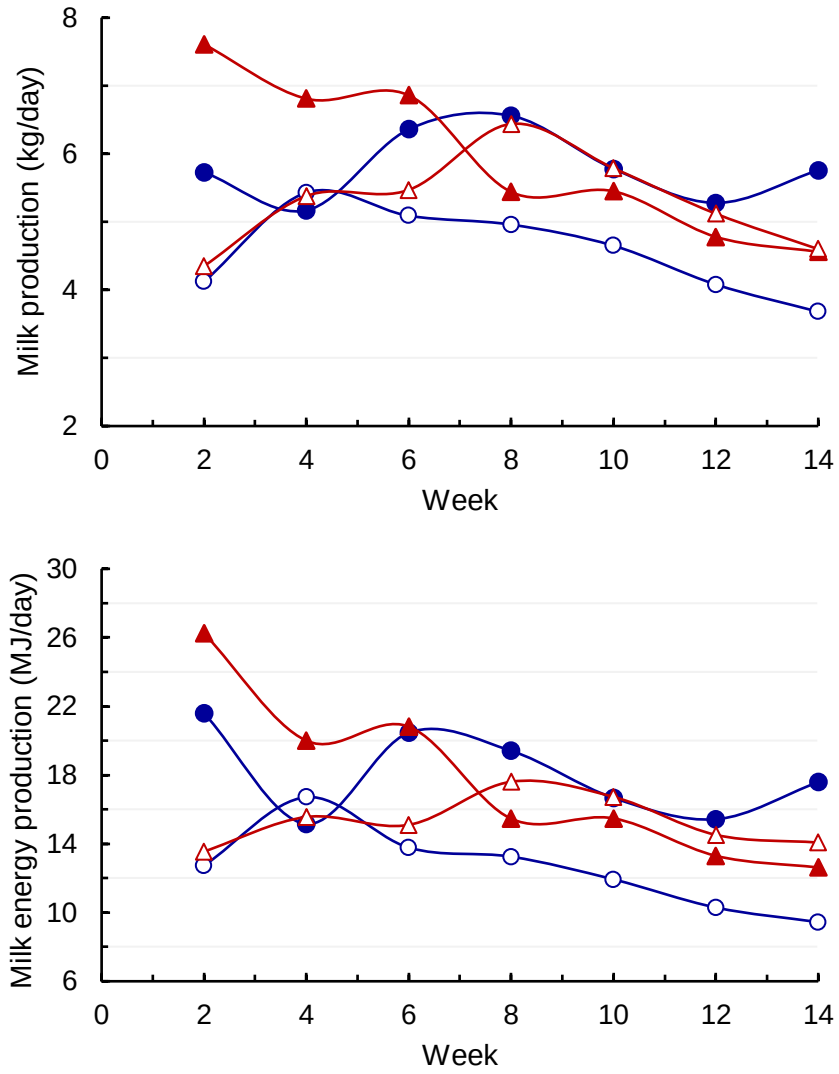


Figure C-3. Pens_C. Milk production (kg/day) (Fig C-3A) and milk energy (MJ/day) (Fig C-3B) of cows fed the four diets comprising HP-HCa (●), LP-HCa (○), LP-LCa (▲) and the LP-LCa-D diets (Δ). Milk output was not affected by diet (P>0.05) but decreased with Time (P<0.05) and there was a diet x time interaction (P<0.05). Milk energy output was affected by diet (P<0.05) and decreased with Time (P<0.001). Diet x time interaction was not significant (P>0.10).

Milk production and milk quality

Both the amount and composition of milk measured at fortnightly intervals changed during the 14 weeks of lactation (Figure C-3A and 3B, Table C-5). The amount of milk (kg/day) did not differ due to the main effect of diet (P>0.05) but decreased during lactation (P<0.01), and there was a diet x time interaction (P<0.05). Averaged across diet treatments milk output was in the range 5.4-5.9 kg/day until week 10 and then decreased (P<0.05) to average 4.7 kg/day by week 14. Average milk fat content declined progressively (P<0.001) from 47.1 at week 2 to 35.4 g fat/kg by week 14, and was lower (P<0.05) in cows fed the LP-HCa diet (Table C-5) than the other diets. The mean fat content and the mean energy content of the milk was lower (P<0.05) with the LP-HCa diet. Milk energy output, calculated from the amount and composition of the milk, declined (P<0.05) from 18.5 MJ/day during week 2 to 13.4 MJ/day during week 14, and tended (P=0.051) to be lower (12.6 MJ/day) for the LP-HCa diet than for the other three diets (15.3 – 18.1 MJ/day) (Table C-3A and C-

3B). Calf growth rate was only moderately correlated with milk energy production measured by milking (r = 0.61-0.68).

Changes in rib bone

The CBT and P concentration of cortical bone (P<0.05) and the PSACB (P<0.001) after 14 weeks were both lower in cows fed the LP diet treatments (Table C-2). Also the PSACB was lower (P<0.05) in the LP-LCa-D diet (0.498 mg P/mm^2) than in the other two LP diets (0.528 and 0.566 mg P/mm^2), and these were all lower than the HP-HCa diet (0.671 mg P/mm^2). From calving to weaning the CBT and the PSACB increased by 29% in the HP-HCa diet, but these measurements did not change during lactation for the three Low P diets.

Ultrasound measurements of fat cover, EMA and ovarian activity

Fat depth at the two sites measured decreased during the experimental interval but there were no differences (P>0.05) among the treatments in the changes in fat cover or EMA. However, the observation that EMA tended to increase in the HP-HCa cows was consistent with the greater LW gain and higher BCS in the cows given this treatment (Table C-2).

Plasma P, Ca and Mg

At calving, plasma mineral concentrations (n=32 cows) were in the normal range at 1.93 ± 0.10 mmol/L (PIP), 2.13 ± 0.04 mmol/L (Ca), and 0.88 ± 0.04 mmol/L (Mg) (Figures C-4, C-5 and C-6). During lactation, mean PIP concentrations of cows given the three low P diets were 0.73 – 0.92 mmol/L and clearly indicated that these animals were severely P-deficient. In contrast, PIP concentrations in animals fed the HP-HCa diet during lactation averaged 1.52 mmol/L indicating that these cows were in marginal rather than surplus P status. Overall during lactation there was a diet by

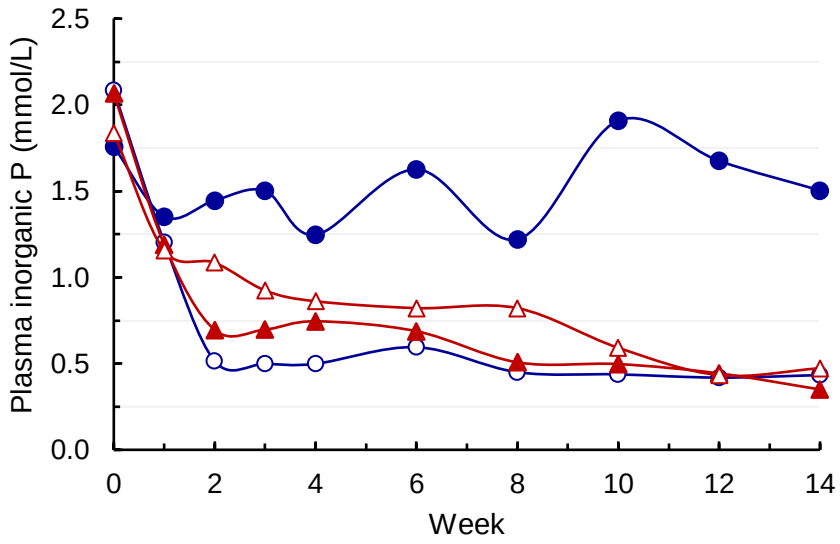


Figure C-4. Pens_C. Plasma concentrations of inorganic phosphorus (PIP) (mmol/L) of cows fed the four diets comprising HP-HCa (●), LP-HCa (○), LP-LCa (▲) and the LP-LCa-D diets (Δ). PIP was affected by diet (P<0.001), time (P<0.001), and diet x time interaction (P<0.001).

time interaction (P<0.001) in PIP concentrations (Figure C-4) with a progressive decrease in PIP concentrations in cows given low P diets. However. there was a slower decline in the LP-LCa-D cows during the first two months of lactation; between weeks 2 and 8 the PIP of the cows fed the LP-LCa-D diet was higher (P<0.05) than for the other two low P diet cows (LP-LCa and LP-HCa).

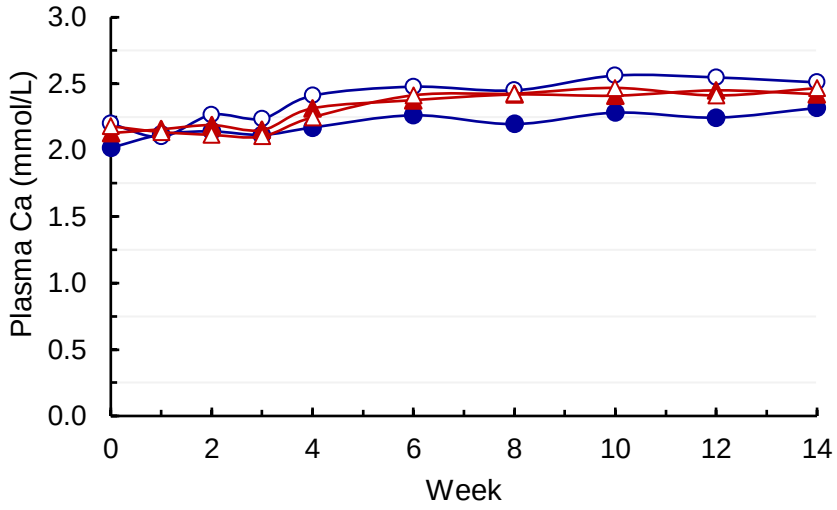


Figure C-5. Pens_C. Plasma concentrations of calcium (mmol/L) of cows fed the four diets diets comprising HP-HCa (●), LP-HCa (○), LP-LCa (▲) and the LP-LCa-D diets (Δ). Plasma Ca was affected by diet (P<0.05) and increased with time (P<0.001). Diet x time interaction was not significant (P>0.10).

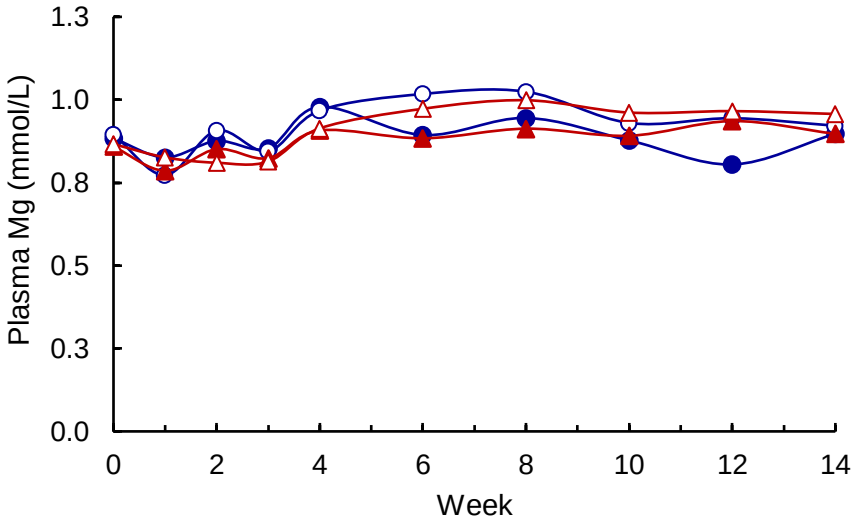


Figure C-6. Pens_C. Plasma concentrations of magnesium (mmol/L) of cows fed the four diets comprising HP-HCa (●), LP-HCa (○), LP-LCa (▲) and the LP-LCa-D diets (Δ). Plasma Mg was not affected by diet (P>0.10) , increased with time (P<0.001). Diet x time interaction was not significant (P>0.10).

In contrast to PIP the plasma Ca concentrations increased during lactation in all of the diet groups (Figure C-5), albeit within the normal range for Ca (2.1 - 2.8 mmol/L). However a significant (P<0.001) diet effect was observed, with lower Ca concentrations in HP-HCa cows (2.18 mmol/L) than in any of the low P diets (2.29 - 2.38 mmol/L). The highest Ca concentrations were observed in the LP-HCa cows. Plasma Mg concentrations also increased during lactation, but no signficant (P>0.10) main effect of diet, or diet by time interaction was observed (Figure C-6). The plasma total Ca to PIP ratio showed little change over time in HP-HCa cows, but exhibited a marked increase in all low P diets. In the third month of lactation substantial significant (P<0.001) differences were noted between the low and high P diets (Figure C-7).

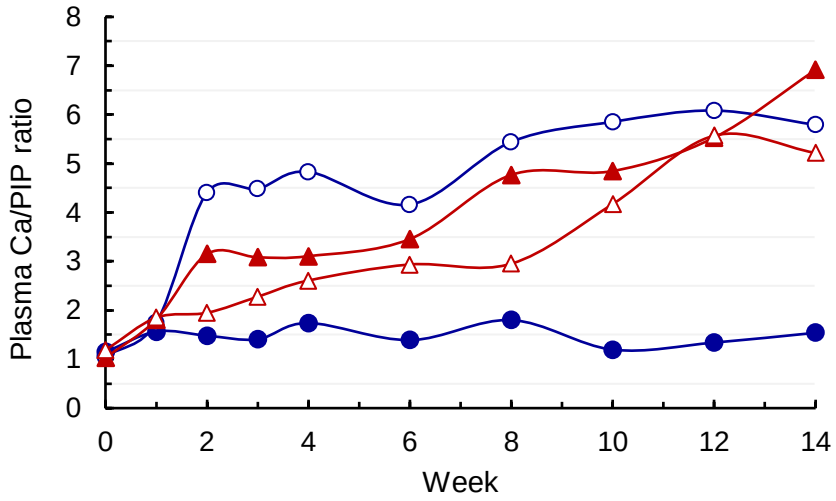


Figure C-7. Pens_C. Ratio of plasma concentrations of total Ca to PIP of cows fed the four diets comprising HP-HCa (●), LP-HCa (○), LP-LCa (▲) and the LP-LCa-D diets (Δ). The ratio of Ca to PIP was affected by diet (P<0.001), time (P<0.001), and a diet x time interaction (P<0.001).

Endocrine markers

Plasma PTH concentrations were highly variable and particularly in early lactation, but nevertheless there was an overall decrease in PTH concentrations during lactation (Figure C-8). There was a significant (P<0.001) diet x time interaction with lower PTH concentrations in the LP-HCa diet than in the other diets at many time points post-calving (Figure C-8). PTH concentrations in LP-HCa cows were low by week 2 of lactation (44 ± 16 pg/ml) and remained low throughout lactation, and these corresponded with the highest plasma Ca concentrations (Figure C-5). In the last month of lactation PTH concentrations generally declined in all diet groups as plasma Ca concentrations increased. At 14 weeks PTH concentrations in both LP-HCa (28 pg/ml) and LP-LCa (19 pg/ml) diets were low and significantly (P<0.05) different from the HP-HCa diet (94 pg/ml).

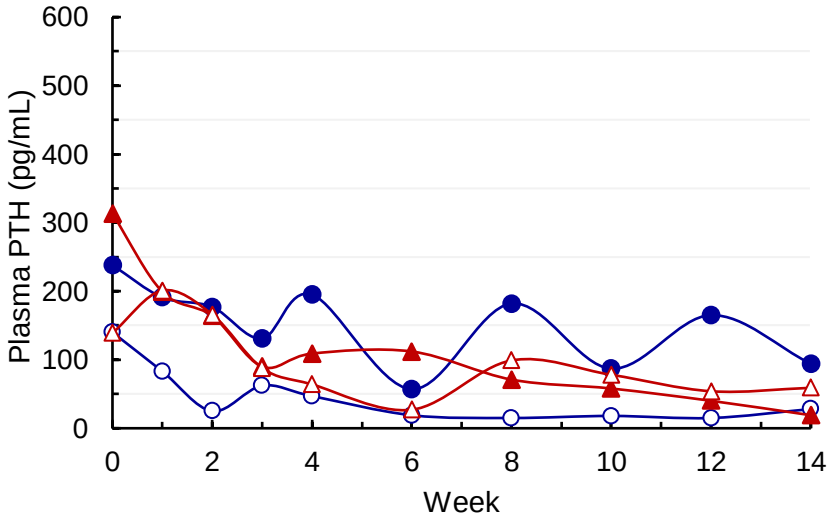


Figure C-8. Pens_C. Plasma concentration of parathyroid hormone (PTH) (pg/ml) of cows fed the four diets comprising HP-HCa (●), LP-HCa (○), LP-LCa (▲) and the LP-LCa-D diets (Δ). Plasma PTH concentration was affected by diet (P<0.001), time (P<0.001). Diet x time interaction was not significant (P>0.10).

Plasma 25-OH Vit D (data not shown) and 1,25-diOH Vit D (Figure C-9) concentrations were high at calving (105 ± 7 ng/ml and 299 ± 38 pg/ml respectively, n=32). Like PTH, plasma 1,25-diOH Vit D concentrations were quite variable in the first two weeks of lactation. However at 2 weeks post-calving there was a significant (P<0.05) increase in 1,25-diOH Vit D concentrations in the LP-LCa-D diet compared to all other diets. This increase was not associated with a diet effect (P=0.41) on plasma 25-OH Vit D concentrations (LP-LCa-D 79 ng/ml; HP-HCa 91 ng/ml; LP-HCa 73 ng/ml; LP-LCa 98 ng/ml). By week 4 of lactation there was no diet effect on 1,25di-OH Vit D concentration, although as lactation progressed the diet differences were again apparent (Figure C-9). At 8 weeks of lactation plasma 1,25di-OH Vit D concentrations were increased (P<0.05) in LP-LCa-D cows compared to all other diets (Figure C-9). In later lactation 1,25diOH Vit D concentrations declined in HP-HCa cows (118 ± 13 pg/ml), whereas LP-HCa remained unchanged (210 ± 14 pg/ml), and both LP-LCa and LP-LCa-D diets had further increased (296 ± 34 and 331 ± 24 pg/ml respectively).

Bone markers

Plasma CTX-1 concentrations were low at calving (0.32 ± 0.02 ng/ml, n=32) but increased in all diets during the first 3 weeks of lactation (Figure C-10). In HP-HCa cows CTX-1 concentrations reached a maximum at 3 weeks lactation (2.04 ± 0.51 ng/ml) and declined thereafter to a low at 14 weeks lactation (0.64 ± 0.16 ng/ml). In contrast, in all the LP diets the CTX-1 concentrations continued to increase during the first 4-6 weeks of lactation (mean > 4.0 ng/ml). From 2 weeks of lactation, CTX-1 was significantly (P<0.001) higher in the LP-LCa-D diet than in the HP-HCa diet and remained different up 14 weeks post-calving (Figure C-10). Between the LP diets there were small, but significant differences in CTX-1 concentrations. From 6 to 14 weeks of lactation the LP-LCa-D diet was higher (P<0.05) than the LP-HCa diet, but only at 14 weeks of lactation was the LP-LCa-D diet higher than the LP-LCa diet (Figure C-10).

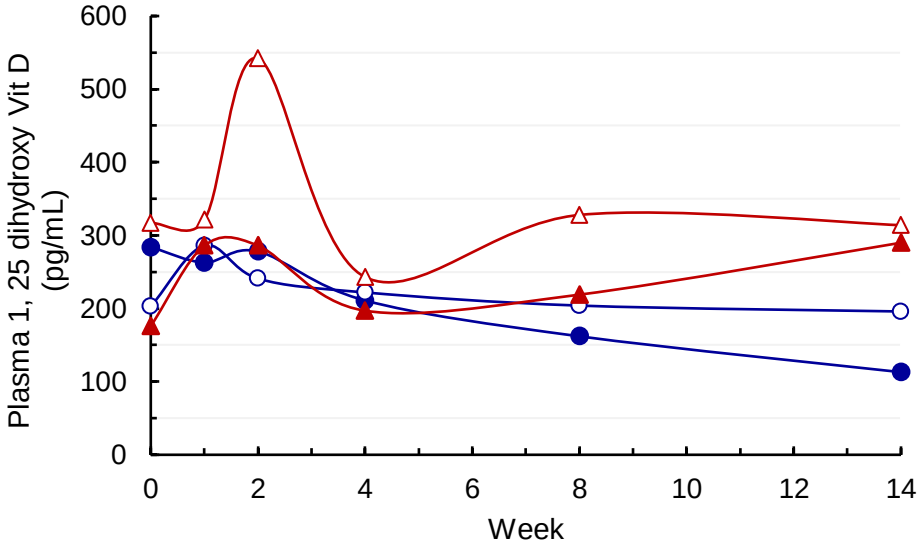


Figure C-9. Pens_C. Plasma concentration of 1,25-dihydroxy Vitamin D (pg/ml) of cows fed the four diets comprising HP-HCa (●), LP-HCa (○), LP-LCa (▲) and the LP-LCa-D diets (Δ). diets comprising HP-HCa (●), LP-HCa (○), LP-LCa (Δ) and the LP-LCa-D diets (□). Plasma 1,25diOH Vit D concentration was affected by diet (P<0.06), time (P<0.01), and diet x time interaction (P<0.05).

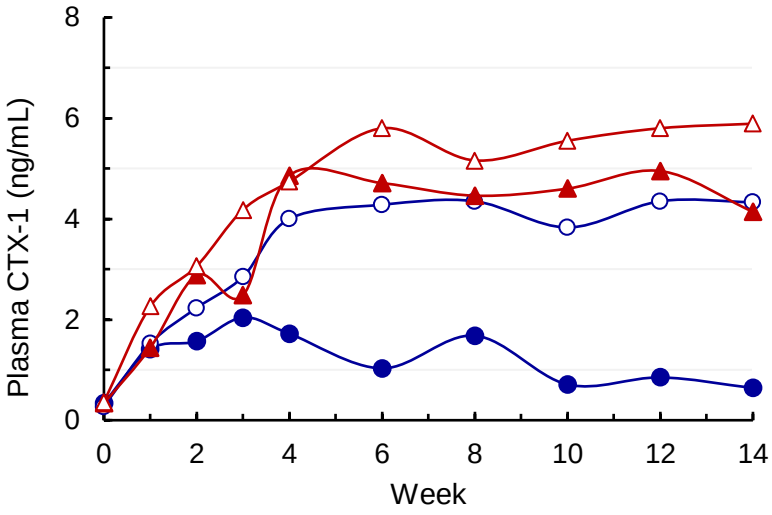


Figure C-10. Pens_C. Plasma concentration of CTX-1 (ng/ml) of cows fed the four diets comprising HP-HCa (●), LP-HCa (○), LP-LCa (▲) and the LP-LCa-D diets (Δ). Plasma CTX-1 concentration was affected by diet (P<0.001), time (P<0.001), and diet x time interaction (P<0.001).

During early lactation plasma OCN concentrations were generally low, and no marked differences between diets were apparent until 6 weeks post-calving (Figure C-11). Nevertheless an overall main effect of diet (P<0.05) and a diet x time interaction (P<0.001) were observed. In general, lower OCN concentrations were observed in HP-HCa cows than in the LP diets from 6 to 14 weeks post-calving. Plasma BAP concentrations followed the same pattern as OCN being low in early lactation, and indeed decreased from calving until week 6 of lactation (Figure C-12). However as lactation progressed BAP concentrations increased (P<0.01) in all LP diets compared to the HP-HCa diet. There were no differences in BAP concentrations between low P diets at any time.

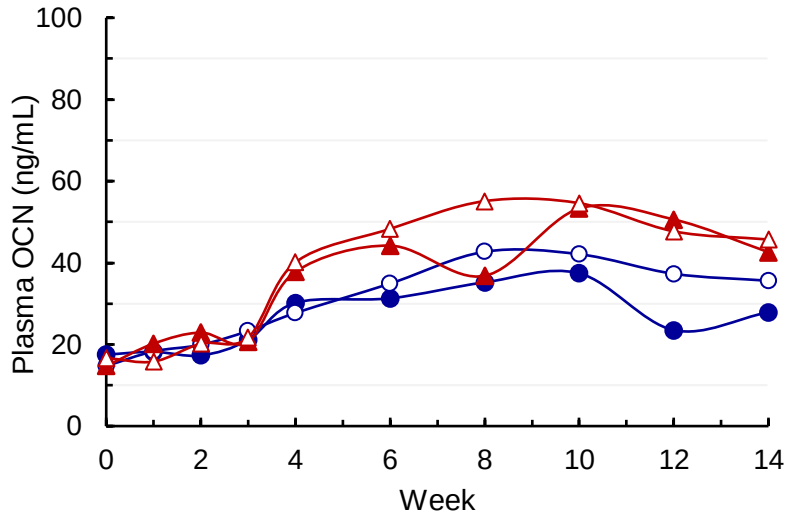


Figure C-11. Pens_C. Plasma concentration of osteocalcin (OCN) (ng/ml) of cows fed the four diets comprising HP-HCa (●), LP-HCa (○), LP-LCa (▲) and the LP-LCa-D diets (Δ). Plasma OCN concentration was affected by diet (P<0.05), time (P<0.001), and diet x time interaction (P<0.001).

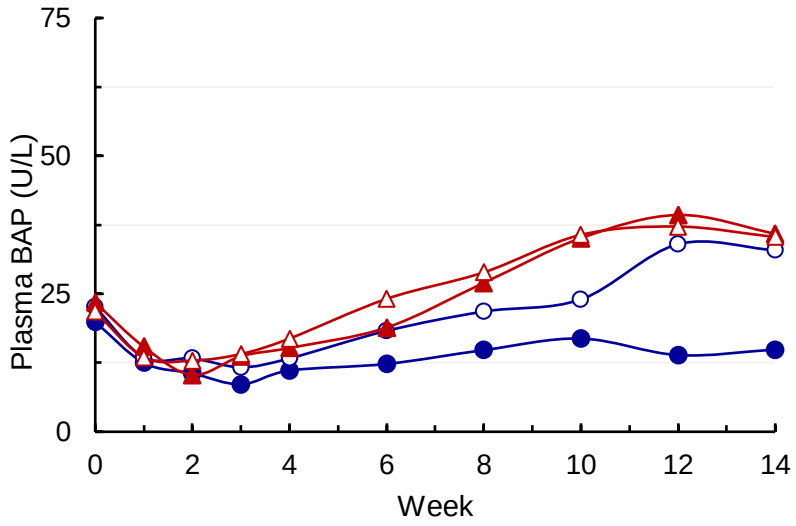


Figure C-12. Pens_C. Plasma concentration of bone alkaline phosphatase (BAP) (U/L) of cows fed the four diets comprising HP-HCa (●), LP-HCa (○), LP-LCa (▲) and the LP-LCa-D diets (Δ). Plasma BAP concentration was affected by diet (P<0.06), time (P<0.001), and diet x time interaction (P<0.01). biopsy measurements and fat cover and eye muscle area (EMA), and the changes in these measurements during the 14 weeks of early lactation, in cows fed four diets (n=7or 8). The cows were fed *ad libitum* on diets of high P (HP-HCa) or low in P (HP –LCa, LP-LCa, LP-LCa-D (see text).

4.2.5 Discussion

Performance of the cows and calves

The HP-HCa diet in the present experiment contained 2.16 g P /kg DM and since the cows ingested 10.22 kg DM per day (Tables C1 and C3) the intake of P averaged 22.1 g P/day during the 14 week experimental interval. This average P intake would be expected to have supplied the needs of breeders cows in terms of liveweight and the milk production. However the DM intake during weeks 1-4 was only about 60% of the intake during weeks 5-14 and consequently during the first four weeks intake of diet P would have been proportionally less than the calculated requirements for P. The average PIP concentrations over the 14 weeks (mean 1.52 mmol/L) also suggested that the P status of these cows was marginal to adequate, rather than in excess. The positive P balance of the HP-HCa cows at weeks 4 and 8 (TC1 and TC2, +5.1 and +6.2 g P/day; Table C-3 and C-6 and after allowing for the amount of P estimated to have been secreted in milk) indicated that there was net deposition of P to body tissues during the first 8 weeks of lactation. Inclusion of this demand for P for net tissue accretion by 5-6 g P would be in addition to the P requirements as calculated, and may explain the lower than expected PIP concentrations particularly during weeks 1-8. The nil P balance in TC3 indicates that P replenishment for this diet was complete by about 3.5 months of lactation. The changes in rib bone PSACB indicating that these cows increased their rib bone P by 29% are in accord with there being net deposition of P during the 14 weeks of lactation. The mean PSACB at calving (0.567 mgP/mm^2) was similar to the PSACB of cows fed the high P diets during the previous Pens_B experiment (0.561 mgP/mm^2), nevertheless there was an increase in PSACB in HP-HCa cows during the current experiment. It appears that the cows had not fully recovered body P reserves during the preparatory (late pregnancy) phase of the experiment and calved in sub-optimal P status.

This may be a reason for the moderate calf growth (0.69 kg/day) and high cow LW gain (0.45 kg/day) in these HP-HCa cows during early lactation.

The most important finding of the present Pens_C experiment was that these breeder cows calving in high body condition (BCS *ca.* 3.5) and with at least reasonable P reserves were able to maintain milk output and calf growth during the first 3 months of lactation even when fed severely P deficient diets. P intake in the three LP diets was on average 4.0 – 4.7 g P/day and thus only *ca.* 20% of the calculated diet requirements in the absence of mobilization of P from body reserves. Despite the differences in P intake, voluntary ME intake and cow LW change between the HP and LP diets neither milk production nor calf growth (0.58-0.64 kg/day) was significantly affected by diet (P>0.10) (Table C-2 and C-5; Figure C-3). The observed decreases in voluntary intake by 14-22% in severe diet P deficiency are in accord with numerous previous reports of decreased intake in cattle and sheep fed P deficient diets. The immediate reduction in voluntary intake following the introduction of the low P diets in the present experiment is in contrast to delays of up to 8-20 weeks often observed in non-pregnant non-lactating animals (Gartner *et al.* 1982; Bortolussi *et al.* 1996; Quigley *et al.* 2015). The most obvious reason for the immediate decreases in voluntary intake and PIP in these LP lactating cows is that the deficit between diet P intake and the P requirements of the cow were, very large. A contributing factor may have been that the cows were in less than replete P status at the start of the experiment. In the present experiment the PIP concentrations rapidly decreased to low concentrations (< 0.8 mmol/L) in the LP-LCa and LP-HCa diet cows by 14 days after parturition and indicate severe P deficiency.

The average calf growth for the HP-HCa treatment (0.69 kg/day) was lower than that often observed in mature Droughtmaster genotype cows (Coates *et al.* 2017; Dixon *et al.* 2016, 2017; Pens_E experiment below). The birth weight of the calves (30.0 kg) was lower than expected for mature breeders of the genotype. One hypothesis is that the HP-HCa cows were in marginal P rather than adequate P status particularly during the first 8 weeks of lactation; the primary evidence is that during lactation the PIP averaged only 1.52 mmol/L whereas in the other experiments reported here it was ≥ 1.8 mmol/L.

Cows fed three LP and severely P deficient diets in the present experiment were able to maintain milk production due torapid mobilization of body tissue reserves. The LP-HCa and LP-LCa cows lost about 20 kg LW and about 1 BCS unit (Table C-2). This was in accord with the estimated P balance averaging -5.7 and -6.0 g P/day across the three total collection intervals when the estimated secretion of P in milk was included in the calculated balance. The absence of change in the P content of rib bone during lactation in these cows suggests that the P for milk was derived primarily from mobilization of the soft tissues, substantial mobilization of trabecular bone not measured by changes in rib bone cannot be excluded.

The measurements of LW change, ME intake and plasma markers, especially when considered together, indicated that the inclusion of ammonium chloride in the diet (LP-LCa-D) markedly increased the capacity of the cows to mobilize bone P. These LP-Ca-D cows were similar to the other two LP diets in milk output, calf growth, P balance and change in rib bone. But importantly these LP-LCa-D cows gained LW rather than losing LW through the 14 week measurement interval, and based on the measurements of PSACB in rib bone mobilized more bone P than the cows fed the other two LP diets. In addition these LP-LCa-D cows tended to have higher DM intake, higher PIP during weeks

2-8, higher plasma CTX-1, BAP and 1,25-dihydroxy vit D, and lower plasma total Ca to PIP ratio, than all other LP diet cows. Further investigation of the effects of negative DCAD diets is important given that such diets can easily be implemented by using molasses based supplements where the rumen degradable N component is partly provided by ammonium chloride rather than urea.

4.2.6 Conclusion

This present experiment provides strong evidence that mature *Bos indicus* cross cows which are in high P status at calving do have major capacity to mobilize body P reserves to meet a deficiency in the diet and to maintain milk production and calf growth. However this was associated with substantial LW loss indicating that milk production was at the expense of body reserves. There was evidence that a low Ca diet and a low Ca - negative DCAD diet ameliorated the severity of the P deficiency and LW loss.

Experimental Section 5. Pens_D experiment

Title. Pens_D. Effects of diet phosphorus deficiency in pregnant Droughtmaster heifers ingesting sub-maintenance energy intakes

4.2.7 Summary of experiment

This study examined the effects of adequate or deficient diet P on the performance of heifers in late pregnancy fed three intakes of metabolisable energy (ME), and then the carryover effects of these diets into the following lactation when all the animals were fed a P deficient diet. Diet treatments during pregnancy comprised a 3x2 factorial design with heifers offered three restricted levels of wheat straw and molasses-urea (LowE, MedE, HighE) calculated to provide for severe, moderate or nil conceptus-free liveweight (CF-LW) loss, with each P adequate or P deficient (HighP or LowP) during pregnancy. Droughtmaster heifers (n = 42) initially (mean ± sd) 419 (±31) kg CF-LW and 3.9 (±0.27) body condition score (BCS) in mid-pregnancy were housed in individual pens during the last 14-18 weeks of pregnancy. Plasma inorganic P (PIP) concentrations confirmed that LowP and HighP cows were severely P deficient and P adequate, respectively, during pregnancy, and all cows were P deficient during lactation. In the LowE and MedE energy treatments where the amount of ME offered was restricted and less than maintenance energy requirements the HP heifers were losing CF-LW; however there was no effect of low diet P on ME intake or CF-LW change (P>0.05) (means -49 and -31 kg CF-LW, respectively). The HighE-HighP heifers ingested all of the feed offered and maintained CF-LW (-4 kg). Heifers given the HighE-LowP diet had lower voluntary intakes of DM and ME, and CF-LW change (-37 kg) was lower than that of the HighE-HighP diet heifers (P<0.05). Diet treatment did not affect calf birth weight (P>0.05). During early lactation when fed a P deficient diet all cows provided sufficient milk for moderate calf growth (0.6 – 0.7 kg/day).Calf growth was reduced (P<0.05) by some of the diet treatments imposed during pregnancy. During early lactation when fed a P deficient diet cows lost LW and this LW change was negatively correlated with cow LW at calving (P<0.05). As a consequence of these LW losses some cows had to be withdrawn from the experiment to avoid compromising the welfare of the cows. The change in P in rib bone during late pregnancy was increased (P<0.01) by feeding the HP and decreased by higher ME intake (P<0.05). During early lactation heifer fed HP in pregnancy mobilized more rib bone P than those fed LP diets. It was concluded that severe P deficiency in late-pregnant heifers may reduce voluntary intake of diets otherwise adequate for CF-LW maintenance. Also P deficient diets during late pregnancy may have adverse effects on heifer bone P reserves at calving, and thus on cow LW, LW change and calf growth during early lactation.

4.2.8 Background and onjectives

In the northern Australian rangelands breeders are often in mid to late pregnancy during the late dry season, but due to the low quality and often low availability of pastures their ME intake is usually insufficient to maintain CF-LW. Such pastures may also be very low in P concentration. Thus the losses in CF-LW and body condition are often severe. The effects of diet P deficiency during late pregnancy in such circumstances and during rapid losses in liveweight and body condition are not well understood. For optimal breeder herd management it is important to know whether breeders in these circumstances will benefit from P supplements. Current industry recommendations (McCosker

and Winks 1994) are that P supplementation during the dry season for breeders will have no benefit unless they are in late pregnancy or lactating. However, non protein N supplements are often fed during the late dry season when senesced tropical grasses are deficient in N and therefore supplementary P can be fed at low marginal cost. Many cattle managers do include some P in dry season supplements and there is extensive anecdotal evidence that this is beneficial in P deficient rangelands. In the Pens_A experiment of this project P supplementation of a P deficient diet which contained sufficient ME content for slow LW gain was found to substantially increase voluntary intake during late pregnancy and to increase cow LW at calving. However the diet in Pens_A was of sufficient quality to allow P adequate heifers to gain substantial CF-LW while P deficient heifers maintained CF-LW, but this result may not be applicable to situations where late pregnant heifers of breeders are rapidly losing CF-LW since mobilization of body tissues during LW loss will mobilize some P and potentially provide the P needs of the conceptus.

The objectives of the present experiment were to: (i) determine the effects of P-deficient versus P-adequate diets on P metabolism and P balance in heifers when ME intakes provided for severe maternal LW (CF-LW) loss through to maternal LW maintenance, and (ii) determine the magnitude of body P mobilization in heifers pre-calving and (iii) the effects of P nutrition during pregnancy on performance of the cows post-calving. The experiment comprised two phases, pregnancy (Period 1) and early lactation (Period 2). Six diet treatments were imposed during the last 4 months of pregnancy with the heifers housed in individual pens. Following parturition the cows and their calves were group-fed a diet with moderate P concentration diet during 12 weeks of lactation to investigate the potential carryover effects of the diet treatments during pregnancy.

4.2.9 Materials and Methods

Experimental design, animals and diets

Bos indicus x *Bos taurus (ca. 5/8 x 3/8)* crossbred Droughtmaster heifers (year #13) were selected from Spyglass Cattle Research Facility, Charters Towers, Queensland. The heifers had been mated at *ca.* 2 years of age from the 23 December 2014 for three months and pregnancy tested by rectal palpation (10 May 2015). Heifers were selected on the criteria of stage of pregnancy, body condition score (BCS; a 5-point scale as described by CSIRO 2007) and with temperament suitable for intensive experimentation. The heifers had grazed native pastures expected to be marginal to adequate in P status during the previous 12 months . The management of the heifers before mating included vaccination for tick fever, botulism and clostridial diseases. During the experiment the heifers were vaccinated for bovine ephemeral fever. The heifers were relocated to the Brian Pastures Research Facility, Gayndah. Following recovery from transport heifers were, group-fed in yards a low P concentration diet (wheat straw and molasses with 39 g/kg urea *ad libitum*) and handled frequently by stockmen to identify heifers with less than suitable behaviour. Forty-two heifers were selected, blood was sampled on 22 June 2015 when the heifers were consuming the common low P diet, and the heifers were then allocated by stratified randomization based on LW and stage of pregnancy to seven blocks each of six diet treatments. The heifers were housed in individual pens (*ca.* 33 m^2) in an animal complex with the heifers in each block allocated to adjacent pens within the animal research complex. Thus the allocation blocks included potential effects of the animal stratification criteria and pen position within the animal complex. Approximately half of each pen had a concrete floor and a roof to protect the feed bunks, and shade sails provided additional cover in the remainder of the pen. The heifers were housed in these individual pens until shortly after calving (Pregnancy, Period

1). On the day of calving or the subsequent day, the cows and the calves were weighed, the cows blood sampled, and the calves were tagged. About one week after calving (mean 9 days) the heifers and calves were moved to a small (1.8 ha) paddock where they were group-fed a common diet for 14 weeks (Lactation, Period 2). Period 1 commenced on 29 June 2015 (Day of year (DOY) 180). The heifers calved from the 7 October 2015 (DOY 280) until the 4 December 2015 (DOY 338) (mean calving date 26 October 2015) and thus the diet treatments were imposed on individual heifers for between 14 and 22 weeks with the interval varying with the actual date of parturition.

The six diet treatments (during pregnancy; Period 1) comprised of a factorial combination of three intakes of ME (LowE, MedE and HighE) and two concentrations of P (LowP and HighP) in the diet. The intakes of ME were achieved by offering restricted amounts of a diet comprising chopped straw and molasses-urea mix (40/60 ratio) fed separate feed troughs. The amounts of straw and molasses-urea were calculated from nutritional standards (CSIRO 2007) to provide the ME intakes required for conceptus-free liveweight (CF-LW) change of -0.4 (LowE), -0.2 (MedE) and nil kg/day (HighE) during the last 14 weeks of pregnancy. The chopped wheat straw was mixed with a small amount of mixed molasses-water (2:1, 60 kg/t) in a feed mixer wagon to reduce dust. The molasses-urea mixture offered to the animals comprised a mixture (g/kg as fed) 954 molasses, 42 urea, 2.4 sodium chloride, 1.4 Rumigrow, 0.478 rumensin. Heifers allocated to the HighP diets were given additional P (58 g/d sodium phosphate) and Ca (40 g calcium chloride) to maintain an approximate 2:1 Ca/P ratio. The sodium phosphate and calcium chloride were dissolved with water and mixed into the molasses-urea. Heifers allocated to the LowP diets were given no additional P or Ca. Sodium chloride was added to provide the same amount of sodium in the LowP and HighP diets. The LowP and HighP diets were calculated (CSIRO 2007) to provide diets with a deficit of 1.1 – 5.6 g P/day or a surplus of 8.9 – 13.3 g P/day, respectively, of average P requirements for the LP and for the HP diets during the last 12 weeks before parturition, respectively.

During lactation (Period 2, paddock) the heifers with calves were offered *ad libitum* hay *(Bothriochloa insculpta*) fed as large round bales in hay feeders, and were also offered *ad libitum* molasses-urea containing (g/kg as fed) 930 molasses, 60 urea, 10 sodium chloride, mineral premix and 0.465 rumensin *ad libitum* in three separated troughs. No source of P was included. The heifers were mated with two bulls from the 11 Jan 2015 (DOY 376).

Sentinel weaner steers (n = 10) of nominally the same genotype as the heifers were included with the group-fed cows and calves during lactation (Period 2) from 26 November 2015 until 4 February 2015 (i.e. for 70 d). From the 20 August (DOY 232) until 16 September 2015 (DOY 259) these steers had grazed senesced pasture and were fed molasses-urea without or with additional P (LowP and HighP diet treatments, n = 5 each, respectively), as described in detail below for the pregnancy phase of the Pens_E experiment (Periods 1B). Then from the 16 September 2015 (DOY 259) until the 26 November 2015 (DOY 330) these steers were fed in small (4 ha) paddocks on wheat straw offered *ad libitum* and the LowP or HighP molasses-urea as described in detail below for the Pens_E experiment (Periods 1C). On DOY 330 the steers were moved to the 1.8 ha paddock with the heifers and calves which had already commenced Period 2. Thus two sub-groups of sentinel steers were expected to be in LowP or HighP status at their entry to Period 2 of the experiment.

Procedures and measurements

During Period 1 samples of feeds offered were obtained on each occasion a batch of straw or molasses-urea feed was prepared. Orts of straw and molasses were collected and sampled on a weekly basis and also at the end of each five day total faecal collection interval described below. Samples of straw and molasses-urea were dried (60°C) and feed DM intake on a weekly basis was calculated. During pregnancy (Period 1) total collection (TC) of faeces for two, five day intervals were done at nine and three weeks (TC1 and TC2, respectively) before the expected parturition. The heifers were constrained in the front half of the pen with the concrete floor and the total faeces excreted were collected, weighed and mixed. A 10% subsample of the daily faeces was stored frozen. These samples were later mixed, subsampled, dried (60°C), and then ground through a 1 mm sieve (Christie and Norris laboratory mill, Chelmsford, UK). During lactation (Period 2) grass hay was sampled from 10 bales using a hay corer, dried (60°C) and ground through a 1 mm sieve. Straw, grass hay and molasses mixture samples were bulked on a fortnightly or monthly basis for subsequent analyses. Other feed ingredients (sodium phosphate, calcium chloride and sodium chloride) were sampled fortnightly and bulked across Period 1 for subsequent analysis.

Liveweight (i.e. total LW, T-LW) (without fasting) was measured and BCS estimated at weekly intervals through pregnancy. Jugular blood samples were obtained fortnightly throughout using vacutainers (BD Diagnostics, Plymouth, UK) with lithium heparin as an anticoagulant to provide plasma, and also with vacutainers without anticoagulant to provide serum. Plasma was separated by centrifugation (3,500 **g** x 15 min) and the plasma and serum were stored frozen (-20 C). In the days preceding and following the TC2 faecal collections samples of urine were obtained by manual stimulation of the vulva, acidified (pH < 3) by addition of 4 M sulphuric acid and stored frozen (-20^{o}C) for subsequent analysis of P concentration. During lactation (Period 2) the LW and BCS of the cows and sentinel steers and LW of calves were measured, and blood and faeces sampled, on a fortnightly basis. Faecal samples were obtained from six cows selected at random and from the steers and were pooled to represent each group.

Bone biopsy samples of external cortical rib bone were obtained surgically in mid-pregnancy (12th rib), shortly after calving (both the 11th and 12th ribs), and at the end of the lactation Period 2 (11th rib) as described in Appendix 3. In addition biopsy samples of the *tuber coxae* bone were obtained in mid-pregnancy and shortly after calving (Appendix 3). Realtime ultrasound scanning (Esaote Pie Medical Aquila with a 3.5 Mhz linear array transducer (Pie Medical Imaging, Maastricht, The Netherlands)) was used to measure fat depth at the rib and P8 rump sites, and of eye muscle area (EMA), on two occasions early and late in lactation. Also ultrasonography (Honda HS200V with a 7.5 MHz linear array transducer (Honda Electronics Co. Ltd, Toyohashi, Japan) was used to examine ovarian activity from six weeks after calving.

Laboratory procedures

Samples of feeds and feed ingredients which included straw, grass hay and molasses, and feed refusals in pregnancy (Period 1), were bulked on a fortnightly basis. Organic matter (OM) was determined by incineration (550^{o}C for 8 h). Samples of feed offered throughout the experiment, and of feed offered, feed refused and faeces during the total collection (TC) intervals, were digested in a nitric-perchloric acid mixture, and the concentrations of P, Ca, Mg and S analysed using an inductively coupled plasma spectrometer (Optima 7300 DV, Perkin Elmer, Waltham, MA, USA).

Samples of feed offered were also analysed for total N, neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin and crude fat by a commercial laboratory (Dairy One, Ithaca, USA). The concentration of P in urine was measured colormetrically (Goodwin 1970) and that of creatinine in by high-performance liquid chromatography (George *et al.* 2006).

Plasma samples were analysed colormetrically for plasma inorganic P (PIP), Ca and Mg (Beckman Coulter Au680 analyser with OSR6122, OSR 6189 and OSR6189 assay kits respectively). The concentrations of these hormones and markers was analysed in plasma samples obtained at fortnightly intervals on four occasions during pregnancy and four occasions during early lactation. Plasma concentrations of hormones and bone metabolism markers were determined using commercial assay kits and according to the manufacturer's instructions as follows: parathyroid hormone (PTH; A11930, Beckman Coutler), 25OH Vit D and 1,25-diOH Vit D (AA-35F1 and AA-54F2, Immunodiagnostic Systems), CTX-1 (Crosslaps AC-02F1, Immunodiagnostic Systems), bone alkaline phosphatase (BAP; MicroVue 8012, Quidel), and osteocalcin (OCN; MicroVue 8002, Quidel).

Rib bone biopsy samples were carefully scraped to remove trabecular bone before measurement of cortical bone thickness (CBT) using Vernier callipers and specific gravity (SG) by gravimetric procedures. Bone samples for histology were fixed in 10% neutral buffered formalin for at least 8 weeks. Samples were decalcified in 14% EDTA for at least 12 weeks and then embedded in parrafin. Sections (5 µm) were stained with Masson's trichrome and were photographed on a microscope at 2, 10 and 20 x magnification. Measurments of cortical thickness, trabecular volume, trabecular thickness, osteoid % and trabecular separation were performed using the image analysis software ImageJ (Rasband *et al.* 2016) with the additional BoneJ (Doube *et al.* 2010).

Calculations and statistical analyses

During Period 1 the week of pregnancy at each measurement or sampling date was recalculated retrospectively from the actual calving date of each heifer. The results obtained through pregnancy and lactation are presented on the basis of weeks before or after the actual calving date. CF-LW of individual heifers at each total liveweight (T-LW) measurement was calculated by subtracting the conceptus weight from total cow LW (O'Rourke *et al.* 1991) and the estimate of conceptus weight was scaled to the measured mean calf birth weight (28 kg). The LW change of the animals was calculated using two approaches; (i) from the linear regression of T-LW and CF-LW with time during the pregnancy or lactation interval (LWG(R)), and (ii) the difference between the LW measured at the beginning and the end of the interval (LWG(D)). Since the cows lost substantial LW shortly after parturition (on average 13 (sd 18) kg T-LW from Day 1 to Day 9 of lactation) the T-LW changes during lactation were calculated for the interval from Day 9 to Day 84 of lactation.

During pregnancy (Period 1) when the heifers were fed in pens the intakes of DM and OM were calculated on a weekly basis. The apparent digestibility of DM and organic matter (OM) during the three total collection intervals were calculated by conventional procedures. The ME content (M/D) of the mixed diets fed in the pens during pregnancy was calculated from the organic matter digestibility (OMD) measured during the two total collection intervals as: M/D = 0.169 (%OMD) – 1.986 where M/D was MJ ME/kg DM (CSIRO 2007, p. 8). The volume of urine excreted during TC2 was calculated from the creatinine concentration and assuming a daily creatinine of 0.91 mmol/kg $W^{0.75}$ (Chen and Gomes 1995). The difference between intake and faecal excretion (I-F) of P and Ca during the TC intervals was calculated from these measurements. P balance during pregnancy was

calculated from P intake minus the excretion of P in faeces and urine, the latter measured from samples obtained shortly before and after the TC2 total collection. Also during the TC intervals the differences between intake and faecal excretion (I-F) of P and Ca were calculated. The P and Ca requirements of the pregnant and later lactating heifers with measured intakes, LW and LW change were calculated following CSIRO (2007) and using the associated spreadsheets. The proportion of P and Ca requirements provided by the restricted amounts of the diets fed through pregnancy P intakes of the heifers measured in the pens could then be calculated.

During lactation (Period 2) when the heifers with their calves and the sentinel steers were fed as a group in a small paddock the intakes of molasses-urea supplements (g DM/kg LW) were calculated from the total intake of supplement and the LW of the animals. Intakes hay ME and DM were estimated using QuikIntake (Version 5, S. McLennan and D. Poppi, unpublished) from the calculated ME requirements of the animals (lactating cows and the steers) minus the ME ingested as molasses-urea supplement. The intake of hay and molasses by the lactating heifers and the sentinel steers was assumed to be the same per kg LW, and the intakes of the calves was assumed to be negligible. The ME content of the hay was estimated from the laboratory analyses. P requirements were calculated as described above; milk P secretion was estimated from the calf growth rate and and the milk required per kg calf gain as described for experiment Pens_C.

The concentration of P in cortical rib bone was calculated as: Pconc (mgP/cc) = 228.8 x (specific gravity) – 261. Also an index of rib bone P, the P in cortical bone per unit surface area of cortical bone (PSACB, mgP/mm^2), was calculated as the product of P concentration in external cortical bone and CBT (Dixon et al. 2018).

A reproductive score of ovarian activity was calculated for individual cows for each the monthly ultrasonography measurement; an animal was given a score 1 if follicles were observed, 2 if follicles ≤6 mm observed, 3 if follicles were 6-10 mm, 4 if follicles > 10 mm, and score 5 for the presence of a CL or CA. Post-partum anoestrus interval (PPAI) was estimated as the days from calving until the first observation of a CL or CA at the monthly ultrasonography measurement, or in cows for which a CL or CA had not been observed by the last measurement the PPAI was calculated as the interval to this measurement date plus 30 days.

The differences among diet treatments during pregnancy were examined separately during the Period 1 (pregnancy) and Period 2 (lactation) by ANOVA in a 2 x 3 factorial design using Genstat (release 16.1, VSN International Ltd, Hemel Hemstead, UK). The effects of the initial animal allocation blocks, of heifer LW, BCS and calving date as potential covariates were examined. Also the initial measurements of rump fat depth, rib fat depth, eye muscle area, rib bone CBT, P concentration of cortical bone and PSACB were examined as potential covariates for these specific variables. Block and covariate effects were included in the statistical model when appropriate and significant at $P<0.10$. Separate ANOVA models were used to examine in the sentinel steers the effects of the LowP and HighP diets imposed before their introduction into Period 2 of the experiment. Where measurements were made at intervals (LW, intake, digestibility, bone biopsies, concentrations of blood minerals and hormones) repeated measures ANOVA models with main effects of diet and time were used. Pair-wise comparisons between means were made using the protected LSD procedure.

4.2.10 Results

Animal health

The animals were generally in good health throughout the experiment. During Period 1 (pregnancy) two heifers were removed from the experiment; one calved two months earlier than expected while another calved two weeks later than any other heifer. Two additional heifer-calf pairs were also rejected from the Period 2 (lactation) measurements due to the neo-natal death of one calf and a prolapse at calving in a second heifer. Due to the extensive LW loss and low body condition of some heifers by late in the lactation interval (Period 2) five heifer-calf pairs were removed from the experiment between 9 and 11 weeks of lactation rather than continuing to the planned 12 weeks of lactation. Nevertheless, because the changes in LW of these heifers and of their calves, as well as those measured during the first 14 weeks of lactation, were all closely described by linear regression of LW with time, the results from these five heifer-calf pairs were considered representative of their treatments and were included in the Period 2 data set.

Table D-1. Pens_D. Pregnancy and lactation. The composition (g as-fed/kg for dry matter concentration and g/kg DM for the other constituents; mean and SD in parenthesis) of the mixed diets containing low or high concentrations of P (Period 1) and that fed to the lactating cows and the weaner heifers during Period 2.

Composition	Pregnancy (Period 1)		Lactation (Period 2)	
	Wheat straw	Molasses-urea (LowP)	*Bothriochloa* hay	Molasses-urea (LowP)
Dry matter (g/kg as fed)	931 (2.3)	794 (7.3)	917	808
Organic matter (g/kg DM)	885 (6.6)	859 (5.6)	894	840
Crude protein	47 (2.6)	190 (3.6)	67	273
NDF	681	-	724	--
ADF	497 (10.4)	-	499	--
Lignin	68 (5.5)	-	55	--
Crude fat	8.3 (0.8)	23 (0.2)	16	18
Ca	2.6 (0.12)	10.1 (0.38)	3.0	8.8
P	0.55 (0.08)	0.93 (0.04)	1.0	1.0
Mg	1.4 (0.11)	8.1 (0.37)	1.1	6.18
S	1.6 (0.14)	10.1 (0.48)	1.3	8.4
Diet Ca/P ratio	4.7	10.9	3.0	8.8
ME content (MJ/kg DM)	-	-	5.7	10.6

Composition of the diets

The wheat straw and molasses-urea fed during pregnancy contained 0.55 and 0.93 g P/kg DM and 2.6 and 10.1 g Ca/kg DM, respectively (Table D-1.) and thus the heifers fed the LP treatments ingested diets containing *ca.* 0.77 g P/kg DM and 7.46 g Ca/kg DM. Since the *Bothriochloa* hay and the molasses-urea fed in Period 1 both contained 1.0 g P/kg DM this was also the content of the diet ingested during Period 2. The *Bothriochloa* hay was estimated to contain 5.8 MJ ME/kg DM.

Liveweight change, intake and faecal excretion of heifers through pregnancy (Period 1)

At the allocation of the heifers (22 June 2015) the T-LW and CF-LW were (mean ± sd) 425 ± 32 kg and 419 ± 31 kg, respectively, while BCS was 3.9 ± 0.27 units. The T-LW, CF-LW and BCS of the heifers 14 weeks before calving (calculated retrospectively) were mean 426 ± 30 kg, 412 ± 30 kg and 3.9 ± 0.26 units and thus were similar to those at allocation. The changes in CF-LW and BCS of the heifers during the last 14 weeks of pregnancy are shown in Figure D-1 and D-2, and Table D-2. Heifers fed the LowE-LowP and LowE-HighP diets lost the same CF-LW (-49 kg) and also lost 1.2-1.4 BCS units. The CF-LW and BCS changes of heifers fed the MedE-LowP and MedE-HighP (-37 and -24 kg CF-LW, -1.1 and -0.5 BCS units, respectively) did not differ (P>0.05).

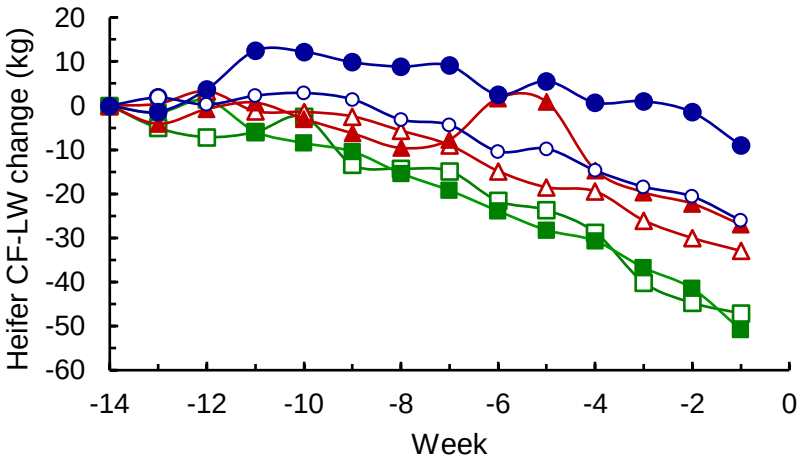


Figure D-1. Pens_D. The changes in CF-LW during the last 14 weeks of pregnancy in heifers given the 6 diet treatments before calving. The treatments were LowE-LP (□), LowE-HP (■), MedE-LP (Δ), MedE-HP (▲), HighE-LP (○) and HighE-HP (●)

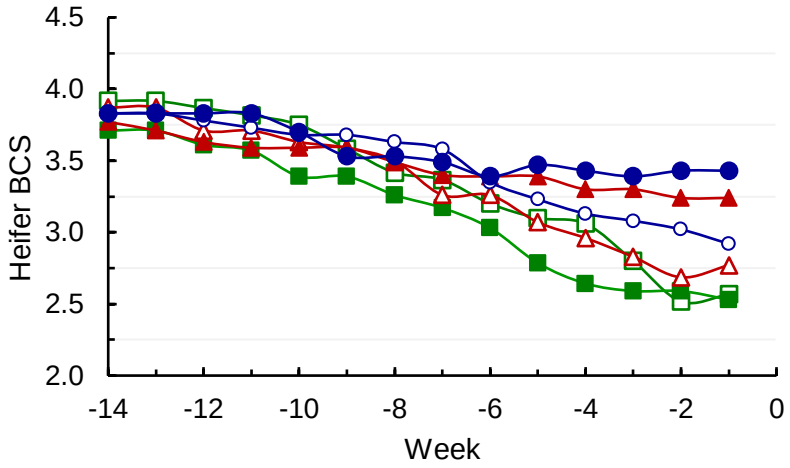


Figure D-2. Pens_D. The body condition score (BCS) during the last 14 weeks of pregnancy in heifers given the 6 diet treatments before calving. The treatments were LowE-LP (□), LowE-HP (■), MedE-LP (Δ), MedE-HP (▲), HighE-LP (○) and HighE-HP (●).

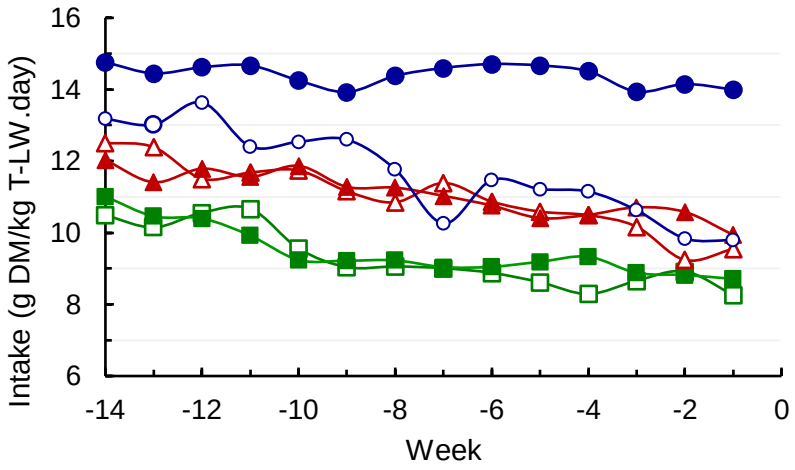


Figure D-3. Pens_D. Intakes of total DM (g DM /kg total LW.day) during the last 14 weeks of pregnancy (Period 1) in heifers given the 6 diet treatments before calving. The diet treatments were LowE-LP (□), LowE-HP (■), MedE-LP (Δ), MedE-HP (▲), HighE-LP (○) and HighE-HP (●).

These CF-LW changes for the LowE and MedE diets were comparable to the planned respective changes of -60 kg and -30 kg, respectively. The CF-LW change for the heifers fed the HighE-HighP diet (-4 kg CF-LW) was comparable with the intended CF-LW change of nil kg, but the HighE-LowP treatment heifers lost substantial CF-LW (-37 kg) which was similar to the CF-LW loss of the MedE diets. This was most obviously because these HighE-LowP treatment heifers did not consume all of the feed offered (Figure D-3) with intake (g DM/kg T-LW.day) being 90%, 77% and 74% of that of the HighE-HighP heifers during the intervals -12 to -9, -8 to -5, and -4 to -1 weeks before parturition, respectively (Table D-2). These intakes by the HighE-LowP treatment heifers during the last 8 weeks of pregnancy were similar to those for the two MedE treatments (Table D-2).

In vivo OM digestibility measured during the two total collection intervals averaged 721 g/kg and did not differ (P>0.05) between diets or between the collection intervals (Table D-2). The ME content of the diets ranged from 9.8 – 10.4 MJ ME/kg. The mean ME intakes during the last 14 weeks of pregnancy estimated from OM digestibility and the DM intakes ranged from 39.2 – 68.0 MJ ME/day, or 94.3 – 149.0 kJ ME/kg T-LW.day. The changes in LW and BCS during pregnancy were associated with decreases in fat cover at the rib and rump sites and in EMA (Table D-3). During pregnancy both fat cover and EMA were reduced by the lower ME intakes (P<0.05). Also EMA was reduced more by the LP than the HP diets (P<0.05, P<0.01).

Table D-2. Pens_D. Pregnancy. Heifer total-LW (T-LW), conceptus-free liveweight (CF-LW), body condition score (BCS) and intake from mid-pregnancy to parturition during Period 1. Heifers were fed restricted amounts of diets based on wheat straw and molasses-urea. DM intake is given for 4 intervals during the last 14 weeks of pregnancy. The organic matter (OM) digestibility was measured during two total collection intervals (TC1 and TC2). The ME intake was estimated from the OM digestibility and the intake during the 14 week interval

Measurement	Diet treatments						s.e.m.			Probability		
	LowE-LP	LowE-HP	MedE-LP	MedE-HP	HighE-LP	HighE-HP	E	P	ExP	E	P	ExP
n	6	7	7	7	6	7						
T-LW (-14 wk) (kg)	418	414	423	434	439	437	8.7	7.1	12.3	0.118	0.958	0.737
T-LW (-1 wk) (kg)	406	400	425	442	443	462	10.8	8.8	15.3	0.011	0.436	0.651
CF-LW (-14 wk) (kg)	403	398	408	418	424	422	8.4	6.9	11.9	0.181	0.891	0.816
CF-LW (-1 wk) (kg)	356	349	376	390	393	412	10.8	8.8	15.2	0.010	0.471	0.665
CF-LW change (Regn) (kg/d)[A]	-0.50[c]	-0.49[c]	-0.38[bc]	-0.22[ab]	-0.38[bc]	-0.04[a]	0.046	0.038	0.065	<0.001	0.006	0.050
CF-LW change (Regn) (kg)[A]	-49	-49	-37	-24	-37	-4	4.5	3.7	6.4	<0.001	0.006	0.050
CF-LW change (Diff) (kg/d)[B]	-0.48	-0.50	-0.33	-0.28	-0.31	-0.10	0.060	0.049	0.084	0.008	0.263	0.373
CF-LW change (Diff) (kg)[B]	-47[b]	-49[b]	-32[ab]	-28[ab]	-31[ab]	-10[a]				0.008	0.263	0.373
BCS (-14 wk)	4.0	4.0	3.6	3.7	3.6	3.9	--	--	--	--	--	--
BCS change	-1.4	-1.2	-1.1	-0.5	-0.9	-0.4	0.11	0.09	0.15	<0.001	0.002	0.461
DMI Wk -14 to-13 (kg DM/d)	4.27	4.44	5.26	5.07	5.81	6.40	0.18	0.14	0.25	<0.001	0.366	0.305
DMI Wk -12 to -9 (kg DM/d)	4.14	4.03	4.92	5.08	5.73	6.50	0.17	0.14	0.24	<0.001	0.172	0.185
DMI Wk -8 to -5 (kg DM/d)	3.68	3.72	4.64	4.85	5.00	6.72	0.21	0.17	0.29	<0.001	0.010	0.013
DMI Wk -4 to -1 (kg DM/d)	3.45	3.60	4.17	4.62	4.57	6.57	0.19	0.15	0.27	<0.001	<0.001	0.003
DMI Wk -14 to-13 (gDM/kg T-LW.d)	10.63	11.23	12.84	12.20	13.70	15.12	0.32	0.26	0.46	<0.001	0.230	0.092
DMI Wk -12 to -9 (gDM/kg T-LW.d)	10.46	10.19	12.09	12.23	13.41	15.17	0.28	0.23	0.39	<0.001	0.103	0.037
DMI Wk -8 to -5 (gDM/kg T-LW.d)	9.55	9.78	11.66	11.77	11.94	15.12	0.40	0.33	0.56	<0.001	0.007	0.005
DMI Wk -4 to -1 (gDM/kg T-LW.d)	9.40	9.89	10.89	11.41	11.38	15.47	0.40	0.33	0.57	<0.001	0.001	0.004
OM digestibility (g/kg) TC1	727	744	728	722	725	752	8.4	6.9	11.9	0.483	0.198	0.369
OM digestibility (g/kg) TC2	726	709	734	703	674	710	12.0	9.8	17.0	0.213	0.778	0.133
OM digestibility (g/kg) mean	727	727	731	713	699	731	8.3	6.8	11.8	0.626	0.648	0.115
ME content (MJ ME/kg DM)	10.3	10.3	10.4	10.1	9.8	10.4	0.14	0.11	0.20	0.626	0.648	0.115
ME intake (MJ ME/d)	39.2	39.8	48.5	49.1	52.9	68.0	1.60	1.31	2.27	<0.001	0.006	0.004
ME intake (kJ ME/kg T-LW.d)	94.3	97.4	114.3	111.3	113.7	149.0	2.60	2.12	3.67	<0.001	<0.001	<0.001
ME intake (kJ ME/kg CF-LW.d)	101.7	105.3	122.7	119.8	121.9	159.4	2.79	2.28	3.95	<0.001	<0.001	<0.001

E, effect of ME intake; P, effect of phosphorus.

A, LW change, calculated by linear regression of LW and time. B, calculated by difference between week -14 and week -1.

Table D-3. Pens_D. Pregnancy and lactation. The initial fat cover at the rib and rump sites, eye muscle area (EMA). The mean calving date was the 26 October 2015

Measurement	Diet treatments during pregnancy						s.e.m.			Probability		
	LowE-LP	LowE-HP	MedE-LP	MedE-HP	HighE-LP	HighE-HP	E	P	ExP	E	P	ExP
n	6	7	7	7	6	7	--	--	--	--	--	--
Heifer rump fat (mm)												
Mid-pregnancy (23 Jun 2015)[A]	11.2	10.9	8.6	9.4	8.7	10.0	0.59	0.48	0.83	0.051	0.367	0.619
Change during late pregnancy (mm)[BE]	-4.4	-5.0	-3.9	-2.4	-2.5	-2.9	0.34	0.28	0.48	<0.001	0.757	0.079
Early lactation (19 Nov15)[CE]	3.1[b]	2.5[b]	2.9[b]	3.4[b]	3.4[b]	5.3[a]	0.42	0.34	0.60	0.016	0.299	0.207
Change during lactation (mm)[DE]	-2.1[ab]	-1.3[ab]	-1.0[a]	-1.9[ab]	-2.3[bc]	-3.6[c]	0.33	0.27	0.47	0.004	0.255	0.106
Heifer rib fat (mm)												
Mid-pregnancy (23 Jun 2015)[A]	7.7	6.4	4.7	5.0	7.2	6.6	0.54	0.44	0.77	0.013	0.400	0.610
Change during late pregnancy (mm)[BF]	-3.4[b]	-3.2[b]	-2.1[ab]	-1.1[a]	-3.1[b]	-1.5[a]	0.34	0.28	0.48	0.043	0.084	0.305
Early lactation (19 Nov15)[C]	2.7	1.7	1.9	2.5	3.1	3.6	0.39	0.32	0.55	0.077	0.909	0.306
Change during lactation (mm)[DE]	-1.7[b]	-0.6[a]	-0.5[a]	-1.4[ab]	-1.6[ab]	-1.8[b]	0.26	0.21	0.37	0.083	0.971	0.038
Heifer EMA (mm^2)												
Mid-pregnancy (23 Jun 2015)[A]	60.6	60.4	63.0	60.0	60.0	60.6	1.73	1.42	2.45	0.874	0.663	0.750
Change during late pregnancy (mm)[B]	-11.2	-4.6	-15.4	-6.4	-5.0	-3.7	1.68	1.37	2.37	0.034	0.007	0.268
Early lactation (19 Nov15)[C]	37.4	42.4	38.6	41.9	41.9	47.0	1.91	1.56	2.70	0.190	0.053	0.930
Change during lactation (mm)[D]	-0.9	-1.9	-0.4	-3.1	-4.1	-6.1	1.43	1.17	2.02	0.120	0.207	0.822

E, effect of ME intake; P, effect of phosphorus.

A, before commencement of the diet treatments during pregnancy; B, from 23 June to 7 October 2015 shortly before any heifers calved; C, 19 November 2015 shortly after the last heifer calved; D, from 19 November 2015 to 11 February 2016 near the end of Period 2 (Lactation) experimental interval.

E, covariate adjustment for initial LW (P<0.05). F, covariate adjustment for date of birth (P<0.05).

Table D-4. Pens_D. Pregnancy. The requirements, intake and excretion in faeces of P during two total collection intervals (TC1 and TC2, 9 weeks and 3 weeks, respectively, before expected parturition)) during Period 1 when heifers were fed restricted amounts of diets based on wheat straw and molasses-urea. Heifers were fed diets with high (High), medium (Med) and low (Low) energy intakes (EI) combined with H or L phosphorus (P) during the last 3 months of pregnancy. Since diet P deficiency may reduce voluntary DM intake only the requirements for the HP diets were calculated

Measurement	Diet treatments						s.e.m.			Probability		
	LowE-	LowE-	MedE-	MedE-	HighE-	HighE-	E	P	ExP	E	P	ExP
n	6	7	7	7	6	7	-	-	-	-	-	-
P requirement (g/day) -14 to -9	-	2.92	-	6.13	-	8.78	-	-	-	-	-	-
-8 to -5	-	3.89	-	7.21	-	10.21	-	-	-	-	-	-
-4 to -1	-	5.44	-	8.75	-	11.83	-	-	-	-	-	-
mean	-	3.92	-	7.19	-	10.06	-	-	-	-	-	-
P intake (g P/day) TC1	3.27	17.53	4.04	18.27	5.05	18.58	0.27	0.22	0.38	0.003	<0.001	0.556
P intake (g P/day) TC2	2.71	17.28	3.68	18.84	4.21	19.83	0.31	0.25	0.43	<0.001	<0.001	0.486
P intake (g P/day) mean	2.99^{d}	17.40^{b}	3.86^{cd}	18.56^{a}	4.63^{c}	19.21^{a}	0.24	0.20	0.34	<0.001	<0.001	0.917
P faeces (g P/day) TC1	3.59	9.16	4.08	9.74	4.77	9.70	0.39	0.32	0.55	0.299	<0.001	0.774
P faeces (g P/day) TC2	3.44	10.86	5.04	11.47	4.73	12.8	0.66	0.54	0.93	0.220	<0.001	0.671
P faeces (g P/day) mean	3.51	10.01	4.56	10.61	4.75	11.3	0.43	0.35	0.61	0.129	<0.001	0.909
P (I-F) (g P/day) TC1	-0.31	8.37	-0.04	8.53	0.28	8.88	0.38	0.31	0.54	0.600	<0.001	0.993
P (I-F) (g P/day) TC2	-0.72	6.42	-1.35	7.37	-0.51	7.00	0.66	0.54	0.93	0.912	<0.001	0.678
P (I-F) (g P/day) mean	-0.52	7.40	-0.69	7.95	-0.12	7.94	0.42	0.35	0.60	0.730	<0.001	0.812
P intake (mg P/kg LW.day) mean	6.8^{c}	42.2^{a}	9.1^{b}	42.7^{a}	10.2^{b}	43.3^{a}	0.55	0.45	0.77	0.024	<0.001	0.294
P faeces (mg P/kg LW.day) mean	8.8	24.2	10.8	24.5	10.4	25.4	1.10	0.90	1.55	0.618	<0.001	0.832
P (I-F) (mg P/kg LW.day) mean	-2.0^{b}	16.9^{a}	-2.5^{b}	17.5^{a}	-0.5^{b}	16.7^{a}	1.18	0.97	1.67	0.903	<0.001	0.712

TC1, on average 9 weeks before calving; TC2, on average 3 weeks before calving.

Table D-5. Pens_D. Pregnancy. The calculated requirements, intake and excretion in faeces of Ca during two total collection intervals (TC1 and TC2 9, weeks and 3 weeks, respectively, before expected parturition) during Period 1 when heifers were fed restricted amounts of diets based on wheat straw and molasses-urea. Heifers were fed diets with high (High), medium (Med) and low (Low) energy intakes (EI) combined with H or L phosphorus (P) during the last 3 months of pregnancy

Measurement	Diet treatments						s.e.m.			Probability		
	LowE-	LowE-	MedE-	MedE-	HighE-	HighE-	E	P	ExP	E	P	ExP
n	6	7	7	7	6	7	-	-	-	-	-	-
Ca requirement (g/day) -14 to -9	-	3.62	-	8.96	-	13.20	-	-	-	-	-	-
-8 to -5	-	5.35	-	10.91	-	15.63	-	-	-	-	-	-
-4 to -1	-	8.15	-	13.73	-	18.59	-	-	-	-	-	-
mean	-	5.41	-	10.88	-	15.43	-	-	-	-	-	-
Ca intake (g Ca/day) TC01	29.35	46.26	36.66	48.37	45.89	59.70	1.49	1.22	2.11	<0.001	<0.001	0.473
Ca intake (g Ca/day) TC02	25.41	41.29	33.12	50.67	39.94	63.68	1.61	1.32	2.28	<0.001	<0.001	0.210
Ca intake (g Ca/day) mean	27.38	43.77	34.89	49.52	42.92	61.69	1.30	1.06	1.84	<0.001	<0.001	0.535
Ca faeces (g Ca/day) TC01	23.15	26.41	26.72	32.70	31.99	34.30	1.57	1.29	2.23	<0.001	<0.001	0.700
Ca faeces (g Ca/day) TC02	19.00	30.79	24.53	38.48	30.68	45.70	1.67	1.37	2.37	<0.001	<0.001	0.789
Ca faeces (g Ca/day) mean	21.08	28.60	25.62	35.59	31.34	40.0	1.42	1.16	2.00	<0.001	<0.001	0.831
Ca (I-F) (g Ca/day) TC01	6.20	19.85	9.94	15.67	13.90	25.37	1.73	1.42	2.45	0.013	<0.001	0.264
Ca (I-F) (g Ca/day) TC02	6.41	10.49	8.59	12.19	9.26	18.00	1.25	1.02	1.77	0.022	<0.001	0.292
Ca (I-F) (g Ca/day) mean	6.31	15.17	9.27	13.93	11.58	21.68	1.23	1.01	1.74	0.004	<0.001	0.278
Ca intake (mg Ca/kg LW.day) mean	64.7	105.9	82.3	113.6	94.8	138.7	2.33	1.90	3.30	<0.001	<0.001	0.149
Ca faeces (mg Ca/kg LW.day) mean	52.9	69.2	60.4	81.2	68.5	89.5	2.50	2.04	3.54	<0.001	<0.001	0.715
Ca (I-F) (mg Ca/kg LW.day) mean	11.9	36.7	21.9	32.4	26.3	49.2	2.80	2.29	3.96	0.005	<0.001	0.156
Ratio Ca/P mean	9.27	2.51	9.02	2.67	9.69	3.22	0.18	0.15	0.26	0.052	<0.001	0.730

TC1, on average 9 weeks before calving; TC2, on average 3 weeks before calving.

Table D-6. Pens_D. Periods 1 and 2. The carryover effects of 6 diet treatments during late pregnancy on first-calf cow LW, BCS and calf growth during lactation when all the cows and weaner steers were held as a group in a small paddock and offered hay *(Bothriochloa insculpta)* and molasses-urea *ad libitum.* In addition the average concentrations of minerals and metabolites in plasma during pregnancy and during lactation are given

Measurement	Previous diets during pregnancy						s.e.m.			Probability		
	LowE-LP	LowE-HP	MedE-LP	MedE-HP	HighE-LP	HighE-HP	E	P	ExP	E	P	ExP
n	6	7	6	6	6	7	--	--	--	--	--	--
Cow LW (D1 after calving)	362	360	386	403	398	425	10.7	8.7	15.1	0.008	0.269	0.635
Cow LW (D9 lactation)	358	351	367	386	373	419						
Cow LW change (D1-D9) (kg)	-6	-9	-19	-17	-25	-6	4.9	4.0	6.9	0.308	0.313	0.286
Cow LW (D84 lactation)	349	360	364	371	356	387	11.3	9.2	16.0	0.536	0.219	0.726
Cow LW change (kg/day) (Regn)	-0.23^{bc}	+0.05^{a}	-0.11^{ab}	-0.22^{bc}	-0.23^{bc}	-0.40^{c}	0.062	0.050	0.087	0.045	0.987	0.030
Cow LW change (kg) (Regn)	-20^{bc}	+4^{a}	-9^{ab}	-19^{bc}	-19^{bc}	-34^{c}	5.2	4.2	7.3	0.045	0.987	0.030
Cow LW change (kg/day) (Diff)[A]	-0.12^{ab}	+0.11^{a}	-0.04^{ab}	-0.19^{bc}	-0.24^{bc}	-0.45^{c}	0.070	0.057	0.099	0.006	0.608	0.068
Cow LW change (kg) (Diff)[A]	-16	+9	-7	-12	-19	-35	5.9	4.8	8.3	0.006	0.608	0.068
Cow BCS (D9 lactation)	2.4	2.5	2.8	3.3	2.9	3.5	--	--	--	--	--	--
Cow BCS (D84 lactation)	2.3	2.2	2.7	3.0	2.6	2.9	0.161	0.131	0.227	0.032	0.485	0.597
Cow BCS change	-0.1	-0.3	-0.1	-0.3	-0.3	-0.6	0.105	0.086	0.149	0.130	0.106	0.993
Calf birth weight (kg)	27.0	25.4	25.8	30.9	28.8	28.2	1.04	0.85	1.47	0.240	0.436	0.064
Calf LW gain (kg/day)(Regn)[B]	0.733^{ab}	0.585^{c}	0.608^{bc}	0.714^{ab}	0.633^{bc}	0.793^{a}	0.0325	0.0266	0.0460	0.695	0.891	0.004
Calf D 88 (kg)	96	82	83	91	94	96	--	--	--	--	--	--
Plasma mean minerals and markers												
PIP Pregnancy (mmol/L)	0.98	2.15	1.03	2.08	0.83	2.20	0.070	0.057	0.093	0.866	<0.001	0.268
PIP Lactation (mmol/L)	0.74	1.09	0.94	0.97	0.68	0.90	0.054	0.044	0.076	0.090	0.003	0.129
Ca Pregnancy (mmol/L)	2.37	2.15	2.41	2.15	2.43	2.21	0.023	0.019	0.033	0.209	<0.001	0.783
Ca Lactation (mmol/L)	2.54	2.41	2.53	2.41	2.56	2.38	0.024	0.020	0.035	0.979	<0.001	0.639
Ca/PIP ratio Pregnancy	2.77	1.07	2.46	1.04	3.58	1.03	0.257	0.210	0.292	0.314	<0.001	0.294
Ca/PIP ratio Lactation	4.01	2.34	2.96	2.56	4.56	2.85	0.334	0.273	0.370	0.153	0.003	0.303
Mg Pregnancy (mmol/L)	0.92	0.86	0.94	0.83	0.95	0.82	0.015	0.012	0.023	0.935	<0.001	0.238
Mg Lactation (mmol/L)	0.95	0.96	0.96	0.91	1.00	0.96	0.015	0.012	0.023	0.145	0.113	0.391
CTX-1 Pregnancy (ngl/mL)	3.66	1.87	3.04	1.41	3.62	1.21	0.226	0.184	0.319	0.246	<0.001	0.448
CTX-1 Lactation (ng/mL)	3.97	3.97	3.82	3.02	4.07	3.72	0.332	0.271	0.469	0.458	0.330	0.701
OCN Pregnancy (ngl/mL)	51.8	88.8	68.5	70.5	42.2	53.3	5.17	4.22	7.31	0.005	0.006	0.045
OCN Lactation (ng/mL)	55.2	62.8	54.1	51.7	48.4	50.8	4.58	3.74	6.48	0.352	0.639	0.741
BAP Pregnancy (Ul/L)	31.8	18.8	36.3	25.7	44.1	26.4	2.29	1.87	3.23	0.016	<0.001	0.535
BAP Lactation (U/L)	21.9	20.5	21.1	22.4	23.1	19.9	1.84	1.51	2.61	0.983	0.611	0.685

A, covariate adjustment for date of birth of calf (P<0.10), covariate adjustment for date of birth of calf (P<0.024).

Table D-7. Pens_D. Periods 1 and 2. Measurements in rib bone of the cortical bone thickness (CBT) and P concentration (mg P/cc) and the PSACB index (mg P/mm^2) in cortical rib bone, during mid-pregnancy and shortly after calving in the 12th rib, and shortly after calving and at ca. Day 84 of lactation in the 11th rib, in heifers fed one of 6 diets during pregnancy and a common diet during lactation. Thus differences at Day 84 of lactation were carryover effects of the diets fed during pregnancy

Measurement	Diet treatments during pregnancy						s.e.m.			Probability		
	LowE-LP	LowE-HP	MedE-LP	MedE-HP	HighE-LP	HighE-HP	E	P	ExP	E	P	ExP
n												
Changes during pregnancy (12th rib)												
Mid-preg CBT (mm)	3.50	3.59	3.40	3.10	3.26	3.27	0.123	0.101	0.172	0.177	0.655	0.503
P conc (mg/cc)	133.0	139.0	122.8	135.6	133.1	138.3	3.58	2.92	5.06	0.329	0.062	0.716
PSACB (mg/mm^2)	0.465	0.500	0.418	0.417	0.444	0.431	0.0217	0.0177	0.0306	0.111	0.763	0.745
Calving CBT (mm)	3.19	4.52	3.82	4.24	3.87	4.17	0.174	0.142	0.246	0.709	0.002	0.090
P conc (mg/cc)	144.4	148.2	146.0	150.7	139.2	148.9	2.96	2.42	4.18	0.590	0.084	0.750
PSACB (mg/mm^2)	0.459	0.672	0.559	0.640	0.540	0.623	0.0302	0.0246	0.0426	0.729	0.001	0.228
Change (Preg) CBT (mm)	-0.368	+0.890	+0.445	+1.136	+0.525	+0.889	0.141	0.115	0.200	0.028	<0.001	0.095
P conc (mg/cc)	+11.7	+9.1	+25.4	+14.5	+6.9	+10.3	4.41	3.60	6.24	0.171	0.512	0.523
P/SACB (mg/mm^2)	-0.012	+0.167	+0.150	+0.218	+0.095	+0.150	0.0243	0.0198	0.0343	0.016	0.001	0.161
Changes during early lactation (11th rib)												
Calving CBT (mm)	3.08	3.86	3.50	3.52	3.20	3.67	0.125	0.102	0.177	0.900	0.006	0.119
P conc (mg/cc)	146.0	154.8	142.9	156.7	146.3	155.4	3.31	2.70	4.68	0.979	0.010	0.837
PSACB (mg/mm^2)	0.452	0.598	0.502	0.548	0.485	0.567	0.0211	0.0172	0.0298	0.999	<0.001	0.250
Lact, D84 CBT (mm)	2.91	3.48	3.09	2.77	2.90	3.12	0.131	0.107	0.185	0.358	0.312	0.070
P conc (mg/cc)	142.5	158.1	148.7	159.5	148.9	153.2	2.57	2.10	3.64	0.557	0.002	0.309
PSACB (mg/mm^2)	0.414	0.551	0.460	0.443	0.434	0.477	0.0223	0.0182	0.0315	0.572	0.043	0.063
Change (Lact) CBT (mm)	-0.172	-0.380	-0.405	-0.747	-0.028	-0.550	0.1133	0.0925	0.1603	0.126	0.011	0.621
P conc (mg/cc)	-3.1	+3.3	+5.6	+2.7	+4.6	-2.3	2.79	2.28	3.94	0.565	0.726	0.245
PSACB (mg/mm^2)	-0.037	-0.047	-0.042	-0.106	+0.010	-0.091	0.0196	0.0160	0.0278	0.408	0.016	0.267
Difference (12th rib – 11th rib)												
CBT (mm)	+0.10	+0.65	+0.33	+0.72	+0.67	+0.52	0.160	0.131	0.227	0.624	0.162	0.271
P conc (mg/cc)	-1.6	-6.6	+3.1	-6.0	-8.4	-6.4	3.15	2.57	4.46	0.417	0.280	0.469
PSACB (mg/mm^2)	+0.008	+0.074	+0.057	+0.078	+0.076	+0.056	0.0277	0.0226	0.0391	0.683	0.363	0.604

CBT, cortical bone thickness; P conc, concentration of P in cortical bone; PSACB, phosphorus per unit surface area of cortical bone.

During the total collection intervals P intake ranged from 2.7 - 5.1 g P/day (7.1 - 10.2 mg P/kg T-LW) in the three LowP diets and 17.3 - 19.8 g P/day (42.2 - 43.3 mg P/kg T-LW/day) in the three HighP diets (Table D-4). Since the P requirements increased markedly during the 14 weeks as the heifers approached parturition with little change within diets in the LP diets the magnitude of the P deficiency ranged from approximately providing the requirement in the LowE-LP diet in TC1, to being only ca. 36% of the requirement in the HighE-LP diet in TC2. Thus the LP diets were P deficient during the last 8 weeks of pregnancy and the HP diets provided much more P than the requirements. The differences between P intake and P excretion in faeces (I-F) were consistently small negative (-0.7 to -0.1 g P/day) in the three LowP diets and positive (7.4 – 8.0 g P/day) in the three HighP diets. All the diets provided Ca greatly in excess (*ca.* 2-4 times) the expected requirements (Table D-5). Ca intakes ranged from 29.4 – 45.9 g Ca/day in the three LowP diets and 41.3 – 63.7 g Ca/day in the three HighP diets. The difference between Ca intake and faecal excretion (I-F) was always positive ranging from 6.2 - 13.9 g Ca/day in the LowP diets and 10.5 – 25.4 g Ca/day in the HighP diets. Diet Ca/P ratios were 9.0-9.7 in the LowP diets and 2.5-3.2 in the HighP diets.

Liveweight and LW change of heifers and calves, and group intake through lactation (Period 2)
During lactation *ca.* 10 kg per animal (heifers and steers) was offered per day and most of this was consumed. The intake of the molasses-urea averaged 3.12 (sd 1.22) kg as-fed/day (7.3 g DM/kg LW.day) across all the animals in the herd and tended to decrease for several days following rain events (total 106 mm in January 2015). This corresponded to intakes of 2.7 kg and 1.7 kg molasses-urea DM in the heifers and the steers, respectively. The total intake of the lactating heifers calculated using QuikIntake was 10.7 kg DM/day (8.0 kg hay DM and 2.7 kg molasses-urea supplement), while the total intake of the steers was calculated to be 8.7 kg DM/day (7.0 kg hay DM and 1.7 kg molasses-urea DM). Since the hay and the molasses-urea both contained 1.0 g P/kg DM (Table D-1) this was the concentration of P in the diet ingested. The average intake of P by the heifers during the lactation phase was calculated to be 10.7 g P/day and 56% of the calculated P requirements of 19.3 g P/day (equivalent to 1.80 g P/kg DM of the diet fed). The average intake of the steers was calculated to be 8.7 g P/day and 69% of the calculated P requirements of 12.6 g P/day (equivalent to 1.45 g P/kg DM of the diet fed). The Ca and ME contents of the hay and molasses-urea components of the diet during lactation differed and there was presumably variation among animals within the group in the proportions of hay and supplement consumed. The hay and molasses-urea supplement were estimated to contain 5.7 and 10.6 MJ ME/kg DM, respectively.

From Day 1 to Day 9 after parturition the heifers lost (mean ± sd) 13 ± 19 kg LW due presumably to the change of diet, changes in gut fill and hydration. Calculated by regression of LW with time the cows on average lost 0.19 kg LW/day during the following 13 weeks (calculated on the interval Day 9 to Day 100 of lactation) and this was affected (P<0.05) by the diet treatments previously fed during pregnancy (Table D-6). Among heifers previously fed the MedE and HighE diets the LW change averaged -0.12 and -0.35 kg/day, respectively, and the LW loss was greater in the animals previously fed the HighE diets. The LW change in the heifers previously fed the LowE-LowP diet was -0.12 kg/day which was not different (P>0.05) to the small LW gain (+0.11 kg/day) in the heifers previously fed the LowE-HighP diet. These LW changes were associated with an average decrease in BCS (-0.3 BCS units) over the 13 weeks. This BCS change was not affected (P>0.10) by the previous diet treatment during pregnancy. The ultrasound measurements indicate that all of the heifers continued to lose fat cover at the rump and rib sites, and EMA, during lactation (Table D-3). The change in heifer LW during lactation was correlated (P<0.05) with the LW at Day 9 of lactation (Figure D-4) with each additional kg of LW on Day 9 of lactation associated with an increased loss of 0.43 kg during the measured interval. Calf birth weight (mean 27.8 kg) was not affected by the diet treatment during pregnancy. Calf LW gain calculated by regression averaged 0.68 kg/day and corresponded to an average calf LW of 90 kg after 12 weeks. The calf growth rate was affected (P<0.05) by diet treatment during pregnancy; in cows fed ModE or HighE during pregnancy the calf growth was higher (P<0.05) in the cows fed the HP rather than the LP diet. However in the cows fed LowE during pregnancy the reverse occurred with calves of LowE-LP heifers growing more rapidly than the calves of LoE-HP heifers (0.733 and 0.585 kg/day, respectively). Faecal P concentration was (mean ± sd) 2.9 ± 0.63 g P/kg DM during DOY 288 – 386.

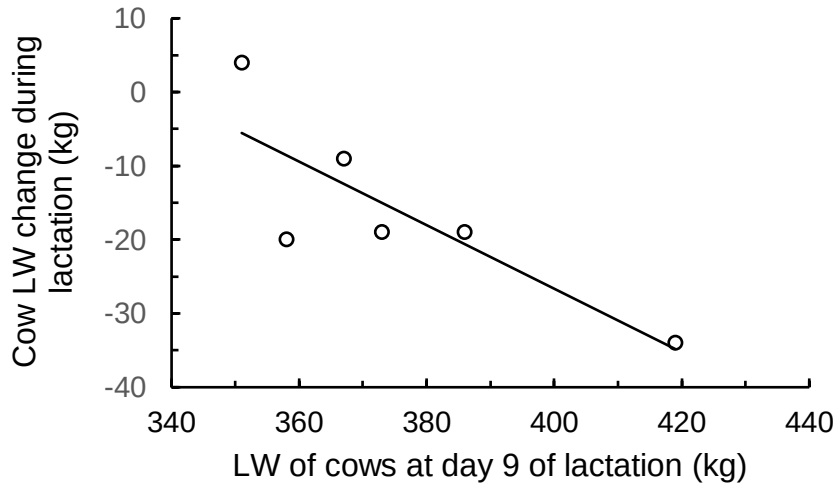


Figure D-4. Pens_D. Correlation of the change in cow LW during lactation (Period 2: Day 9 to Day 84) with the LW at Day 9 of lactation. The means of the six diet treatments imposed during pregnancy are shown. The regression relationship was as follows: Y = 0.43 X + 146 (r=0.83, P<0.05).

Changes in rib bone and tuber coxae bone in cows sampled by biopsy

The mean CBT, P concentration of external cortical bone (Pconc) and PSACB at mid-pregnancy in the heifers were 3.35 mm, 133.6 mg P/cc and 0.445 mg P/mm^2 (Table D-7). Both CBT and PSACB increased during late pregnancy and to calving, but at calving both CBT and PSACB were increased by both HP diets and by increasing amounts of ME fed (P<0.001 and P<0.01, respectively). On average the LowP diet heifers had 16% lower CBT (3.63 mm) and 20% lower PSACB (0.645 mg P/mm^2). These were also increased (P<0.05) by higher ME intakes during pregnancy, but the 'diet energy x diet P interaction' was not significant (P>0.05). There was no effect (P>0.05) of late pregnancy or of diet treatments on P concentration of cortical bone. There was a carryover effect of diet P content fed during pregnancy on the change in CBT and PSACB during lactation (P<0.05); heifers fed HighP diets during pregnancy increased (P<0.05) the loss of CBT and PSACB during the 14 weeks lactation, the latter from 0.490 to 0.436 (11%). Across all treatments the PSACB decreased on average by 0.052 mg/mm^2; also the decrease was greater (P<0.05) in the heifers previously fed the HighP diets than in those fed the LowP diets (-0.081 and -0.023, respectively). The mean trabecular bone volume (BV/TV) and trabecular thickness (Tb.Th) at mid pregnancy in the heifers were 13.1% and 118 µm (Table D-8). At calving BV/TV had been reduced by 29%, and Tb. Th had been decreased (P<0.001) by 21%, by feeding the LowP diets during late pregnancy. However there was no effect of diet ME intake on trabecular bone changes during late pregnancy.

Table D-8. Pens_D. Period 1. Measurements of bone volume (BV/TV) and trabecular thickness (Tb.Th) of trabecular bone in *tuber coxae* biopsies during mid-pregnancy and shortly after calving in heifers fed one of six diets during pregnancy

Measurement	Diet treatments during pregnancy						s.e.m.			Probability		
	LowE-LP	LowE-HP	MedE-LP	MedE-HP	HighE-LP	HighE-HP	E	P	ExP	E	P	ExP
n	6	7	6	7	7	7						
Mid-pregnancy												
BV/TV (%)	13.35	11.57	12.4	13.54	13.29	12.57	0.42	0.34	0.59	0.24	0.97	0.043
Tb.Th (µm)	118.5	115.2	118.8	117.0	117.3	122.9	0.27	0.22	0.38	0.68	0.96	0.45
Calving												
BV/TV (%)	9.99 [b]	12.57 [a]	9.68 [b]	13.39 [a]	8.66 [b]	13.51 [a]	0.54	0.44	0.76	0.84	<0.001	0.34
Tb.Th (µm)	98.2 [b]	121.6 [a]	95.6 [b]	121.9 [a]	91.8 [b]	120.8 [a]	0.78	0.64	1.11	0.64	<0.001	0.76

Plasma P, Ca and Mg

During pregnancy, PIP concentrations were related to diet P treatments (Figure D-5, Table D-5) being higher (P<0.01) on the HighP diets (means 2.17, 2.07 and 2.12 mmol/L) than on LowP diets (means 0.88, 0.97 and 0.82 mmol/L). The PIP concentrations indicated that heifers given each of the LowP diets were severely P-deficient. Also PIP concentrations were decreasing in all heifers as pregnancy progressed (P<0.05), although this effect was larger and more obvious in heifers fed the HighP diets (Figure D-5A). During lactation the PIP concentrations in heifers previously given the HighP diets rapidly declined with the change to LowP diets shortly after calving. Mean PIP concentrations in these heifers previously fed HP diets in pregnancy remained significantly (P<0.05) higher during lactation than in heifers fed LowP diets during both pregnancy and lactation (Figure D-5, Table D-5). There was no significant effect of diet ME on PIP concentrations during pregnancy, nor any carry-over effect into lactation (P>0.05).

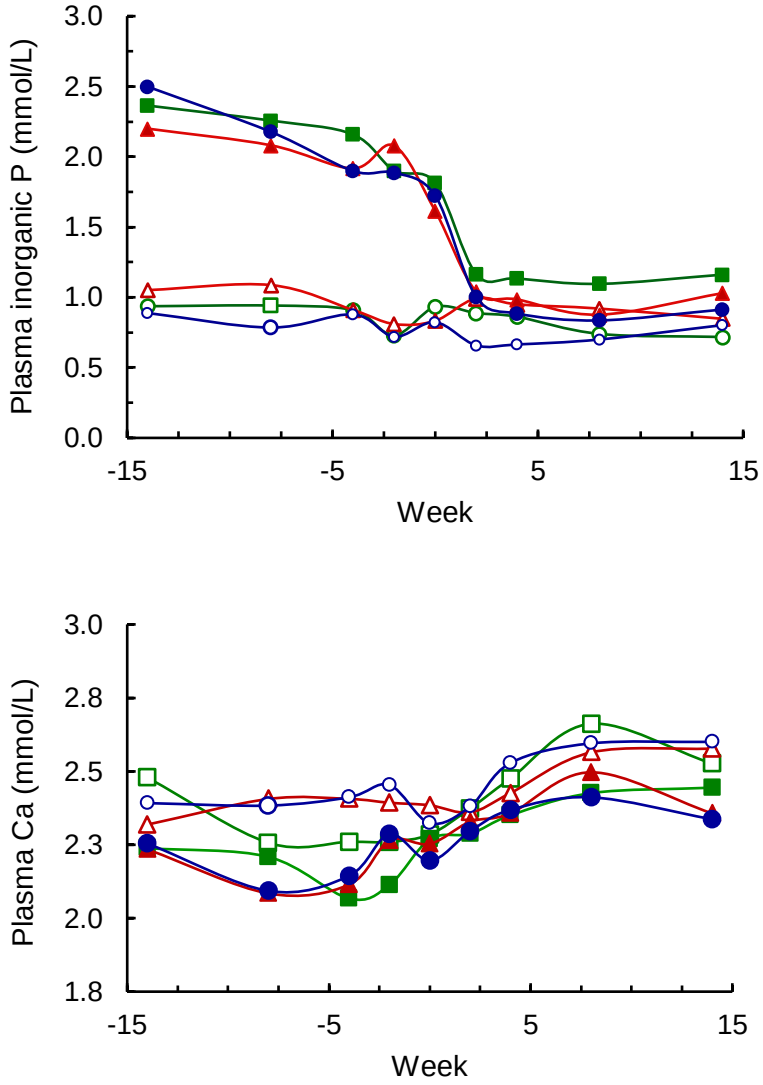


Figure D-5A and 5B. Pens_D. Concentrations of plasma inorganic phosphorus (PIP) (mmol/L) (Fig. 5A) and plasma total Ca (mmol/L) (Fig. 5B) in heifers during the last 14 weeks of pregnancy (Period 1) and the first 14 weeks of lactation (Period 2) relative to calving. The diet treatments were LowE-LP (□), LowE-HP (■), MedE-LP (Δ), MedE-HP (▲), HighE-LP (○) and HighE-HP (●).

Plasma total Ca exhibited opposite diet responses to PIP. During pregnancy most animals exhibited total Ca concentrations within the normal homeostatic range. There was a significant (P<0.05) main effect of diet with increased total Ca concentrations in LowP diets compared to HighP diets (Table D-5, Figure D-5B). In lactation plasma total Ca concentrations increased in each of the diets and was higher(P<0.001) in heifers previously given LowP diets during pregnancy (Figure D-5B). Plasma Mg concentrations exhibited similar diet and time effects to plasma total Ca, being higher in pregnancy in heifers given LowP diets than HighP diets and increasing further in lactation in all heifers (Table D-5). There was no significant effect of diet ME on plasma Ca or Mg concentrations during pregnancy, nor any carry-over effect in lactation. The plasma Ca/P ratio was high (>2.0) during pregnancy in heifers on LowP diets, and low (*ca* 1.0) in heifers on HighP diets (Figure D-6). There was an increase in the Ca/P ratio in very late pregnancy (2 and 4 weeks prior to calving) in heifers on HighP diets, whereas in heifers on LowP diets in general the Ca/P ratio remained did not change but remained high (Figure D-6). In lactation (Period 2), when LowP diets were provided to all heifers, the Ca/P ratio in heifers fed the LowP diets in pregnancy (Period 1) continued to be significantly higher than in heifers fed HighP diets in Period 1. The differences between the groups was markedly reduced and the Ca/P ratio was high in association wiith the LowP diets fed to all heifers post-calving.

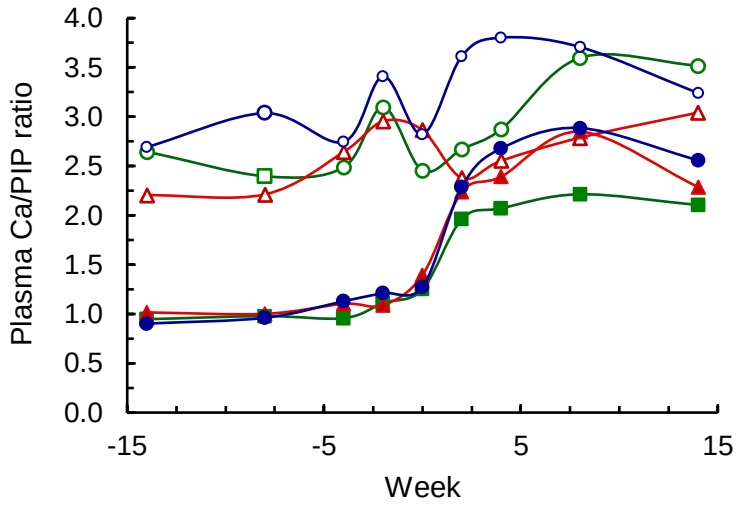


Figure D-6. Pens_D. The plasma Ca/P ratio of the concentrations of plasma total Ca and plasma inorganic phosphorus (PIP) (mmol/L) in heifers during the last 14 weeks of pregnancy (Period 1) and the first 14 weeks of lactation (Period 2) relative to calving. The diet treatments were LowE-LP (□), LowE-HP (■), MedE-LP (Δ), MedE-HP (▲), HighE-LP (○) and HighE-HP (●).

Bone markers

During pregnancy plasma CTX-1 concentrations were much higher (P<0.01) in heifers fed LowP diets than in heifers fed HighP diets (Table D-6, Figure D-7). Towards calving plasma CTX-1 concentrations steadily increased in heifers on HighP diets and at calving but were still lower (P<0.01) than the LowP diets (Figure D-7). Around calving (from -2 to +2 weeks) there were marked fluctuations in plasma CTX-1 concentrations within LowP diets, although mean CTX-1 concentrations remained quite high in all LowP diet groups (>3.0 ng/ml). After calving, plasma CTX-1 concentrations were high in all diet groups and remained high throughout lactation (Figure D-7). No carry-over effects of diet in pregnancy were observed in lactation as there were no effects (P>0.05) of either diet P or diet E during pregnancy on plasma CTX-1 concentrations during lactation (Table D-6).

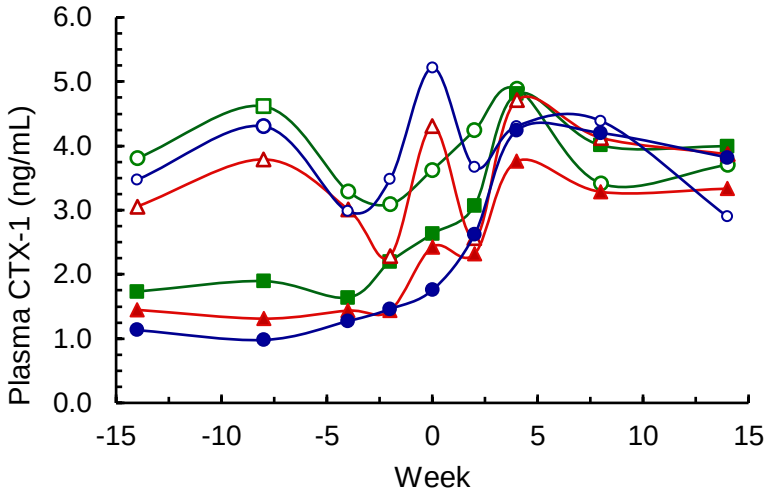


Figure D-7. Pens_D. The concentrations of CTX-1 (ng/mL) in plasma of heifers during the last 14 weeks of pregnancy (Period 1) and the first 14 weeks of lactation (Period 2) relative to calving. The diet treatments were LowE-LP (□), LowE-HP (■), MedE-LP (Δ), MedE-HP (▲), HighE-LP (○) and HighE-HP (●).

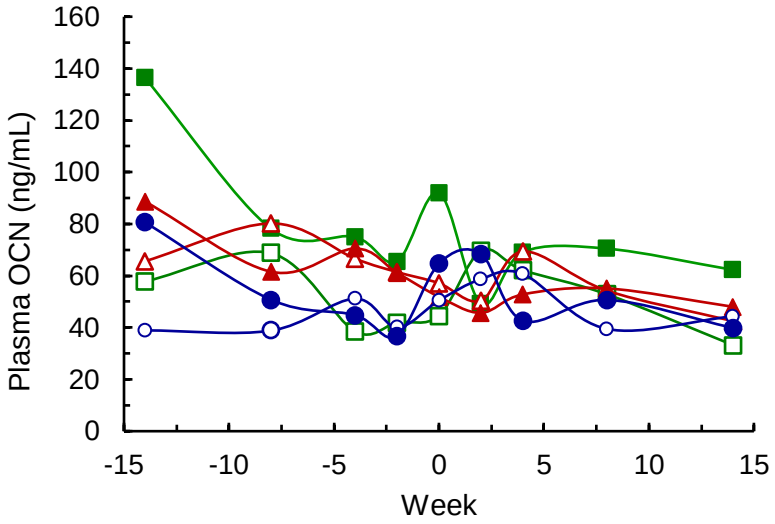


Figure D-8. Pens_D. The concentrations of OCN (ng/mL) in plasma of heifers during the last 14 weeks of pregnancy (Period 1) and the first 14 weeks of lactation (Period 2) relative to calving. The diet treatments were LowE-LP (□), LowE-HP (■), MedE-LP (Δ), MedE-HP (▲), HighE-LP (○) and HighE-HP (●).

Plasma OCN concentrations differed widely among diet groups at the start of the experiment (Figure D-8, Table D-5). Over time with pregnancy and into lactation the variability between diets reduced. There were differences among diets in OCN during pregnancy with both main effects of both diet P and ME and an interaction between diet P and ME (Table D-5). There were no significant diet effects during lactation. Plasma BAP concentrations during pregnancy declined as pregnancy progressed and there were also differences due to diet P and diet ME (Figure D-9; Table D-5); BAP concentrations were higher ($P<0.01$) in cows fed LowP diets than HighP diets and also increased with increasing diet ME (Table D-5). In early lactation plasma BAP concentrations were low across all treatment groups. There were no effects of the diet fed during pregnancy on plasma BAP concentrations during lactation (Table D-5).

Resumption of ovarian activity in the lactating cows

The proportion of cows across all treatments which were cycling (as indicated by presence of corpus luteum) increased from 26, 38, 60 and 60% at *ca.* 3, 4, 5 and 6 months, respectively, post-partum (Table D-9).

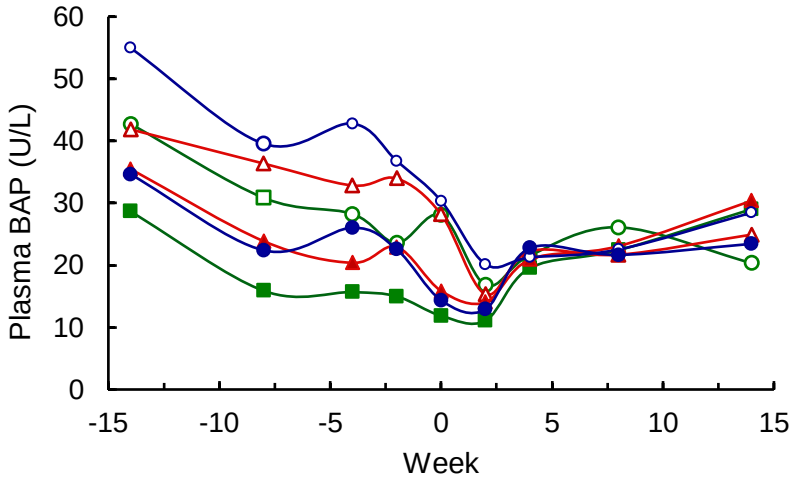


Figure D-9. Pens_D. The ratio of the concentrations of BAP (U/L) in plasma of heifers during the last 14 weeks of pregnancy (Period 1) and the first 14 weeks of lactation (Period 2) relative to calving. The diet treatments were LowE-LP (□), LowE-HP (■), MedE-LP (Δ), MedE-HP (▲), HighE-LP (○) and HighE-HP (●).

Table D-9. Pens_D. Lactation. The resumption of ovarian activity in lactating cows measured by ultrasound scanning. The mean calving date was the 26 October 2015 and ultrasound measurements were made monthly. Follicles were classed as small if ≤6 mm, medium if 6-10 mm, large if > 10 mm.

Measurement	Diet treatments during pregnancy						s.e.m			Probability		
	LowE-LP	LowE-HP	MedE-LP	MedE-HP	HighE-LP	HighE-HP	E	P	ExP	E	P	ExP
14 Dec 15 With small follicles	0.17	0.00	0.50	0.50	0.33	0.00	0.107	0.088	0.152	0.024	0.188	0.554
(~2 mo lact) With medium follicles	0.77	0.71	0.16	0.27	0.33	0.43	0.111	0.091	0.157	0.008	0.686	0.850
With large follicles	0.00	0.00	0.17	0.00	0.00	0.000.00	0.043	0.035	0.610	0.302	0.273	0.302
With CL/CA	0.00	0.29	0.19	0.13	0.19	0.14	0.084	0.069	0.119	0.940	0.452	0.224
Reprod score	2.5	3.6	3.0	2.5	2.7	2.4	0.30	0.24	0.42	0.541	0.810	0.150
11 Jan 16 With small follicles	0.04	0.29	0.29	0.37	0.29	0.29	0.117	0.095	0.165	0.567	0.436	0.730
(~3 mo lact) With medium follicles	0.67	0.43	0.17	0.50	0.50	0.43	0.139	0.113	0.196	0.551	0.961	0.337
With large follicles	0.00	0.00	0.00	0.00	0.00	0.00	-	-	-	-	-	-
With CL/CA	0.28	0.29	0.50	0.12	0.17	0.29	0.120	0.098	0.170	0.886	0.530	0.313
Reprod score	3.5	3.3	3.7	2.9	3.0	3.3	0.294	0.240	0.416	0.844	0.413	0.434
11 Feb 16 With small follicles	0.00	0.14	0.33	0.33	0.17	0.43	0.116	0.095	0.165	0.241	0.323	0.730
(~4 mo lact) With medium follicles	0.33	0.43	0.00	0.33	0.33	0.14	0.121	0.099	0.171	0.453	0.574	0.323
With large follicles	0.17	0.00	0.17	0.17	0.00	0.00	0.075	0.661	0.106	0.302	0.524	0.664
With CL/CA	0.42	0.43	0.53	0.09	0.53	0.43	0.134	0.110	0.190	0.660	0.266	0.480
Reprod score	4.0	3.7	3.9	3.0	3.9	3.4	0.34	0.28	0.48	0.706	0.168	0.850
10 Mar 16 With small follicles	0.23	0.14	0.19	0.06	0.19	0.00	0.080	0.065	0.113	0.723	0.157	0.897
(~5 mo lact) With medium follicles	0.13	0.29	0.34	0.29	0.17	0.14	0.107	0.087	0.151	0.567	0.825	0.758
With large follicles	0.00	0.00	0.00	0.00	0.00	0.00	-	-	-	-	-	-
With CL/CA	0.64	0.57	0.47	0.64	0.64	0.86	0.028	0.105	0.181	0.562	0.475	0.688
Reprod score	4.1	4.0	3.8	4.2	4.1	4.7	0.308	0.251	0.435	0.590	0.335	0.710
18 Apr 16 With small follicles	0.00	0.00	0.00	0.17	0.07	0.14	0.075	0.061	0.106	0.357	0.587	0.624
(~6 mo lact) With medium follicles	0.36	0.43	0.54	0.20	0.20	0.14	0.118	0.096	0.167	0.365	0.421	0.469
With large follicles	0.00	0.00	0.00	0.00	0.00	0.00	-	-	-	-	-	-
With CL/CA	0.65	0.57	0.43	0.65	0.60	0.71	0.128	0.105	0.181	0.819	0.576	0.704
Reprod score	4.3	4.1	4.0	4.1	4.1	4.7	0.287	0.234	0.406	0.655	0.532	0.654
PPAI	148	137	141	134	123	120	14.1	11.5	19.9	0.550	0.647	0.979
Pregnant	0.67	0.57	0.44	0.67	0.44	0.57	0.138	0.113	0.196	0.845	0.585	0.704

Table D-10. Pens_D. Measurements in sentinel steers included with the lactating cows during Period 2. For four months before steers entered the present Pens_D experiment they had been ingesting low quality forage (senesced pasture or wheat straw) and molasses-urea supplements containing no additional P (LowP) or additional P (HighP) as described in the Pens_E experiment

Measurement	Previous diet		s.e.m.	Prob
	LowP	HighP		
n	5	5		
Liveweight and body condition score				
LW initial (26 Nov 2015) (kg)	218	213	8.4	0.690
LW final (4 Feb 16) (kg)	252	249	6.9	0.751
LW change (kg/d)	0.50	0.53	0.042	0.682
BCS (26 Nov 2015) initial	3.7	3.6	0.23	0.724
BCS final (4 Feb 16)	3.9	3.7	0.18	0.456
BCS change	0.2	0.1	0.15	0.719
Plasma mineral concentrations				
PIP Period 1 (mmol/L)(Previous to Pens_D	2.14	2.42	0.066	0.018
PIP Period 2 (mmol/L)(During present study)	1.78	1.89	0.082	0.398
Ca Period 1 (mmol/L))(Previous to Pens_D	2.38-	2.35	0.028	0.473
Ca Period 2 (mmol/L))(During Pens_D)	2.56	2.55	0.030	0.867
Ca/P ratio Period 1 (Previous to Pens_D diets)	1.13	0.99	0.034	0.020
Ca/P ratio Period 2 (During Pens_D)	1.46	1.39	0.075	0.528
Mg Period 1 (mmol/L))(Previous to Pens_D	0.93	0.87	0.016	0.039
Mg Period 2 (mmol/L))(During Pens_D)	0.97	0.95	0.016	0.408
Rib bone				
Initial CBT (mm)	3.80	3.71	0.192	0.750
Initial P concentration (mg/cc)	126.9	124.6	3.13	0.642
Initial PSACB	0.482	0.461	0.026	0.597
Final CBT (mm)	3.56	3.49	0.213	0.834
Final P concentration (mg/cc)	123.9	131.2	1.93	0.040
Final PSACB	0.442	0.458	0.028	0.056
Change CBT (mm)	-0.24	-0.22	0.304	0.956
Change P concentration (mg/cc)	-3.0	+6.6	2.99	0.071
Change PSACB	-0.041	-0.003	0.045	0.597
Fat depth and eye muscle area				
Rump fat (mm) 19 Nov15	1.00	1.20	0.14	0.347
11 Feb 16	1.60	1.60	0.25	1.000
change	0.60	0.40	0.25	0.580
Rib fat (mm) 19 Nov15	1.00	1.00	-	-
11 Feb 16	1.40	1.20	0.22	0.545
change	0.40	0.20	0.22	0.545
EMA (mm^2) 19 Nov15	38.2	40.2	2.07	0.514
11 Feb 16	46.2	44.8	2.37	0.688
change	8.0	4.6	1.02	0.045
Hip Height(cm) 10 Dec 2015	120.0	119.3	1.97	0.755
22 Feb 16	121.2	121.8	1.86	0.841
Change	1.2	2.5	0.63	0.187

Intake, liveweight and LW change and other measurements of the sentinel steers through Period 2
The requirements of the steers were 12.6 g P/day and 19.2 g Ca/day, and for the estimated intake this was equivalent to 1.45 g P/kg DM and 2.20 g Ca/kg DM. The estimated P intake was 8.7 g P/day and was therefore 69% of the expected requirements. At the start of Period 2 LW of the steers previously given a LowP diet for four months were (mean ± sd) 218 ± 22 kg and BCS 3.7 ± 0.4 , while the LW of steers previously given a HighP diet for four months were 213 ± 15 kg and BCS 3.6 ± 0.6(Table D-10). There were no discernible effects (P>0.05) of the previous diet treatments described in the Pens_E report on the LW or BCS of the two groups of steers at the commencement of Period 2. During Period 2 there was no carryover effect (P>0.05) of the previous treatments on the LW or BCS gains (means 0.50 kg/day and 0.2 units), fat cover or EMA during Period 2 interval. The mean PIP concentrations during the four weeks before entering Period 2 were 1.8 and 2.3 mmol/L for the LowP and HighP steers, respectively, and were maintained in the range 1.8-2.0 mmol/L (Table D-10). Before entry to Period 2 of the present experiment and during Pens_E the faecal P concentration was higher (P<0.05) in the steers fed the HighP diet (4.4 ± 1.32 g P/kg DM) than in those fed the LowP diet (3.2 ± 0.76 g P/kg DM). During Period 2 the faecal P concentration was not affected by the previous diet and tended (P>0.10) to decline from 3.3 to 2.6 g P/kg DM.

The CBT, Pconc and PSACB of the steers at the commencement of Period 2 (3.76 mm, 125.8 mg P/cc and 0.472 mg P/mm^2, respectively; Table D-10) were comparable with these respective measurements in the heifers in mid-pregnancy (3.35 mm, 133.6 mg P/cc and 0.445 mg P/mm^2; Table D-6). In the steers there were no effects of previous diet on rib bone CBT, P concentration of cortical bone or PSACB index. During Period 2 of the present experiment both CBT and PSACB tended to decrease by *ca.* 5% during the 10 weeks which was a lesser decrease than occurred in the lactating heifers (Table D-6). The steers were able to accommodate the LowP diet fed during Period 2 with lesser effects on bone P mobilization than the heifers and were able to maintain a moderate LW gain despite the LowP diet. However the steers were estimated to be ingesting 69% of their P requirements while as described above the lactating heifers were estimating to be ingesting only 56% of their P requirements.

4.3.11 Discussion

The calculations of P intake in relation to P requirements (Table D-4) indicated that the intake of P by the HP heifers was 1.8 – 4.4 times that required, while the LP heifers provided between 0.83 and 0.50 of requirements as the ME intake was increased from low to medium to high. This is in part because animals losing LW can obtain P from mobilization of body tissues as a direct consequence of the LW loss. The heifers given the LE-LP diet approached their P requirements whereas this did not occur for the heifers given sufficient ME for approximate LW maintenance only.

The restricted intakes of the straw – molasses based diet fed through late pregnancy caused CF.LW loss during the last 14 weeks of pregnancy. The CF.LW changes ranged from -4 kg in the HE-HP diet, -24 kg in the ME-HP diet to -49 kg in the LE-HP diet (Table D-2). In the heifers fed the LP diets the effect of the P deficiency was to increase the rate of CF.LW loss from -4 to -37 kg in the HE diet. Since this was associated with a large reduction in VI and reduced ME intake (from 159.4 to 121.9 kJ ME/kg CF.LW) the difference in LW loss was most obviously a consequence of the lower VI of the diet. The LP diets also decreased PIP during pregnancy from 2.20 to 0.83 mmol/L (Table D-8) and is consistent with the results reported here (Pens A, B, C and E). However the absence of an effect of

the LP diets on the DM or ME intakes of the Low energy and the Medium energy diets (although PIP reduced from 2.1-2.2 mmol/L to 0.98-1.03 mmol/L) indicates that the heifers could maintain an intake of DM and ME at the lower range despite the decrease in P intake and PIP. The ME intake of the heifers fed the two medium energy diets (122.7 and 119.8 kJ ME/kg CF.LW) was similar to that for the HE-LP diet (121.9 kJ ME/kg CF,LW).

A key finding in the present experiment was that the P balance was increased from about nil (-0.69 - -0.12 g P/day) in the LP diets to 7.4 – 7.95 g P/day with the HP diets (Table D-4). Thus these heifers were able to store in bone some of the diet P in excess of their immediate requirements. Presumably the additional stored bone P would be available during lactation and later in the annual cycle. This indicates that P plus N supplements for these heifers, and probably for older animals, and for pregnant breeders during the dry season are likely to have beneficial effects. Both the CTX-1 concentrations (mean 3.44 mmol/L) and the Ca/P ration (mean 2.94) in blood indicated that the LP heifers were mobilizing substantial bone P during late pregnancy to alleviate the P deficiency. In contrast in the HP heifers these values were 1.57 and 1.50, respectively. OCN and BA, as markers of bone formation, were also affected by both the ME intake and the P intake (Table D-4) and support the concept that bone formation increased with the HP diets.

The general absence of carryover effects of the diet treatments during pregnancy on the FCC performance during lactation were unexpected. The principal effect appeared to be an effect of the level of ME intake with heifers calving in higher body condition losing more liveweight in lactation. During lactation PIP and Ca/P (but not CTX-1) were increased, and the change in PSACB also indicated increase in P mobilization associated with the previous provision of HP during late pregnancy.

This experiment provides evidence that provision of supplements providing P and N to breeders grazing dry season pastures during late pregnancy is likely to have beneficial effects by replenishing previously mobilized bone P reserves and to alleviate the effects of P deficiency when the breeder is lactating during the subsequent wet season.

4.3 Experimental Section 6. Pens_E experiment

Pens_E. Mobilization and deposition of diet phosphorus into body reserves in Bos indicus cross mature cows during late pregnancy and early lactation

4.3.1 Summary of the experiment

An experiment examined P deficiency during late pregnancy and/or early lactation in mature Droughtmaster breeders. During the last 14-18 weeks of pregnancy the breeders were group-fed either P deficient (LowP)(ca. 60-70% of P requirements) or P adequate (HighP) diets. During the first 14 weeks of lactation the cows and their calves were fed P deficient or P adequate diets in individual pens. There were four diet treatments: LowP-LowP, HighP-LowP, LowP-HighP and HighP-HighP. During pregnancy the cows grazed small paddocks and were fed molasses-urea supplements (± P). In addition growing sentinel weaners initially 7-10 months of age grazed with the LowP and HighP treatment groups of cows (n = 4 heifers and n=5 steers per treatment) during their pregnancy phase. The heifers were fed the LowP diet in pens during the lactation phase. Cows were initially (mean ± sd) 465±44 kg CF-LW and 3.6 ± 0.59 BCS. During pregnancy PIP concentrations were lower in LowP than HighP cows. Cows lost on average 29 kg CF-LW during pregnancy and the LW change was not affected by diet treatment. During lactation the PIP concentrations indicated that the cows fed the LowP were severely P deficient; these cows had a lower voluntary intake and lost on average 33 kg LW. Conversely the cows fed the HighP diet during lactation were P adequate and gained 23 kg. The HighP diet during lactation increased calf growth from 0.76 to 0.93 kg/day ($P<0.05$) so that calves of HighP cows were 17 kg heavier after 14 weeks. Diet treatment during pregnancy did not affect ($P>0.05$) cow performance during lactation. During lactation cows fed a severely P deficient diet reduced voluntary intake but maintained milk output and calf growth by mobilization of LW and P. Sentinel heifers continued to gain LW slowly (0.2 kg/day) even when fed the P deficient diets. In the LowP diet plasma Ca/P ratio and concentrations of CTX-1 indicated a small increase in pregnancy, and a substantial increase in lactation, in bone P mobilization. Mobilization of bone was also observed during lactation. These mature breeders were able to maintain milk production during severe P deficiency by mobilizing body P reserves.

4.3.2 Background

The Pens_C experiment indicated that the early lactational performance of mature breeder cows was not markedly reduced by severely P deficient diets despite a decrease in voluntary feed intake. Cows fed P deficient diets accommodated the deficiency by extensive mobilization of body reserves. In the Pens_C experiment the cows had been fed a high P and high ME diet during the last 2-4 months of pregnancy and calved in good body condition and were thus expected to be in high P status. These results contrasted with the results observed in the Pens_A experiment with first-calf cows where heifers fed P deficient diets in late pregnancy and/or in early lactation demonstrated severe LW losses during lactation and reduced milk production. Apparently first-calf cows have a much lesser capacity than mature cows to mobilize body reserves to maintain early lactation when fed a P deficient diet; the likely reasons are that the first-calf cows are still growing both bone and soft tissue.

It was considered essential to obtain more information on the extent of mobilization of body energy and P reserves by P-deficient mature cows in early lactation before general management recommendations can be made to the cattle industry. From other experiments (e.g. at Lansdown and Springmount; Miller *et al.* 1998; Dixon *et al.* 2016; Coates *et al.* 2017) and industry observation it is obvious that mature breeders in P deficiency during early lactation do not always maintain satisfactory calf growth. Even when calf growth is maintained the severe losses of body reserves of the breeders must be addressed within annual breeder management strategies to maintain satisfactory re conception of lactating cows and to avoid excessive mortality. It is clearly necessary to avoid the adverse effects of low body condition and low body P reserves of breeders late in the dry season and the associated risks for breeder mortality and reduced fertility.

Experiment Pens_E was designed to examine the consequences in mature cows of diet P deficiency during the last four months of pregnancy and/or in the first three months of lactation. The experimental design was similar to the Pens_A experiment but with two differences. First, the breeders were mature cows rather than heifers / first-calf cows. Second, although the ideal experimental protocol would have been to hand-fed the cows in individual pens during both pregnancy and lactation the project resources were not available to do this and to also undertake the Pens_D experiment examining the consequences of severe LW loss with P-deficient or P-adequate diets during late pregnancy. During the present Pens_E experiment the P-deficient and P-adequate diets in late pregnancy were achieved by grazing the breeders on senesced native pasture expected to be low in P, and by providing a substantial part of the diet (*ca.* about half the ME requirement) as molasses-urea either low or high in P concentration. The breeders grazed native grass paddocks on hillsides with shallow soils at Brian Pastures Research Facility and where the senesced pasture was expected from previous records (Quirk 1988; Burrows *et al.* 1988) to be low in P concentration.

4.3.3 Material and Methods

Experimental design, animals and diets

Bos indicus x *Bos taurus (ca.* 5/8 x 3/8) crossbred Droughtmaster cows 4.5 - 6.5 years of age were selected from the herd on Spyglass Cattle Research Station (100 km N Charters Towers, Queensland, Australia) in the seasonally dry tropics. This herd had been mated from January - April 2015 and pregnancy was diagnosed by rectal palpation in June 2015. Cows were selected on the criteria of stage of pregnancy, body condition score (BCS; 5-point scale, CSIRO 2007) and with temperament suitable for intensive experiments in pens. These breeders had grazed northern Spear grass native pastures expected to be deficient to moderate in P, and had been mated annually from *ca.* 2 years of age. The selected breeders were relocated to the Brian Pastures Research Facility, Gayndah, Australia) in the sub-tropics. Following recovery from the transport, the herd (n = 54, mean ± sd) was 493 ± 38 kg LW, 3.7 ± 0.4 and 23 ± 1.2 weeks pregnant. In addition sentinel heifers (n = 8) and steers (n=12) of nominally the same genotype were included to compare mature breeder cows with young growing cattle. These cattle from the Pens_C experiment on Brian Pastures Research Facility had been weaned in July 2015, fed a high concentrate diet in yards for a month post-weaning, and were 7-10 months of age and 193 ± 16 kg LW at entry to the experiment on the 20 August 2015 (day of year from 1 January 2015; DOY 232).

In brief, there were four diet treatments in a 2x2 factorial design. These treatments comprised low P (LowP) or high P (HighP) intakes during about the last four months of pregnancy, and/or during the first three months of lactation. During pregnancy the treatments were imposed as the cattle grazed as two separate herds and were given *ad libitum* molasses-urea supplement containing low or high P concentrations. During early lactation the cow-calf pairs were housed in individual pens and fed mixed diets either low or high in P. Thus the diet treatments were LowP-LowP, LowP-HighP, HighP-LowP and HighP-HighP with one factor comprising low or high diet P concentration, and the other factor the imposition of these diets in pregnancy and/or lactation.

On 9 July 2015 (DOY 190) the cows (n=54) were allocated by stratified randomization based on stage of pregnancy and LW to two diet treatments comprising LowP and HighP which were managed as 2 herds through pregnancy. These herds grazed two paddocks (each *ca.* 30 ha; Paddocks A and B) of senesced naturalized pasture comprising principally black Spear grass *(Heteropogon contortus)* and Indian couch *(Bothriochloa pertusa)* from 9 July 2015 (DOY 190) until 16 September 2015 (DOY 259) (Periods 1A and 1B). Molasses-urea supplement containing either low or high P concentrations were fed *ad libitum* from the 9 July 2015 (DOY 190) to the LowP and HighP treatment groups, respectively. The herds were exchanged between the two paddocks fortnightly to reduce any effects of paddock differences. The sentinel heifers and steers were allocated to the two groups by stratified randomization based on sex and LW, and were included with each paddock group of breeders from the 20 August 2015 (DOY 232); the interval from 9 July 2015 to the 20 August 2015 was considered Period 1A, and the interval from 20 August 2015 to 16 September 2015 Period 1B. On the 16 September 2015 (DOY 259), two weeks before the expected commencement of calving, the two herds were moved to two paddocks (4.3 ha) close to the pen facilities and offered *ad libitum* baled wheat straw and the low or high P molasses-urea supplements. There was negligible pasture available in these small paddocks. The animals remained in these small paddocks (Period 1C) until transfer of cows and their calves to individual pens about one week after calving. Sixteen cows from each diet treatment during pregnancy were used during Period 2 during the first three months of lactation of the cows. These cows calved from 3 October 2015 (DOY 276) through to 22 November 2015 (DOY 326) and the mean calving date was 25 October 2015 (DOY 298). Thus the pregnancy treatments were imposed for on average 108 days. The cows which calved after the 22 November 2015 were rejected from the experiment from this DOY. At the end of Period 2 after 14 weeks in pens the cows and their calves were moved to small paddocks where they grazed with bulls. The heifers (n = 8) were moved from the small paddocks where they were held with the cows during Period 1C to individual pens with the cows and calves in three groups between 29 October 2015 (DOY 302) and 26 November 2015 (DOY 330). Thus the two diet treatments were imposed on the heifers for on average 84 days during Period 1B and 1C. The steers were removed from the present experiment on the 26 November 2015 (DOY 330) when they entered the Pens_D experiment as described.

During Periods 1A, 1B and 1C the 2 paddock groups were offered *ad libitum* mixtures of molasses-urea supplement (Table E-1) in which the proportion of urea in the molasses-urea supplement was increased gradually over 7 weeks from an initial concentration (on an as-fed basis) of 29 g/kg to 60 g/kg. The LowP supplement contained no additional source of P, while the high P supplement contained sodium orthophosphate (initially 7.5 g/kg increasing to 17.5 g/kg). Sodium chloride (7.5 – 10.2 g/kg) and monensin (Elanco rumensin 100, Elanco) (0.338 – 0.465 g/kg) were included in both molasses-urea supplements. During Period 1C wheat straw was fed as large bales in hay feeders and

was provided in excess of the voluntary intake. The wheat straw and molasses-urea were sampled weekly.

Table E-1. Pens_E. Period 2. The ingredients (g as-fed/kg) and the composition (g as-fed/kg for dry matter concentration and g/kg DM for the other constituents; mean (sd) of the mixed diets containing low or high concentrations of P and fed to the lactating cows and the weaner heifers during Period 2

	Diets offered in pens in Period 2	
Ingredient	LowP	HighP
Wheat straw	512.1	506.3
Wheat flour	282.2	279.1
Sugar	140.9	139.3
Canola oil	22.2	22.0
Urea	22.2	22.0
Calcium phosphate (Kynophos)	0.0	12.9
Limestone	7.53	5.82
Ammonium sulphate	4.71	4.65
Sodium chloride	4.68	4.63
Magnesium oxide	2.34	2.32
Rumigro premix	0.767	0.758
Elanco rumensin 100	0.267	0.264
Composition		
Dry matter (g/kg as fed)	934 (2.2)	927 (3.0)
Organic matter (g/kg DM)	926 (1.2)	916 (3.4)
Crude protein	118 (3.2)	117 (6.1)
NDF	412 (22)	446 (42)
ADF	294 (6.4)	287 (41)
Lignin	32.3 (3.9)	33.5 (8.2)
Crude fat	30.0 (5.2)	27.3 (5.5)
Ca	4.58 (0.22)	5.56 (0.49)
P	0.88 (0.13)	3.05 (0.13)
Mg	1.75 (0.13)	1.85 (0.24)
S	2.33 (0.05)	2.35 (0.13)

At calving the cow and calves were weighed, the calf ear tagged, and a blood sample obtained from the cow. Between 5-13 days after calving (on average 8 days) the cow and calf were moved from the small paddock to the pens facility where they were housed together in individual pens during the next 14 weeks of lactation (Period 2). Cows given LowP or HighP diet treatments during pregnancy were allocated to the two diet treatments during lactation (Table E-1) in blocks of four cows and calves to represent each of the four treatments. The eight heifers were allocated to the LowP diet in pens; thus the treatments of these heifers during Period 2 comprised only the LowP-LowP and the HighP-LowP diets. The pens facility used has been described above for previous experiments.

The cow-calf pairs in the individual pens were offered their designated diets (LowP and HighP) at *ca.* 10% in excess of the actual intakes to achieve *ad libitum* intakes. Feed refusals were collected weekly. The main ingredients of the total mixed ration diets consisted of coarsely chopped wheat

straw, wheat flour and sugar (Table E-1). Diets low or adequate in P were prepared by the exclusion or incorporation, respectively, of calcium phosphate (Kynophos).

Measurements and sampling

Assessment of pasture grazed during pregnancy (Period 1A): The pasture available in the two paddocks during Period 1A was measured on two occasions (21 Aug 2015 and 9 Oct 2015) during Periods 1A and 1B, respectively, using the Botanal procedure (Tothill *et al.* 1992). Herbage mass was estimated from 100 quadrats (0.25 m^2 quadrats, 2 transects each with 50 estimations) in each paddock. Visual estimations of herbage mass were calibrated by cutting 14 quadrats at ground level on each occasion. In addition, in each paddock and at each sampling date five quadrats of ungrazed plant material of each of the major grass species were measured and collected for pasture quality measurements. The extended tiller height was measured in four random tillers per quadrat. The plant material in the upper (U) and the lower (L) fractions of the pasture, defined as less than or greater than 50% of the average extended height, were then cut at ground level for pasture quality analyses. The plant material was dried (60°C), ground through a 1 mm sieve, and the plant material from the five quadrats per plant species and stratum were bulked for subsequent analyses.

Animal measurements during pregnancy (Period 1A,1B and 1C)

The intakes of molasses-urea was measured twice weekly in each paddock and samples of molasses-urea offered were obtained each occasion a batch was mixed. The animals were mustered to yards fortnightly and LW (without fasting) was measured and BCS evaluated. Blood was sampled from the jugular vein using vacutainers (BD Diagnostics, Plymouth, UK) with no anti-coagulant and with lithium heparin as an anti-coagulant to provide serum and plasma, respectively. Samples were centrifuged (3,000 g x 15 min) to separate serum or plasma which were stored frozen. Faeces was obtained from the rectum of the six cows and six sentinel animals selected randomly from each treatment group at each fortnightly measurement, bulked for each of the subgroups, and were stored frozen. The straw offered during Period 1B was sampled using a hay corer. Straw and molasses mix samples were bulked on a monthly basis and retained for analysis.

Animal measurements during lactation (Period 2)

Samples of feed offered were obtained on each occasion a batch of diet was mixed and samples of feed refusals of individual animals were collected weekly. Straw or feed mix samples were dried (60°C) and ground through a 1 mm screen (Christie and Norris laboratory mill, Chelmsford, UK). Feed offered samples were bulked fortnightly or monthly and retained for analysis. Feed residues from each pen were collected and weighed weekly and DM determined. Total collections of faeces for two 5-day intervals were conducted during weeks 6 and 14 (TC1 and TC2, respectively) with the cows and calves constrained in the front half of the pen with a concrete floor. Samples of feed offered and feed refused by each pen were bulked over the collection interval, subsampled, dried and ground as described above and samples retained for analyses. Faeces from cows were collected daily for each individual pen, weighed, mixed and a subsample (10% of the total weight) was retained and frozen. These samples were later mixed and a subsample dried at 60°C, ground through a 1 mm screen and retained for analysis. In addition, shortly before and after the TC2 total collection of faeces samples of urine were obtained from the majority of the cows and heifers by stimulation of the vulva. These samples were acidified to pH < 3 by addition of 10 M sulphuric acid and stored frozen for subsequent analysis of purine derivatives and phosphorus. Cow and calf LW were

measured, cow BCS was evaluated, and blood was sampled from cows at fortnightly intervals through Period 2 for both plasma and serum.

Bone biopsy samples
Biopsies of the cortical bone of the 12th rib and of the *tuber coxae* were obtained shortly after calving and after 14 weeks of lactation following the procedures of Little (1972) and Kidd *et al.* (2014).

Fat cover, eye muscle area and ovarian activity.
Realtime ultrasound scanning (Esaote Pie Medical Aquila with a 3.5 Mhz linear array transducer (Pie Medical Imaging, Maastricht, The Netherlands) was used to measure fat depth at the rib and P8 rump sites, and of eye muscle area (EMA). Also ultrasonography (Honda HS200V with a 7.5 MHz linear array transducer, Honda Electronics Co. Ltd, Toyohashi, Japan) was used to determine ovarian activity in the cows. Measurements of fat depth and eye muscle area were made in mid-pregnancy and shortly before calving commenced, and then at monthly intervals until *ca.* five months lactation. Ovarian activity was indicated by the presence of corpus luteum (CL) or corpus albicans (CA) (Johnston *et al.* 2014).

Laboratory procedures
Feed samples from Periods 1 and 2 (molasses mixtures, wheat straw and the mixed diets fed in Period 2) were bulked monthly. Samples of pasture and molasses-urea supplements from Period 1, and of feed offered, feed refused and faeces collected during the total collection intervals during Period 2, were analysed for organic matter (OM) by incineration (550°C for 8 h). These samples were also digested in a nitric-perchloric acid mixture, and the concentrations of P, Ca, Mg and S analysed using an inductively coupled plasma spectrometer (Optima 7300 DV, Perkin Elmer, Waltham, MA, USA). Samples of feed offered were also analysed for total N, neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin and crude fat by a commercial laboratory (Dairy One, Ithaca, USA). Creatinine in urine was measured by high-performance liquid chromatography (George *et al.* 2006) and the concentration of P colorimetrically (Goodwin 1970). Plasma samples were analysed colormetrically for plasma inorganic P (PIP), Ca and Mg (Beckman Coulter Au680 analyser with OSR6122, OSR 6189 and OSR6189 assay kits respectively). Rib bone biopsy samples were carefully scraped to remove trabecular bone before measurement of cortical bone thickness (CBT) using Vernier callipers and specific gravity (SG) by gravimetric procedures.

Plasma concentrations of hormones and bone metabolism markers were determined using commercial assay kits and according to the manufacturer's instructions as follows: parathyroid hormone (PTH; A11930, Beckman Coutler), 25OH Vit D and 1,25-diOH Vit D (AA-35F1 and AA-54F2, Immunodiagnostic Systems), CTX-1 (Crosslaps AC-02F1, Immunodiagnostic Systems), bone alkaline phosphatase (BAP; MicroVue 8012, Quidel), and osteocalcin (OCN; MicroVue 8002, Quidel).

Calculations and statistical analyses
Voluntary intake (VI) of molasses-urea supplements (g DM/kg LW) during Period 1A, 1B and 1C were calculated from the total intake of supplement and the LW of the animals. It was assumed that the cows and weaners consumed the same amounts of supplement per kg LW and average intakes (kg/day) were calculated for the cow and sentinel groups from their respective LW. The estimated ME contents (M/D) of the pastures grazed during Periods 1A and 1B, and straw fed during Period 1C, were calculated as: M/D = 16.65 – 0.024 (ADF) where M/D was MJ ME/kg DM and the acid detergent

fibre (ADF) content was g/kg DM (CSIRO 2007, p. 9). The voluntary intake of pasture during Periods 1A and 1B, and wheat straw hay during Period 1C, were estimated from the calculated ME required for each class of animal minus the ME ingested as molasses-urea supplement, and the ME content of the pasture or hay using QuikIntake (Ver 5, McLennan and Poppi, unpublished). During Period 2 when the cows and heifers were fed in pens the voluntary intakes and the apparent digestibilities of DM and OM were calculated by standard procedures, while the differences between intake and faecal excretion (I-F) of P and Ca were calculated these measurements. The P and Ca requirements of the various classes of animals (i.e. as pregnant and later lactating cows, growing heifers and steers, each with measured LW and LW change) were calculated following CSIRO (2007) using the associated spreadsheets. P intake was calculated from the estimated or measured intakes of pasture and supplement during Period 1A – 1C, the measured intakes during Period 2, and the measured concentrations of P in diet in pasture and supplement or in the mixed diet. During Period 2 the P balance was calculated from P intake minus the excretion of P in faeces and urine (measured from samples obtained shortly before and after the TC2 total collection), and minus the estimated P in milk . The milk P was estimated from the calf LW gain and the milk required per kg calf gain in Experiment Pens_C. Since the calculation of P requirement included the DM intake, and voluntary intake of diet DM may be reduced during P deficiency, the calculated requirements for the HighP diets are the appropriate value to estimate the P requirements for the animal for maximum productivity. The estimated ME content (M/D) of the mixed diets fed in the pens during Period 2 was calculated from the organic matter digestibility measured during the total collection intervals as: M/D = 0.169 (OMD) – 1.986 where M/D was MJ ME/kg DM and the OMD content was organic matter digestibility percent (CSIRO 2007, p. 8). The volume of urine excreted during TC2 was calculated from the creatinine concentration and assuming a daily creatinine of 0.91 mmol/kg $W^{0.75}$ (Chen *et al.* 1995).

The conceptus-free LW (CF-LW) of cows during pregnancy was calculated by subtraction of the estimated conceptus weight from the measured LW as described by O'Rourke *et al.* (1991) for *Bos indicus* cattle. The age of the conceptus was calculated retrospectively from the actual calving date. LW change of animals during Period 2 were calculated using 2 procedures: (i) linear regression of the LW of individual animals and time was used to calculate LW change and the total change over the interval as the product of LW change and the interval, and (ii) by diffference between the LW at the commencement and the end of the period. Because during pregnancy the cows were at about LW maintenance the LW change was calculated only by difference. The cows calved over an interval of 50 days and the measurements made in individual animals were expressed as the weeks before calving or the week of lactation. The concentration of P in cortical rib bone was calculated as: Pconc (mgP/cc) = 228.8 (SG) – 261 (Dixon *et al.* 2018). An index of rib bone P, the P in cortical bone per unit surface area of cortical bone (PSACB, mgP/mm^2), was calculated as the product of P concentration in external cortical bone and CBT (Dixon *et al.* 2018). A reproductive score of ovarian activity was calculated for individual cows for each the monthly ultrasonography measurement; an animal was given a score 1 if follicles were observed, 2 if follicles ≤6 mm observed, 3 if follicles were 6-10 mm, 4 if follicles > 10 mm, and score 5 for the presence of a CL or CA. Post-partum anoestrus interval (PPAI) was estimated as the days from calving until the first observation of a CL or CA at the monthly ultrasonography measurement, or in 3 cows for which a CL or CA had not been observed by the 18 April 2016 the PPAI was calculated as the interval to this measurement date plus 30 days.

Data were analysed using Genstat (release 16.1, VSN International Ltd, Hemel Hemstead, UK). ANOVA models examined the effects of treatments on the cows in both a 2x2 factorial design (with the factors being low or high P concentration in diets fed in pregnancy and/or lactation and) and as a one-way ANOVA to compare the four treatments during pregnancy (Periods 1A and 1B) or the six treatments during lactation (Period 2); the latter comprised the 4 treatments ascribed to the cows during lactation and the two treatments (Low and high P) of the sentinel heifers. The data for the sentinel animals during Period 1 were analysed as a separate 2x2 factorial design with the effects of sex (heifers *versus* steers) and diet treatment. The effects of blocking in relation to date of calving and position within the pen complex, and also potential covariates (cow age, actual day of calving, LW at the start of pregnancy or the start of lactation, initial measurements of fat cover and EMA) were examined and consideration given to inclusion in the ANOVA when they approached statistical significance ($P<0.10$). This is indicated in the respective tables. Repeated measures models were examined when sequential measurements were made for intake, the total collection intervals during Period 2 and the consitiuents of plasma. Protected LSD test was used to compare means.

Table E-2. Pens_E. Period 1A. Total pasture on offer (kg/ha) and distribution of species across two grazed paddocks during the early (21 August 2015) and late (9 October 2015) phases of Period 1A

Date	Paddock	*Heteropogon contortus*	*Bothriochloa pertusa*	Other grasses	*Stylosanthes scrabra*	Total
21 Aug 2015	A	2178	430	280	58	2946
	B	1243	838	118	51	2250
9 Oct 2015	A	1137	138	92	0	1367
	B	504	385	60	0	950

Table E-3. Pens_E. Period 1A. The height of tillers (mm) and proportion of two major grass species (g/g) in the DM present in the upper part of the sward across two grazed paddocks. Measurements were made in plant material harvested in early (21 August 2015) and late (9 October 2015) phases of Period 1A

Date	Paddock	Fraction	*Heteropogon contortus*	*Bothriochloa pertusa*
21 Aug 2015	A	Height (mm)	1146	597
		DM-Upper (g/g)[#]	0.465	0.408
	B	Height (mm)	910	580
		DM-Upper (g/g)	0.306	0.389
9 Oct 2015	A	Height (mm)	1006	533
		DM-Upper (g/g)	0.426	0.368
	B	Height (mm)	967	506
		DM-Upper (g/g)	0.457	0.544

[#], the proportion of the total DM in the upper fraction of the sward.

4.3.4 Results

Pasture and molasses-urea supplement fed in Period 1A and 1B

The forage DM on offer in the upper and lower fractions of the *Heteropogon contortus* and *Bothriochloa pertusa* grasses, which comprised ≥ 89% of the sward DM, are given in Table E-2 and Table E-3. Forage DM on offer declined from 2.6 t/ha in August to 1.2 t/ha in October as the pastures were grazed. The P concentrations did not vary markedly between the grass species or between the upper and lower parts of the sward, and averaged 1.5 and 1.3 g P/kg DM in August and October, respectively (Table E-4). The mean estimated ME content of the pasture was 6.0 and 6.9 MJ ME/kg DM and the N concentration 5.64 and 5.40 g N/kg DM, in August and October, respectively. The wheat straw fed in Period 1B contained 4.9 MJ ME/kg DM and 7.5 g N/kg DM.

Table E-4. Pens_E. Period 1A, 1B and 1C. Concentrations (g/kg DM) of DM, OM, nitrogen (N), sulphur (S), acid detergent fibre (ADF), lignin (Lig), phosphorus (P) and calcium (Ca) in the upper and lower fractions (U and L, respectively) of sampled pasture in paddocks A and B (g/kg DM) during Periods 1A and 1B. Paddocks were grazed from 9 July to the 16 September 2015. Composition of the wheat straw fed for 2-6 weeks before calving (Period 1C) included

	Paddock	Spp	Fract	Ash	N	S	ADF	Lig	M/D	P	Ca
Grazed pasture											
21 August 2015	A	Hc	U	86	5.76	0.6	439	44	6.1	1.15	3.47
			L	74	4.48	0.4	454	43	5.8	1.12	2.13
		Bp	U	92	5.60	0.7	435	24	6.2	1.40	5.08
			L	130	5.60	0.6	374	31	7.7	1.78	6.11
	B	Hc	U	93	6.08	0.5	386	25	7.4	1.22	3.91
			L	98	4.80	0.3	425	38	6.5	1.72	3.01
		Bp	U	83	5.76	0.6	441	32	6.1	1.58	7.07
			L	119	7.04	0.8	396	39	7.2	1.71	9.78
		Mean		97	5.64	0.6	419	35	6.6	1.46	5.07
9 October 2015	A	Hc	U	88	5.12	0.5	433	31	6.3	0.99	3.63
			L	88	4.00	0.4	461	45	5.6	1.19	2.47
		Bp	U	100	5.44	0.6	465	21	5.5	1.45	5.14
			L	125	6.72	0.7	438	33	6.1	1.60	7.08
	B	Hc	U	104	5.12	0.5	414	23	6.7	1.02	3.44
			L	86	4.32	0.4	470	43	5.4	1.37	2.50
		Bp	U	122	6.72	0.5	444	47	6.0	1.64	8.60
			L	108	5.76	0.6	436	27	6.2	1.50	6.45
		Mean		103	5.40	0.5	445	34	6.0	1.34	4.91
Straw		Mean		115	7.5	1.57	491	67.8	4.9	0.52	2.62
		sd		6.6	0.42	0.15	10.4	5.5	0.25	0.04	0.08

Spp., species of grasses; *Hc, Heterpogon contortus; Bp, Bothriochloa pertusa.* Fract., Fraction. U and L, Upper and lower fraction of grasses at half the tiller height. M/D, ME content.

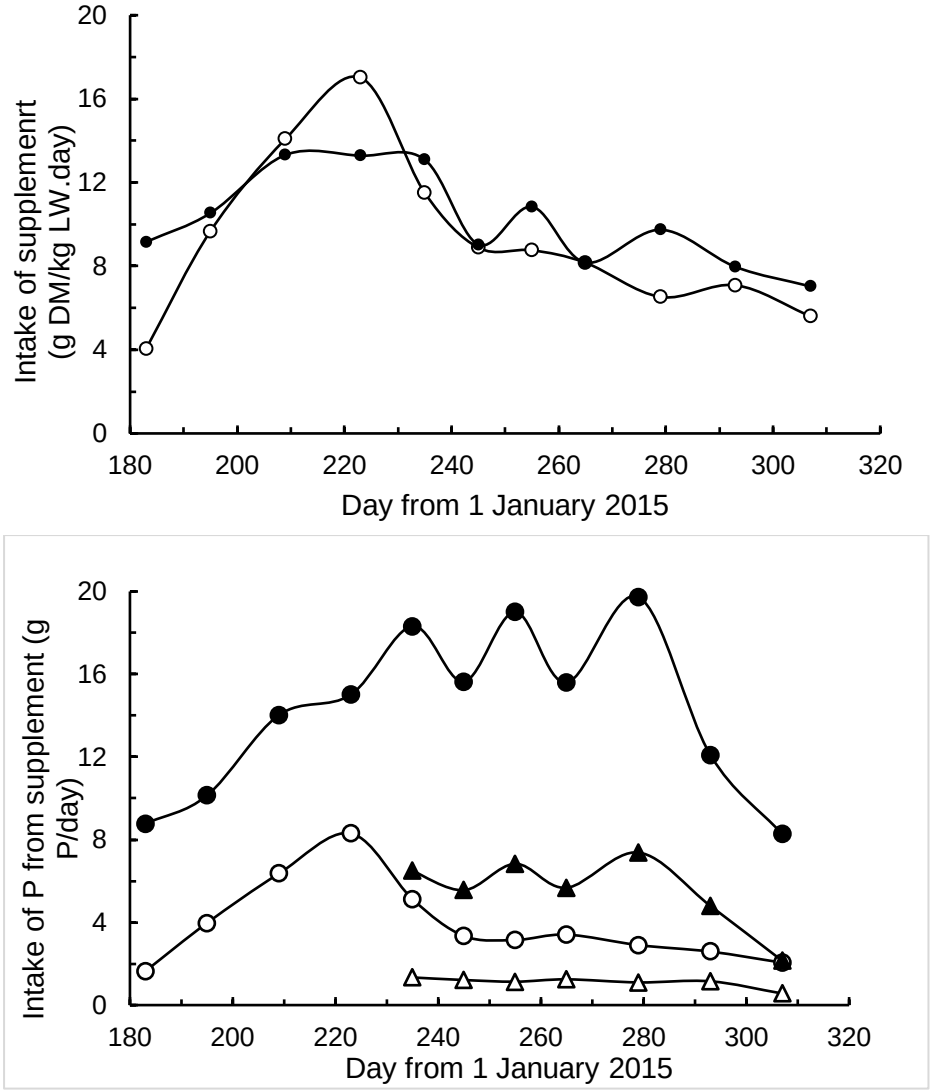


Figure E-1A and 1B. Period 1. Supplementation during Periods 1A, 1B and 1C during pregnancy of the cows. Fig. 1A shows the voluntary intake of molasses-urea supplement DM (g DM/kg LW.day) not containing (LowP)(○) or containing additional P (HighP)(●). Fig. 1B shows the intake of supplement P (g P/day) by cows (○) and sentinels (Δ) offered supplement containing no additional P, or by cows (●) and weaners (▲) offered supplement containing P (●). On Day 259 both the LowP and HighP herds of cattle were moved from grazing 30 ha paddocks to 4 ha paddocks where they were offered wheat straw *ad libitum* during Period 1C.

Intakes of pasture and molasses-urea supplement DM and P during pregnancy (Period 1A, 1B and 1C)
The average voluntary intakes (VI) of the molasses-urea mixes (Figure 1) were 4.6 and 4.1 kg as-fed /head.day for the LowP and the HighP herds, respectively (Figure E-1). Supplement intake increased gradually during the first 6 weeks to *ca.* 14 g DM/kg LW.day and then declined to *ca.* 8 g DM/kg LW late in Period 1B. The concentration of P in the LowP and HighP molasses-urea mix fed in Period 1A and 1B (Table E-5) averaged 0.78 and 2.80 g P/kg DM. With these assumptions the intakes of supplement P by the LowP treatment animals were estimated to range from 3-5 g P/day and 1-2 g P/day in the cows and the weaner animals, respectively (Figure E-2). In the HighP treatment animals intakes were estimated to be in the range 14-19 g P/day in the cows and 5-7 g P in the weaners. In

addition the intakes of P from both pasture and supplement were calculated from the estimated intake of pasture and the P concentration determined in pasture samples. These intakes of P in the cows and the weaners, and also the calculated P requirements are given in Table E-6. The HighP cows ingested 153, 144 and 91% of their P requirements during Periods 1A, 1B and 1C, respectively, while the LowP cows ingested only 67, 50 and 39% of their expected P requirements during Periods 1A, 1B and 1C, respectively (Table 6). The HighP weaners ingested 133 and 83% of their expected P requirements, and the LowP weaners 84 and 52% of their expected P requirements during Periods 1B and 1C, respectively.

Table E-5. Pens_E. Period 1A, 1B and 1C. Concentrations (g/kg as fed) of DM, and (g/kg DM) of ash, nitrogen (N), sulphur (S), phosphorus (P) and calcium (Ca) in molasses-urea mixtures fed to the breeder cows and weaners during mid and late pregnancy

Molasses-urea mix		DM	Ash	N	S	P	Ca
Without additional P	Mean	811	155.0	34.4	7.20	0.78	8.20
	sd	19.6	5.5	7.48	0.42	0.01	0.28
With additional P	Mean	796	176	33.6	7.53	2.80	8.65
	sd	9.1	19.0	19.6	0.021	0.78	0.01

Animal total LW, CF-LW and BCS during pregnancy

During late pregnancy the cows in both LowP and HighP treatments both gained total LW (0.46 and 0.48 kg/day, and the sentinel heifers and steers from 0.20 – 0.24 kg/day, and the LW gain did not differ between the P treatments (Table E6). Cows did not change in conceptus-free LW or BCS during Period 1, with average decreases of 2 kg CF-LW and 0.2 BCS units. Fat cover measured at the rump and rib sites (initially averaging 9 and 6 mm, respectively) decreased by 1-2 mm while eye muscle area increased from 63 to 65 mm^2, but there were no differences ($P>0.05$) due to P treatment or change during the last 14 weeks of pregnancy.

Table E-6. Pens_E. PREGNANCY. Period 1A, 1B and 1C. The responses of mature cows and weaner heifers and steers during the interval from mid-pregnancy to parturition in the cows. Both cows and weaners ingested Low P or high P diets while grazing senesced pasture and given molasses-urea supplements low or high in P. The calculated P requirement is for given for the HighP diet where voluntary intake should not have been constrained by diet P intake. For calculation of P intakes and P requirements the heifer and steer sentinel animals were considered as a singe group given the absence of differences in LW or LW gain. Separate ANOVA analyses were used for the mature cows and for the weaners with the latter in a 2x2 factorial of provision of additional diet P and the groups of weaners (heifers and steers)

Measurement	Mature cows				Weaner heifers		Weaner steers		s.e.m.			Probability		
	LowP	HighP	s.e.m.	Prob	LowP	HighP	LowP	HighP	P	G	PxG	P	G	PxG
n	16	16	--	--	4	4	6	6	--	--	--	--	--	--
Period 1A. P required (g P/day)	-	14.3	-	-	NA	NA	NA	NA	-	-	-	-	-	-
P intake (g P/day)	9.6	21.9	-	-	NA	NA	NA	NA	-	-	-	-	-	-
P (Intake/Required)%	67	153	-	-	NA	NA	NA	NA	-	-	-	-	-	-
Period 1B. P required (g P/day)	-	15.0	-	-	-	8.5	-	8.5	-	-	-	-	-	-
P intake (g P/day)	7.6	21.5	-	-	7.1	11.3	7.1	11.3	-	-	-	-	-	-
P (Intake/Required)%	50	144	-	-	84	133	84	133	-	-	-	-	-	-
Period 1C. P required (g P/day)	-	19.0	-	-	-	9.9	-	9.9	-	-	-	-	-	-
P intake (g P/day)	7.4	17.3	-	-	5.2	8.2	5.2	8.2	-	-	-	-	-	-
P (Intake/Required)%	39	91	-	-	52	83	52	83	-	-	-	-	-	-
T-LW. Week -14 from parturition (kg)	523	514	13.0	0.61	193	191	194	193	5.5	6.1	8.7	0.87	0.90	0.93
T-LW. Week -1 from parturition (kg)	562	554	13.2	0.67	218	215	216	213	5.2	5.9	8.3	0.66	0.84	0.97
T-LW change (kg)	39	41	5.8	0.88	+24	+23	+22	+20	2.8	3.2	4.5	0.62	0.51	0.81
T-LW change (Regression) (kg/day)	0.46	0.48	0.069	0.85	+0.24	+0.23	+0.22	+0.20	0.282	0.032	0.045	0.62	0.51	0.81

P, effect of inclusion of phosphorus in the diet; G, group.

Table E-7. Pens_E. Period 2. LACTATION. The responses of mature cows and heifers during the interval when cows were lactating. Cows ingested Low P or high P diets during mid-late pregnancy while grazing senesced pasture and given supplements, and from calving the cows in each of these pregnancy treatments were fed Low or High P diets in pens for 13 weeks (treatments LowP-LowP, HighP-LowP, LowP-HighP and HighP-HighP). Heifers ingested Low or High P diets while grazing with the pregnant cows, and were fed a LowP diet in pens when the cows were lactating

Measurement	Mature cows										Weaner heifers			
	LowP-LowP	HighP-LowP	LowP-HighP	HighP-HighP	s.e.m.[A]			Prob.[A]			LowP-LowP	HighP-LowP	s.e.m.[B]	Prob.[B]
					Preg	Lact	PxL	Preg	Lact	PxL				
n	8	8	8	7	--	--	--	--	--	--	4	4	--	--
LW. Start of lactation (kg)	476[a]	468[a]	467[a]	453[a]	9.6	9.6	13.6	0.437	0.382	0.796	211[b]	210[b]	20.5	<0.001
LW. Wk 14 of lactation	442[ab]	437[b]	490[a]	468[ab]	12.6	12.6	17.9	0.536	0.032	0.730	244[c]	240[c]	25.04	<0.001
LW change (Regn) (kg)	-0.46[b]	-0.41[b]	+0.22[a]	+0.24[a]	0.056	0.056	0.079	0.669	<0.001	0.821	+0.38[a]	+0.35[a]	0.111	<0.001
LW change (kg)	-34[b]	-31[b]	+23[a]	+19[a]	5.2	5.2	7.37	0.955	<0.001	0.721	+33[a]	+30[a]	9.2	<0.001
BCS. Start of lactation	3.7	3.7	3.5	3.6	0.130	0.130	0.184	0.668	0.400	0.621	3.1	3.4	0.30	0.630
BCS. Wk 14 of lactation	2.9	2.8	3.6	3.6	0.183	0.183	0.259	0.707	0.010	0.736	2.9	3.5	0.34	0.080
BCS change	-0.8[b]	-1.0[b]	+0.1[a]	-0.1[a]	0.118	0.118	0.167	0.298	<0.001	0.983	-0.3[a]	+0.1[a]	0.21	<0.001
Calf birth weight (kg)	33.4	34.6	36.2	35.4	0.99	0.99	1.40	1.000	0.276	0.597	m	m	m	m
Calf LW gain (Regression) (kg)	0.72	0.80	0.94	0.92	0.041	0.041	0.058	0.643	0.007	0.376	m	m	m	m
Calf LW at 14 weeks (kg)	108	117	129	130	4.5	4.5	6.4	0.451	0.017	0.550	m	m	m	m
DM intake (kg/d)[A]	7.76[b]	7.86[b]	10.11[a]	9.82[a]	0.33	0.33	0.47	0.907	<0.001	0.744	4.33[c]	4.03[c]	0.58	<0.001
DM intake (g/kg LW.d)	16.7[d]	17.2[cd]	20.9[ab]	21.3[a]	0.43	0.43	0.61	0.391	<0.001	0.946	19.0[bc]	17.7[cd]	0.76	<0.001
OM digestibility (g/kg)TC-1	678	657	685	695	11	11	16	0.748	0.160	0.332	675	678	22	0.657
OM digestibility (g/kg)TC-2	662	633	640	655	9	9	13	0.758	0.766	0.257	695	690	112	0.838
OM digestibility (g/kg)_mean	682	662	680	689	9	9	12	0.709	0.279	0.220	700	688	16	0.392
NDF digestibility (g/kg)_mean	532	501	532	532	14	14	20	0.481	0.434	0.408	552	525	26	0.676

A, sem and probability calculated from the 2x2 factorial of low or high diet P during pregnancy and lactation. B, sem and probability calculated from the one-way ANOVA of all six treatments. Lower case superscript letters indicate differences between means based on the one-way ANOVA.
LW, liveweight; BCS, body condition score; OM, organic matter; NDF, neutral detergent fibre.

Table E-8. Pens_E. Period 2. LACTATION. The fat cover at the rump and rib sites, and the eye muscle area (EMA) of mature cows during lactation. The fat cover at the rump and rib sites, and eye muscle area (EMA), shortly after calving and then at monthly intervals was measured by ultrasound scanning. Cows were fed diets low or high in P during pregnancy, and low or high in P during lactation. In addition the comparative fat cover and EMA of the sentinel heifers previously fed Low or High P diets during the pregnancy phase (Period 1A and 1B) and fed the LowP or High P diet in the pens during the lactation (Period 2) period is also given. The mean calving date was the 23 October 2015

Measurement	Mature cows										Weaner heifers			
	Low-Low	High-Low	Low-High	High-High	s.e.m.[A]			Prob.[A]			Low-Low	High-Low	s.e.m.[B]	Prob.[B]
					Preg	Lact	PxL	Preg	Lact	PxL				
n	8	8	8	7	--	--	--	--	--	--	4	4	--	--
Rump fat (mm)														
Initial (19 Nov 2015)	10.1	13.4	7.3	9.5	--	--	--	--	--	--	1.3	1.5	1.55	0.006
14 Dec15 (1.7 mo	4.6	3.6	5.8	5.6	0.74	0.74	1.04	0.656	0.127	0.616	1.5	1.8	1.41	0.066
11 Jan16 (2.6 mo lactation)	4.0	2.6	4.5	5.0	0.79	0.79	1.11	0.743	0.191	0.376	1.8	1.3	1.39	0.157
11 Feb 16 (3.6 mo	3.5	2.2	3.9	5.0	0.80	0.80	1.14	0.959	0.167	0.283	2.0	2.0	1.41	0.310
10 Mar 16 (4.5 mo	3.2	2.5	4.0	4.1	0.82	0.82	1.15	0.820	0.296	0.718	2.0	2.3	1.35	0.625
Rib fat (mm)														
Initial (19 Nov 2015)	6.6	9.5	6.0	7.0	--	--	--	--	--	--	--	--	--	--
14 Dec15 (1.7 mo	2.6	2.4	3.3	3.0	0.31	0.31	0.43	0.636	0.140	0.924	1.0	1.3	0.58	0.016
11 Jan16 (2.6 mo lactation)	2.3	2.0	2.5	3.0	0.31	0.31	0.45	0.771	0.171	0.402	1.5	1.3	0.59	0.160
11 Feb 16 (3.6 mo	2.3	1.7	2.3	2.3	0.30	0.30	0.42	0.568	0.458	0.458	1.5	1.8	0.53	0.656
10 Mar 16 (4.5 mo	2.3	1.9	2.3	2.4	0.41	0.41	0.59	0.813	0.692	0.616	1.5	1.8	0.70	0.860
EMA (mm^2)														
Initial (19 Nov 2015)	64.6	61.8	60.2	64.0	--	--	--	--	--	--	--	--	--	--
14 Dec15 (1.7 mo	55.8	52.1	57.0	56.6	2.18	2.18	3.08	0.579	0.321	0.548	35.8	40.2	4.52	0.001
11 Jan16 (2.6 mo lactation)	52.6	49.0	60.0	57.6	2.22	2.22	3.14	0.398	0.016	0.773	39.0	34.5	4.64	<0.00
11 Feb 16 (3.6 mo	52.8	47.4	57.9	56.7	2.04	2.04	2.89	0.341	0.015	0.386	34.8	41.0	4.42	<0.00
10 Mar 16 (4.5 mo	51.0	47.9	54.5	57.7	2.18	2.18	3.08	0.936	0.037	0.286	35.8	39.5	4.28	0.001

A, sem and probability calculated from the 2x2 factorial of low or high diet P during pregnancyt and lactation. B, sem and probability calculated from the one-way ANOVA of all six treatments. Lower case superscript letters indicate differences between means based on the one-way ANOVA.
LW, liveweight.

LW, BCS and intake of DM and P during lactation

During lactation the cows fed the LowP diets experienced a LW decline of 0.41-0.46 kg/day while the cows fed the HighP diets gained LW at 0.22-0.24 kg/day (Figure E-2 and Table E-7). These differences due to diet during lactation were significant (P<0.001). BCS did not change during lactation in cows fed the HighP diets but those fed the LowP diets lost on average 0.9 BCS units. There were no differences among the treatments in the fat depth at the rump and the rib sites. EMA was reduced (P<0.05) in the cows fed the LowP diets from the measurement on the 11 January 2016 after *ca.* 2.6 mo lactation (Table E-8). The sentinel heifers given either the LowP or HighP treatments during pregnancy, and later all fed the same LowP diet in the pens, gained 0.35 and 0.38 kg LW/day (P>0.05). There was no effect of the diet P deficiency during Period 1B and 1C (when the sentinels were offered the same forage and supplements as the pregnant cows) on the subsequent LW, or the changes in LW, of the sentinel heifers when they were fed a severely P deficient diet for 3 months in pens during Period 2 and corresponding to lactation in the cows.

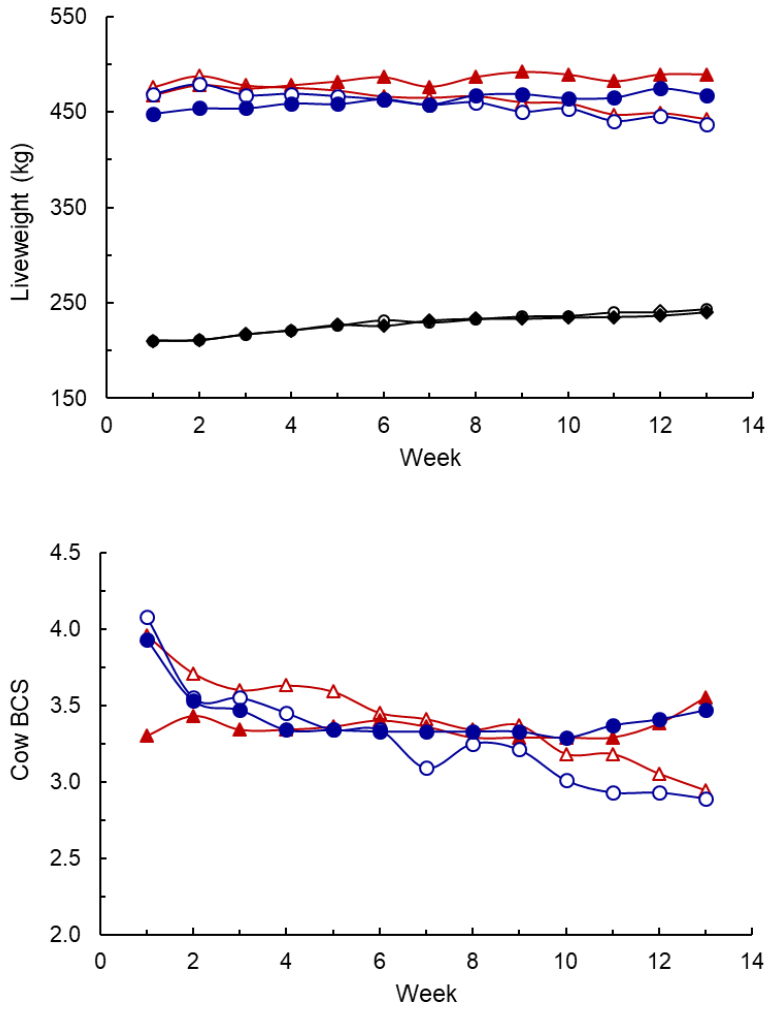


Figure E-2. Pens_E. Changes in cow LW (E-2A) and body condition score (E-2B) during Period 2 when the cows were lactating and were fed in pens. The diet treatments were LP-LP (Δ), HP-LP (○), LP-HP (▲) and HP-HP (●) in the cows, and LP-LP (◊) and HP-LP (♦) in the heifers.

The HighP lactating cows ingested about 30.41 g P/day which provided 123% of the calculated P requirements of 24.67 g P/day for these lactating cows. The LowP lactation cows ingested only 6.87 g P equivalent to 28% of their P requirement to achieve the same production as milk output and LW gain as that observed in the HighP cows (Table 6). The sentinel heifers fed the High P diet ingested 12.29 g P/day (163% of calculated requirements) while the LowP diet heifers ingested 3.81 g P/day which was 50% of their expected P requirements. The performance of these heifers in pens during Period 2 was not affected by their nutritional treatments during Period 1.

DM intake of the cows averaged across diet treatments (Table E-8; Figure E-3) increased progressively ($P<0.05$) from 11.4 g DM/kg LW in week 1 to 21.5 g DM/kg LW in week 12 of lactation. Cows fed the HighP diet had higher ($P<0.001$) DM intakes than those fed the LowP diet (21.3 and 17.1 g DM/kg LW, respectively, but there was no carryover effect ($P>0.05$) of the diet during pregnancy. DM intake of the sentinel heifers (average 18.4 g DM/kg LW) was intermediate between the intakes of the cows fed the two diets and also was not affected by the previous diet treatment during Period 1.

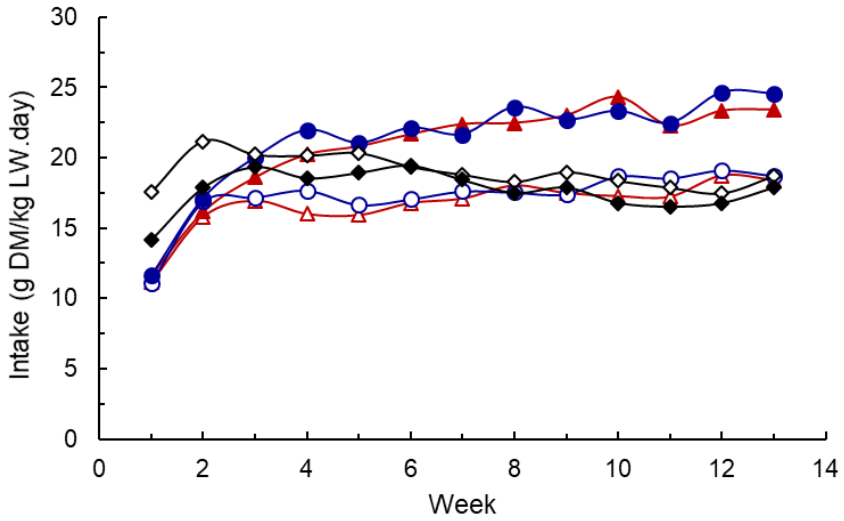


Figure E-3. Pens_E. Lactation. Voluntary intake of cows and sentinel heifers during Period 2 when the cows were lactating and the animals were fed in pens. The diet treatments were LP-LP (Δ), HP-LP (○), LP-HP (▲) and HP-HP (●) in the cows, and LP-LP (◊) and HP-LP (♦) in the heifers.

Neither OM nor NDF digestibility measured *in vivo* during lactation were affected by the diets and averaged 678 and 524 g/kg, respectively (Table E-7). The average ME content calculated from the OMD was 9.5 MJ ME/kg DM. During the total collection intervals the ME intake (kJ ME/kg LW.day) of the cows fed the LowP diet was 74% of that by the cows fed the HighP diet (Table E-9). The intake of ME by the weaner heifers was, on a LW basis, 88% of the HighP cows. During these total collection intervals the intake of P averaged in cows fed the HighP diet (33.5 g P) and the LowP diets (8.4 g P/day) (Table E-9).

In both the cows and the weaner heifers fed the LowP diet the difference between P intake and P excretion in faeces (P (I-F)) ranged from -1.0 to +1.7 g P/day during each of the total collection intervals. The amount of P in milk was estimated to be 5.50 and 6.83 g P/day in milk the cows fed

the LowP and the HighP diets, respectively. Thus the LowP diet cows were on average mobilizing substantial body P (P balance -5.2 g P/day) while the HighP cows were in approximate P balance (+0.48 g P/day). However, in the HighP cows there was a substantial decrease in P balance from week 6 (TC1) to week 14 (TC2). It appeared that the HighP cows were replenishing body P reserves earlier in lactation (week 6 and TC1 measurement) and that this replenishment had been completed by week 14 (TC2)(Table E-9). For Ca the differences between the LowP and the HighP diets in intake and in the difference between intake and faecal excretion (Ca (I-F)) were numerically greater but showed similar differences among the treatments during the total collection intervals (Table E-10).

The weaner heifers were in approximate P balance after both 6 and 14 weeks of ingesting the LowP diet in the pens during Period 2, even though the amount of P being ingested was only about 50% of calculated requirements. This P balance suggests that during this interval these young cattle were able to continue slow growth (0.32 kg/day) by reducing the concentration of P in soft tissue and/or bone P as has been reported (Coates *et al*. 2017; Dixon *et al*. 2017).

Table E-9. Pens_E. LACTATION. The responses of mature cows and heifers during the interval when cows were lactating. Cows ingested Low P or high P diets during mid-late pregnancy while grazing senesced pasture and given supplements, and from calving the cows in each of these pregnancy treatments were fed Low or High P diets in pens for 13 weeks (treatments LowP-LowP, HighP-LowP, LowP-HighP and HighP-HighP). Heifers ingested LowP or High P diets while grazing with the pregnant cows, and were fed a LowP diet in pens when the cows were lactating

Measurement	Mature cows										Sentinel heifers			
	LowP-LowP	HighP-LowP	LowP-HighP	HighP-HighP	s.e.m.[A]			Prob.[A]			LowP-LowP	HighP-LowP	s.e.m.[B]	Prob.[B]
					Preg	Lact	PxL	Preg	Lact	PxL				
n	8	8	8	7	--	--	--	--	--	--	4	4	--	--
ME content (MJ ME/kg DM)	9.54	9.20	9.51	9.66	0.145	0.145	0.205	0.709	0.279	0.22	9.85	9.65	0.262	0.392
ME intake (MJ ME/day)_mean	76.8^{b}	74.2^{b}	103.3^{a}	105.4^{a}	3.51	3.51	4.95	0.937	<0.001	0.55	45.7^{c}	43.4^{c}	6.34	<0.001
ME intake (est kJ ME/kgLW.day)	165^{cd}	162^{d}	214^{ab}	229^{a}	4.82	4.82	6.81	0.350	<0.001	0.17	199^{b}	191^{bc}	10.4	<0.001
DM intake (kg/day)_TC1	7.8^{b}	7.9^{b}	10.6^{a}	10.2^{a}	0.40	0.40	0.57	0.708	<0.001	0.62	4.7^{c}	4.8^{c}	0.75	<0.001
DM intake (kg/day)_TC2	8.3^{b}	8.2^{b}	11.3^{a}	12.0^{a}	0.47	0.47	0.67	0.682	<0.001	0.57	4.7^{c}	4.4^{c}	0.90	<0.001
P requirement (g P/day)	15.2	15.2	24.7	24.7	--	--	--	--	--	--	8.1	7.6	--	--
P intake (g P/day)_TC1	6.85^{b}	7.04^{b}	33.95^{a}	33.53^{a}	1.87	1.87	2.65	0.871	<0.001	0.81	4.16^{b}	3.95^{b}	3.65	<0.001
P intake (g P/day)_TC2	8.89^{b}	8.74^{b}	33.28^{a}	33.09^{a}	1.85	1.85	2.62	0.857	<0.001	0.81	5.11^{b}	4.98^{b}	3.55	<0.001
P intake (g P/day)_mean	7.87^{b}	7.89^{b}	33.61^{a}	33.31^{a}	1.33	1.33	1.87	0.991	<0.001	0.99	4.68^{b}	4.35^{b}	2.32	<0.001
P faeces (g P/day)_TC1	6.96^{b}	8.08^{b}	23.74^{a}	22.12^{a}	1.26	1.26	1.78	0.910	<0.001	0.46	5.31^{b}	4.91^{b}	2.29	<0.001
P faeces (g P/day)_TC2	7.22^{b}	7.87^{b}	30.25^{a}	28.25^{a}	1.44	1.44	2.04	0.951	<0.001	0.70	4.24^{b}	4.10^{b}	2.90	<0.001
P faeces (g P/day)_mean	7.09^{b}	7.98^{b}	27.00^{a}	25.18^{a}	1.23	1.23	1.74	0.925	<0.001	0.55	4.47^{b}	4.86^{b}	2.34	<0.001
P (I-F) (g P/day)_TC1	-0.1^{b}	-1.0^{b}	+10.2^{a}	+11.4^{a}	1.64	1.64	2.32	0.922	<0.001	0.76	-1.1^{b}	-1.0^{b}	3.20	<0.001
P (I-F) (g P/day)_TC2	+1.7	+0.9	+3.0	+4.8	1.04	1.04	1.47	0.686	<0.001	0.35	+1.01	+0.74	2.08	0.397
P (I-F) (g P/day)_mean	+0.78^{b}	-0.09^{b}	+6.62^{a}	+8.12^{a}	0.80	0.80	1.13	0.871	<0.001	0.36	0.21^{b}	-0.51^{b}	1.43	<0.001
DM intake (g DM/kg LW)_mean	17.4^{b}	17.9^{b}	22.5^{a}	23.6^{a}	0.54	0.54	0.77	0.292	<0.001	0.68	19.6^{b}	19.2^{b}	1.07	<0.001
P intake (mg P/LW.day)_mean	17.1^{b}	17.6^{b}	68.4^{a}	71.9^{a}	2.02	2.02	2.86	0.451	<0.001	0.56	19.7^{b}	18.6^{b}	3.66	<0.001
P faeces (mg P/LW.day)_mean	15.4^{b}	17.6^{b}	55.0^{a}	55.1^{a}	2.14	2.14	3.03	0.704	<0.001	0.72	18.9^{b}	20.8^{b}	4.28	<0.001
P (I-F) (mg P/LW.day)_mean	+1.7^{b}	+0.0^{b}	+13.4^{a}	+17.7^{a}	1.63	1.63	2.30	0.659	<0.001	0.25	+0.8^{b}	-2.2^{b}	3.10	<0.001

A, sem and probability calculated from the 2x2 factorial of low or high diet P during pregnancyt and lactation. B, sem and probability calculated from the one-way ANOVA of all six treatments. Lower case superscript letters indicate differences between means based on the one-way ANOVA. LW, liveweight.

Table E-10. Pens_E. LACTATION. The responses of mature cows and heifers during the interval when cows were lactating. Cows ingested Low P or high P diets during mid-late pregnancy while grazing senesced pasture and given supplements, and from calving the cows in each of these pregnancy treatments were fed LowP or High P diets in pens for 13 weeks (treatments LowP-LowP, HighP-LowP, LowP-HighP and HighP-HighP). Heifers ingested LowP or High P diets while grazing with the pregnant cows, and were fed a LowP diet in pens when the cows were lactating

Measurement	Mature cows										Weaner heifers			
	LowP-LowP	HighP-LowP	LowP-HighP	HighP-HighP	s.e.m.[A]			Probability[A]			LowP-LowP	HighP-LowP	s.e.m.[B]	Prob.[B]
					Preg	Lact	PxL	Preg	Lact	PxL				
n	8	8	8	7	--	--	--	--	--	--	4	4	--	--
Ca intake (g P/day)_TC1	34.1^{b}	34.1^{b}	62.5^{a}	62.3^{a}	3.15	3.15	4.45	0.899	<0.001	0.896	19.1^{c}	18.6^{c}	5.49	<0.001
Ca intake (g P/day)_TC2	37.5^{bc}	37.9^{b}	53.6^{a}	54.2^{a}	2.59	2.58	3.65	0.543	<0.001	0.608	21.4^{c}	22.9^{bc}	6.59	<0.001
Ca intake (g P/day)_mean	35.8^{b}	36.0^{b}	58.0^{a}	58.2^{a}	2.48	2.48	3.51	0.812	<0.001	0.853	20.3^{c}	20.1^{c}	4.62	<0.001
Ca faeces (g P/day)_TC1	26.4^{bc}	32.4^{b}	42.9^{a}	42.6^{a}	2.55	2.55	3.60	0.438	0.001	0.385	19.3^{c}	17.9^{c}	4.49	<0.001
Ca faeces (g P/day)_TC2	27.8^{bc}	33.0^{b}	50.5^{a}	50.6^{a}	3.01	3.01	4.26	0.446	<0.001	0.661	14.8^{c}	15.2^{c}	5.42	<0.001
Ca faeces (g P/day)_mean	27.1^{bc}	32.7^{b}	46.7a	46.6^{a}	2.37	2.37	3.36	0.370	<0.001	0.457	16.0^{d}	17.3^{cd}	4.28	<0.001
Ca (I-F) (g P/day)_TC1	7.8^{b}	1.7^{b}	19.6^{a}	19.7^{a}	2.89	2.89	4.09	0.412	0.002	0.529	-0.2^{b}	+0.7^{b}	5.02	<0.001
Ca (I-F) (g P/day)_TC2	9.8	4.4	3.1	3.6	1.88	1.88	2.66	0.640	0.344	0.145	6.6	7.7	5.03	0.777
Ca (I-F) (g P/day)_mean	8.8	3.1	11.3	11.6	1.99	1.99	2.81	0.413	0.048	0.249	4.3	2.8	3.74	0.109
Ca intake (mg P/LW.day)_mean	78^{b}	80^{b}	119^{a}	126^{a}	3.90	3.90	5.52	0.309	<0.001	0.586	85^{b}	86^{b}	9.2	<0.001
Ca faeces (mg P/LW.day)_mean	58^{b}	72^{b}	95^{a}	100^{a}	4.13	1.13	5.84	0.109	<0.001	0.510	67^{b}	74^{b}	5.7	<0.001
Ca (I-F) (mg P/LW.day)_mean	19.5	7.3	23.4	25.0	4.16	4.16	5.88	0.445	0.065	0.213	17.7	11.9	9.9	0.513

A, sem and probability calculated from the 2x2 factorial of low or high diet P during pregnancy and lactation. B, sem and probability calculated from the one-way ANOVA of all six treatments. Lower case superscript letters indicate differences between means based on the one-way ANOVA.

Table E-11. Pens_E. LACTATION. The responses of mature cows and heifers during the interval when cows were lactating. Cows ingested Low P or high P diets during mid-late pregnancy while grazing senesced pasture and given supplements, and from calving the cows in each of these pregnancy treatments were fed Low or High P diets in pens for 14 weeks (treatments Low-Low, High-Low, Low-High and High-High). Heifers ingested Low or High P diets while grazing with the pregnant cows, and were fed a LowP diet in pens when the cows were lactating

Measurement	Mature cows										Weaner heifers			
	LowP-LowP	HighP-LowP	LowP-HighP	HighP-HighP	s.e.m.[A]			Probability[A]			LowP LowP	HighP -LowP	s.e.m. [B]	Prob.[B]
					Preg	Lact	PxL	Preg	Lact	PxL				
n	8	8	8	7	--	--	--	--	--	--	4	4	--	--
Calving CBT (mm)	4.59[a]	4.43[a]	4.51[a]	4.67[a]	0.122	0.122	0.173	0.91	0.562	0.31	3.27[b]	3.66[b]	0.293	0.002
SG	1.810a	1.812[ab]	1.801[b]	1.836[a]	0.006	0.006	0.001	0.08	0.509	0.14	1.697	1.653	0.016	<0.00
P concentration (mgP/cc)	153.0[a]	153.6[ab]	151.2[b]	159.1[a]	1.55	1.55	2.19	0.08	0.509	0.14	127.3	117.2	3.73	<0.00
PSACB (mg P/mm^2)	0.702[a]	0.679[a]	0.682[a]	0.742[a]	0.018	0.018	0.025	0.44	0.388	0.11	0.415	0.426	0.043	<0.00
3 month lactation CBT	3.94[ab]	3.91[ab]	3.91[ab]	4.27[a]	0.123	0.123	0.175	0.32	0.325	0.24	3.12[c]	3.42[bc]	0.260	0.016
SG	1.787[b]	1.785[b]	1.819[ab]	1.836[a]	0.007	0.007	0.018	0.39	<0.00	0.30	1.666	1.684	0.018	<0.00
P concentration (mgP/cc)	147.9[b]	147.5[b]	155.1[ab]	159.2[a]	1.85	1.75	2.47	0.39	<0.00	0.30	120.1	124.2	4.14	<0.00
PSACB (mg P/mm^2)	0.583[b]	0.574[b]	0.606[ab]	0.681[a]	0.018	0.018	0.026	0.18	0.018	0.10	0.375	0.424	0.037	<0.00
Change in CBT (mm)	-0.68	-0.56	-0.65	-0.39	0.154	0.154	0.218	0.49	0.766	0.87	-0.18	-0.25	0.323	0.710
Change in SG	-0.018	-0.024	+0.018	+0.000	0.1140	0.1140	0.0161	0.636	0.045	0.924	-0.035	+0.03	0.0245	0.229
Change in P concentration	-4.2	-5.6	+4.1	+0.1	2.61	2.61	3.69	0.63	0.045	0.92	-7.9	+7.1	5.60	0.229
Change in PSAB (mg P/mm^2)	-0.119	-0.109	-0.083	-0.061	0.0227	0.0227	0.0321	0.624	0.216	0.887	-0.044	-0.004	0.049	0.404
%Change in CBT	-17.5	-15.1	-19.0	-9.9	4.59	4.59	6.49	0.46	0.872	0.70	-8.5	-9.1	9.01	0.843
%Change in SG	-1.0	-1.3	+1.0	+0.0	0.631	0.631	0.893	0.62	0.046	0.90	-2.0	+1.9	1.361	0.198
%Change in P concentration	-3.1	-3.7	+3.0	+0.1	1.70	1.70	2.40	0.61	0.036	0.77	-5.6	+6.1	3.59	0.104
%Change in PSAB	-16.3	-14.5	-12.2	-6.0	3.39	3.39	4.79	0.44	0.222	0.69	-9.5	-2.0	7.75	0.614

CBT, cortical bone thickness; SG, specific gravity of cortical bone; P concentration calculated from SG; PSACB, P in cortical bone per unit surface area of cortical bone,A, sem and probability calculated from the 2x2 factorial of low or high diet P during pregnancyt and lactation. B, sem and probability calculated from the one-way ANOVA of all six treatments. Lower case superscript letters indicate differences between means based on the one-way ANOVA. LW, liveweight.

Table E-12. Pens_E. LACTATION. The *tuber coxae* biopsy trabecular bone volume (BV/TV, %), trabecular thikness (Tb.Th, µm) and the separation between trabeculae (Tb.Sp, µm) in mature cows during the interval when cows were lactating. Cows ingested Low P or high P diets during mid-late pregnancy while grazing senesced pasture and given supplements, and from calving the cows in each of these pregnancy treatments were fed Low or High P diets in pens for 14 weeks (treatments Low-Low, High-Low, Low-High and High-High). SD, standard deviation

Measurement	Mature Cows							
	LowP-LowP		HighP-LowP		LowP-HighP		HighP- HighP	
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
Calving								
BV/TV (%)	9.2	1.8	12.0	1.40	10.4	1.3	11.6	2.9
Tb.Th (µm)	120	12.4	123.5	12.1	120.5	16.0	117.8	15.2
Tb.Sp (µm)	988	96	812	71	931	178	843	215
Weaning								
BV/TV (%)	8.7	1.2	9.5	1.0	9.6	2.3	11.0	2.9
Tb.Th (µm)	107	11.3	103	12.6	105	5.7	124	21.4
Tb.Sp (µm)	957	134	880	138	930	225	896	164

Table E-13. Pens_E. The resumption of ovarian activity in cows measured by ultrasound scanning. The months of lactation indicated is from the mean calving date (23 October 2015). Cows ingested Low P or high P diets during mid-late pregnancy while grazing senesced pasture and given supplements, and from calving the cows in each of these pregnancy treatments were fed Low or High P diets in pens for 14 weeks (treatments Low-Low, High-Low, Low-High and High-High). CL, observation of a *corpus luteum* or *corpus albicans*; Reproduction score was on a scale 1-5, PPAI, Post partum anoestrus interval.

Measurement	LowP-LowP	HighP-LowP	LowP-HighP	HighP-HighP	s.e.m.			Probability		
n	8	8	8	7	Preg	Lact	PxL	Preg	Lact	PxL
14 Dec 15 (1.7 mo lact)										
With follicles (%)	93	88	88	82						
With CL (%)	13	0	13	13	0.07	0.07	0.10	0.565	0.527	0.527
Reprod score (1-5)	3.1	2.5	3.0	3.3	0.18	0.18	0.26	0.538	0.201	0.085
11 Jan 16 (2.6 mo lact)										
With CL (%)	50	25	38	61	14	14	19	0.976	0.541	0.217
Reprod score (1-5)	3.8	3.5	3.6	4.4	0.32	0.32	0.45	0.576	0.407	0.274
11 Feb 16 (3.6 mo lact)										
With CL (%)	88	25	88	100	9.3	9.3	13.1	0.071	0.010	0.010
Reprod score	4.6	3.9	4.9	5.0	0.19	0.19	0.27	0.283	0.017	0.109
10 Mar 16 (4.5 mo lact)										
With CL (%)	88	63	88	100	9.1	9.1	12.8	0.714	0.129	0.129
Reprod score	4.9	4.3	4.8	5.0	0.14	0.14	0.20	0.436	0.109	0.033
18 Apr 16 (5.8 mo lact)										
With CL (%)	100	75	88	100	6.6	6.6	9.3	0.572	0.453	0.048
Reprod score	4.8	4.3	4.8	5.0	0.14	0.14	0.20	0.436	0.109	0.033
Estimated PPAI (days)	97	143	105	84	10	10	14	0.405	0.086	0.030
Pregnant (%)	88	38	63	100	11	11	16	0.707	0.237	0.011

Calf growth

There was no effect of the diet treatments during late pregnancy on birth weight of the calves which averaged 34.6 kg. The growth rate of calves suckling cows fed HighP diets was 0.93 kg/day, while growth rate of calves suckling cows fed LowP was decreased (P<0.01) to 0.80 and 0.72 kg/day for HighP-LowP and LowP-LowP treatments, respectively (Table E-7). Since these latter calf growth rates did not differ (P<0.05) there was no statistically significant evidence of a carryover effect of the HighP diet during pregnancy increasing lactation performance of P-deficient cows. The calves suckling the cows fed the LowP diet during lactation were 17 kg lighter at 14 weeks of age than the calves suckling the HighP diet.

Changes in rib and tuber coxae bone

There were no differences among the cows in the cortical bone thickness (CBT), P concentration or PSACB at calving following the Period 1 treatments (Table E-11); thus there was no discernible effect of the LowP and HighP diets fed during pregnancy. Averaged across all treatments the 14 weeks of lactation decreased the CBT by 15.4% and PSACB by 12.2% indicating that there was substantial net mobilization of rib bone P during early lactation in cows fed both the LowP and the HighP diets. However there was also an effect of diet during lactation; after 14 weeks the P concentration in cortical bone was lower (P<0.05) in the cows fed the LowP diet than in cows fed the HighP diet indicating greater bone P mobilization in the cows fed the LowP diet. The weaner heifers at the end of Period 1 (i.e. at calving in the cows) had lower CBT and PSACB than the cows (Table E-11) and both CBT and PSACB tended to decrease during Period 2 in the heifers given the LowP treatments through pregnancy and lactation. There were no differences in these weaner heifers due to the diet P treatment during Period 1.

At calving cows fed LowP in pregnancy had less *tuber coxae* bone volume (mean 9.9%) than cows fed HighP diets (11.9%). Trabecular thickness was very similar between groups but cows fed LowP diets had greater average separation between trabeculae indicating loss of these structural elements (Table E-12). After 14 weeks of lactation all cows had lost bone volume but this was most pronounced in the cows transitioning from HighP in pregnancy to LowP in lactation; these cows fed HighP-LowP diets lost about 20% of their BV/TV over the lactation period. Cows fed HighP in both pregnancy and lactation maintained bone volume, trabecular thickness and trabecular separation over lactation and still had a BV/TV of 11% at weaning. Cows which were given the LowP-LowP treatment had very low mean BV/TV at weaning (8.7%) and thin trabeculae with large separation between them indicating loss of trabecular structures. Once trabeculae have been lost it is difficult to restore bone without the required structural framework. Cows fed the LowP-HighP or HighP-LowP diets finished lactation with similar bone volume (9.5 and 9.6%) and trabecular thickness that was higher than in LowP-LowP but lower than in HighP-HighP treatment groups. Because these cows did not have prolonged periods consuming LowP diets it is likely they were able to maintain enough structural integrity of the trabecular bone to prevent severe loss of bone.

Post-partum anoestrus interval

Follicles were observed in 88% of the cows on the 14 December 2015 (DOY 348) and 50 days after the mean calving date (Table E-13). On the 11 Feb 2016 (109 days after the mean calving date) and near the end of the diet treatments fed during lactation 75% of cows had a CL or a CA. The cows given the HighP-LowP treatment were consistently lower (P<0.05) in showing a CL/CA and in the reproductive score on the 11 February 2016, in reproductive score on the 10 March 2016, and in

each measure of reproductive activity (percent showing a CL, reproductive score, estimate PPAI and percent pregnant) at the last measurement date on the 18 April 2016. This observation of lower reproductive activity in cows fed a higher P diet only during pregnancy needs to be considered with caution given the small number of cows in each group.

Plasma PIP, Ca and Mg during pregnancy and lactation

During pregnancy the PIP concentrations of cows given the LowP treatment were consistently lower (P<0.001) than that of cows given the HighP treatment (1.75 and 2.13 mmol/L, respectively; Figure E-4), and also the PIP concentrations of LowP cows tended to decline (1.93 to 1.75 mmol/L) during the last two months of pregnancy. Thus although the LowP diet did have some effect during pregnancy the PIP concentration was not reduced to <1 mmol/L as occurs during severe and prolonged P deficiency and which is indicative of severe P deficiency. There was no evidence of a difference in the PIP concentrations of the weaner heifers given the LowP and the HighP treatments for almost the same interval.

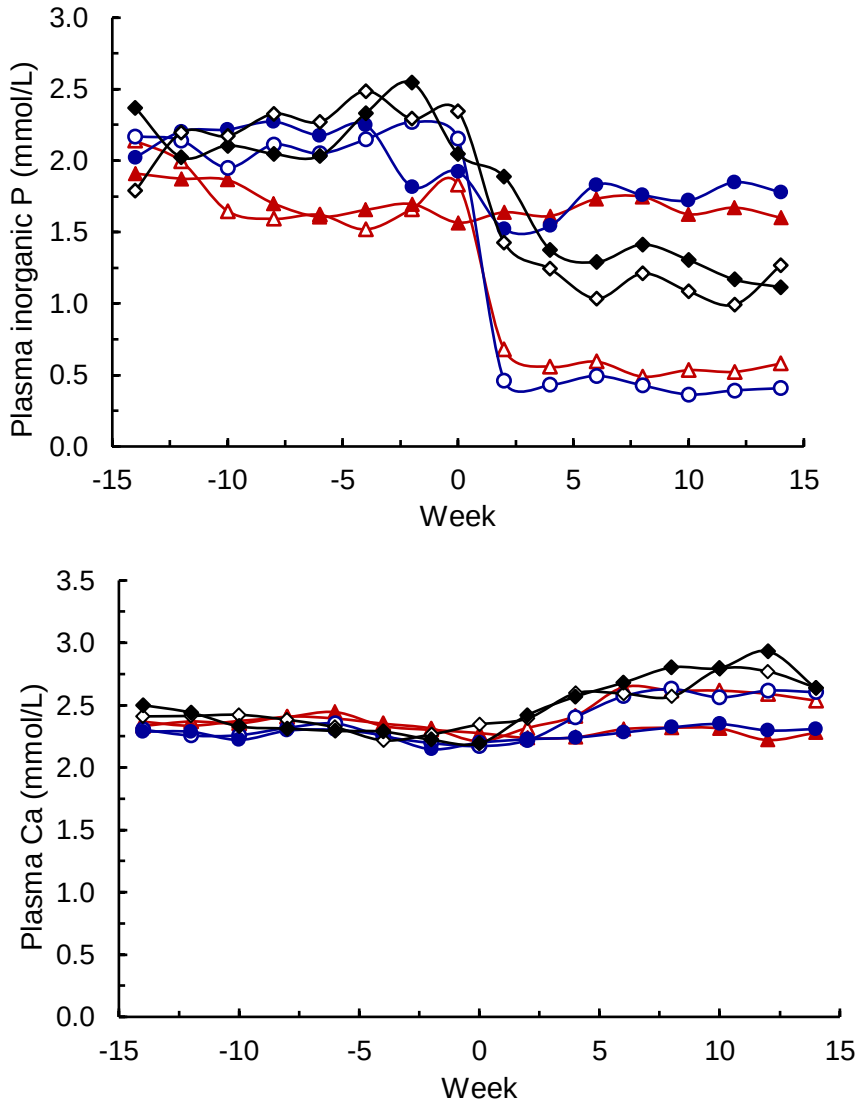


Figure E-4. Pens_E. Plasma PIP (E-4A) and total calcium (Ca) (E-4B) concentrations in the cows during late pregnancy, and in the sentinel heifers during the same interval. The diet treatments were LP-LP (Δ), HP-LP (○), LP-HP (▲) and HP-HP (●) in the cows, and LP-LP (◊) and HP-LP (♦) in the heifers.

At calving the PIP concentration of HighP-LowP cows decreased abruptly from 1.8 mmol/L shortly before calving to 0.4-0.5 mmol/L during early lactation (Figure E-4). PIP concentrations in LowP-LowP cows fed the same low P diet during lactation declined more gradually, and during lactation were often higher ($P<0.05$; 0.5-0.6 mmol/L) than the PIP concentrations in HighP-LowP cows (Figure E-4). Physiological mechanisms, most likely greater bone P mobilization, had initiated in these LowP-LowP cows during pregnancy so that for at least the first 14 weeks of lactation P availability was greater than in the HighP-LowP cows. Cows given HighP diets during lactation maintained similar PIP concentrations in lactation (1.7-1.9 mmol/L) regardless of whether they had been fed LowP or HighP diets in pregnancy (Figure E-4). The weaner heifers given both the LowP and the HighP treatments maintained high PIP (>2 mmol/L) during Period 1 (i.e. when the cows were pregnant). In contrast, there was a large difference between the heifers during Period 2 when the cows were lactating. The LowP diet cased an abrupt decrease in PIP of the lactation cows but a more gradual decline in the heifers and even after 14 weeks the PIP concentrations were *ca*.1.3 mmol/L. There was apparently no effect of diet P during the previous period 1. At all times during lactation the PIP concentrations of LowP-LowP cows were lower ($P<0.01$) than PIP concentrations in LowP-LowP heifers.

During both pregnancy and lactation the cows on LowP diets exhibited higher plasma total Ca concentrations than cows on HighP diets (Figure E-4), suggesting that there was greater mobilization of bone minerals in the LowP diets. Whilst only a modest difference in plasma total Ca concentrations was seen between LowP and HighP diets during pregnancy (2.24 versus 2.19 mmol/L respectively), the effect of diet P on total Ca was more pronounced in lactation (2.28 versus 2.52 mmol/L). It should be noted that these Ca changes were a result of diet P, both within pregnancy and lactation, and were within the normal homeostatic range for plasma total Ca concentrations.

The Ca/P ratio values in this experiment, as a marker of bone mobilisation, further supports the hypothesis that cows mobilise more bone mineral reserves in lactation than pregnancy (irrespective of diet P), and that LowP diets in either physiological state result in increased bone mobilisation. During pregnancy the Ca/P ratio of cows on HighP diets was low (*ca* 1.1) and did not change up to calving, whilst the Ca/P ratio in cows on LowP diets increased during pregnancy (Figure E-5) and overall was higher (*ca* 1.4). In lactation the Ca/P ratio of cows remaining on the HighP diet (HP-HP) increased slightly to 1.4, similar to cows that were changed to the HighP diet in lactation after the LowP diet in pregnancy (LP-HP). In the other diets (LP-LP and HP-LP) there were marked increases in the Ca/P ratio in lactation (Figure E-4), suggesting greater bone mobilisation in cows on these diets.

In weaner heifers fed the LowP diet the plasma total Ca concentration was higher (2.66 mmol/L) than that in lactating cows fed the LowP diet (2.52 mmol/L, Figure E-4). This could suggest that these young animals ingesting the P-deficient diet were able to mobilize relatively more bone than the mature lactating cows. The lower plasma concentrations of Ca and P in mature cows likely reflect mineral loss for lactation demands. A better comparison is obtained by the relative differences in plasma concentrations of both Ca and P, by examining the Ca/P ratio (Figure E-5). These results suggest that the weaner heifers do not mobilise as much bone mineral reserves as lactating mature cows given the same LowP diets.

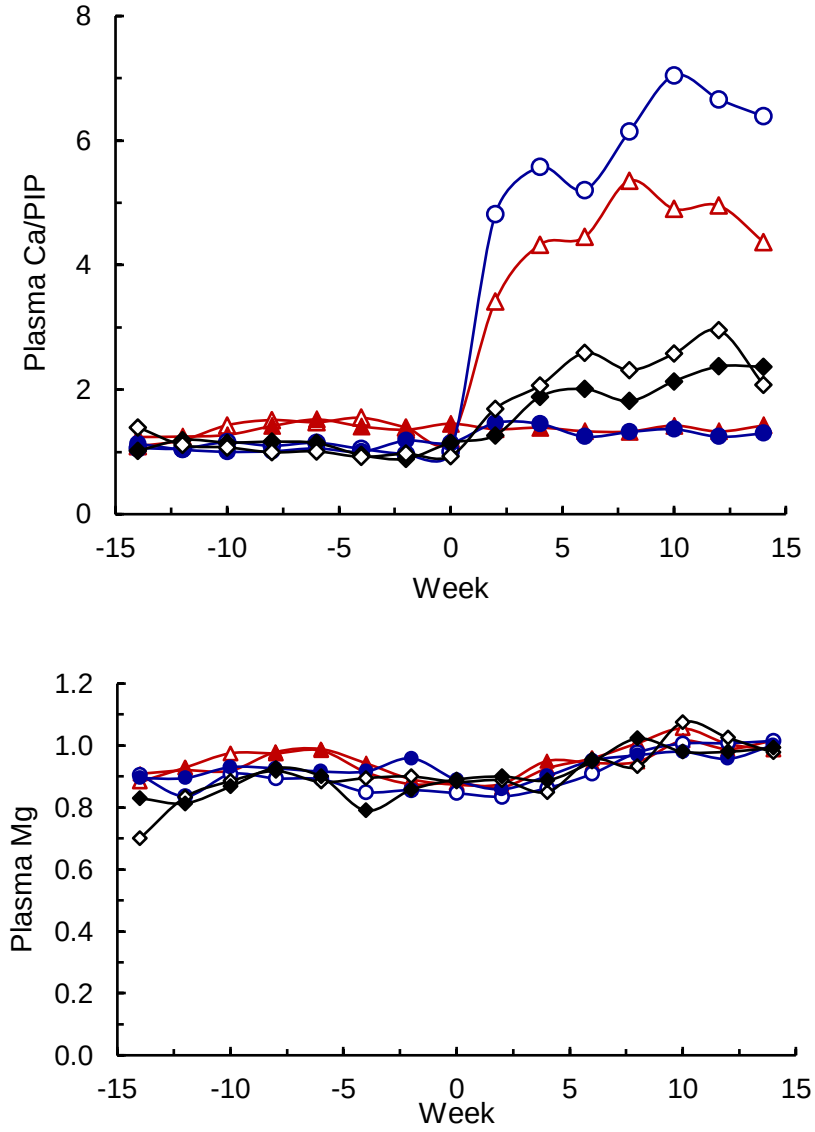


Figure E-5. Pens_E. Plasma Ca/P ratio (E5-A) and plasma Mg (E-5B) in the cows during late pregnancy and early lactation, and in the sentinel heifers during the same interval. The diet treatments were LP-LP (Δ), HP-LP (○), LP-HP (▲) and HP-HP (●) in the cows, and LP-LP (◊) and HP-LP (♦) in the heifers.

Bone markers

During late pregnancy plasma CTX-1 concentrations were significantly (P<0.05) higher in cows given the LowP diet treatment than in cows given the HighP diet treatment (Figure E-7). Plasma CTX-1 concentrations steadily increased in all cows from two weeks prior to calving through to early lactation. In cows given HighP diets in lactation plasma CTX-1 concentrations were significantly (P<0.01) lower than LowP diets; the CTX-1 concentrations in the HighP treatment cows peaked at one month of lactation before slowly declining as lactation advanced. In contrast, cows given LowP diets during lactation (HP-LP and LP-LP) exhibited substantial increases in plasma CTX-1 concentrations in lactation up to eight weeks of lactation, but with no further increase up to 13 weeks of lactation (Figure E-7). In weaner heifers plasma CTX-1 concentrations were higher than in mature cows on the same diets during Period 1 when the cows were pregnant, and there was a small but significant (P<0.05) effect of the LowP diet to increase CTX-1 in the heifers as was observed

in the mature cows. In the lactation phase plasma CTX-1 concentrations increased in both treatment groups of heifers but the increase was larger (P<0.05) in the heifers given the LowP diet treatment during Period 1. The concentration of CTX-1 in the heifers were much lower than in the cows fed the lowP diet. This result further supports the validity of the Ca/P ratio as a marker of bone mobilisation and the hypothesis that heifers do not mobilise as much bone P reserves as lactating mature cows when both are consuming LowP diets.

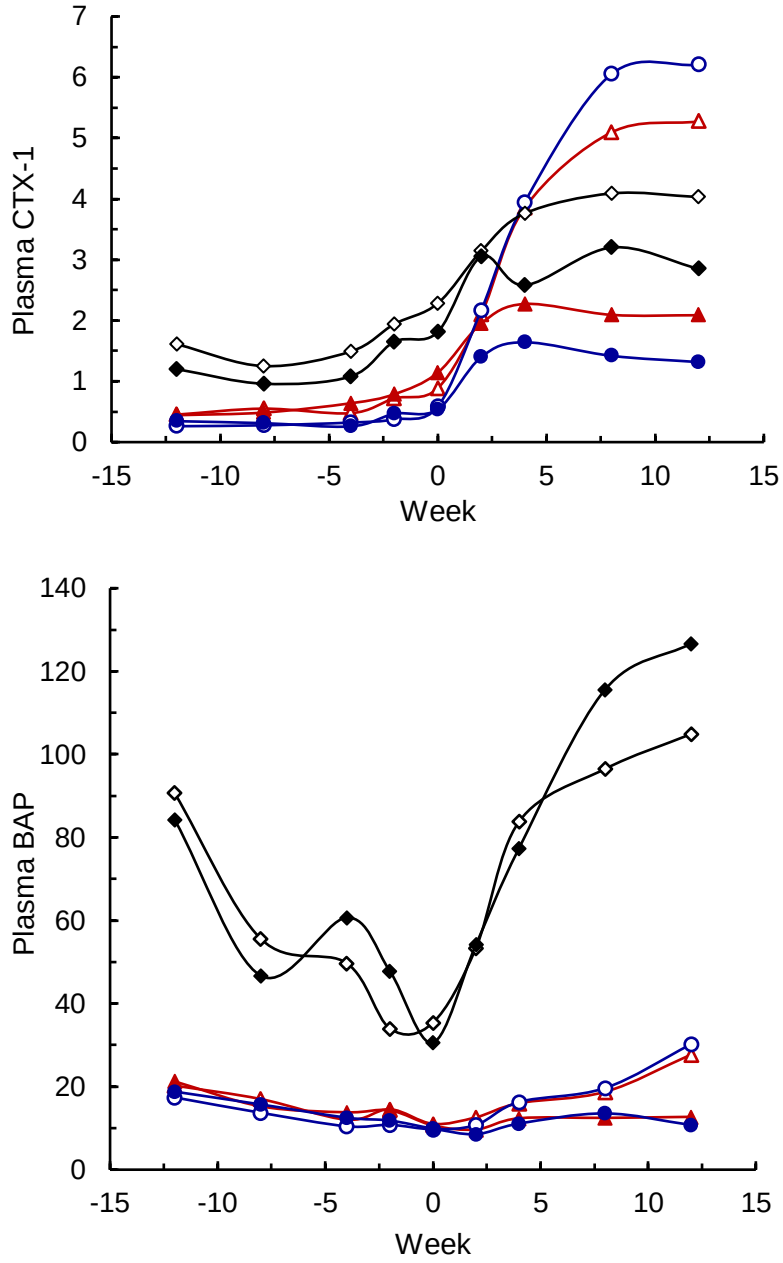


Figure E-6. Pens_E. Plasma CTX-1 (6A) and plasma BAP (6B) concentrations in the cows during late pregnancy and early lactation, and in the sentinel heifers during the same interval. The diet treatments were LP-LP (Δ), HP-LP (○), LP-HP (▲) and HP-HP (●) in the cows, and LP-LP (◊) and HP-LP (♦) in the heifers.

Table E-14. Pens_E. LACTATION. The P intake and losses in faeces, urine and milk of mature cows and during lactation. Cows ingested Low P or high P diets during mid-late pregnancy while grazing senesced pasture and given supplements, and from calving the cows in each of these pregnancy treatments were fed LowP or High P diets in pens for 14 weeks (treatments LowP-LowP, HighP-LowP, LowP-HighP and HighP-HighP)

Measurement	LowP-LowP	HighP-LowP	LowP-HighP	HighP-HighP
P intake (g/day)	6.83	6.92	30.84	29.95
P (I-F) (mean of TC1 and TC2) (g P/day)	+0.78	-0.09	+6.62	+8.12
P urine (g P/day)	0.02	0.02	0.02	0.57
Estimated milk P (g P/day)	5.2	5.8	6.9	6.8
Estimated P retention (g P/day)	-4.5	-5.9	-0.3	+0.8
P intake (mg/kg LW.day)	14.9	15.3	64.4	65.0
P (I-F) (mean of TC1 and TC2) (mg P/kg LW.day)	+1.7	-0.2	13.8	17.6
P urine (mg P/kg LW.day)	0.0	0.0	0.0	1.2
Estimated milk P (mg P/kg LW.day)	11.4	12.7	14.4	14.7
Estimated P retention (mg P/kg LW.day)	-9.8	-12.9	-0.6	+1.7

Plasma BAP concentrations in cows were generally low (<30 U/L) and declined during late pregnancy to reach a nadir (ca 10 U/L) at calving (Figure E-6B). Also during pregnancy there was no significant diet P effect on BAP concentrations. The plasma BAP concentrations were much higher in the weaner heifers (>65 U/L in heifers) than in the cows at the start of Period 1 and declined markedly during Period 1 (pregnancy in the cows) to reach a nadir (*ca* 30 U/L) at the time when cows were calving. There were no diet P effects on BAP concentrations in the heifers. In early lactation (2 weeks post-calving) plasma BAP concentrations were low across all diet groups. However plasma BAP concentrations subsequently increased markedly in both the lactating cows (HP-LP and LP-LP) and the weaner heifers given the same LowP diet (Figure E-6). Also the heifers had higher (P<.0.05) BAP concentrations than the cows fed the HighP diets (HP-HP and LP-HP).

4.5.5 Discussion

During Period 1 when the breeders were pregnant and the cows and the weaner heifers and steers grazed the senesced pasture a substantial proportion of their ME intake would have been from the molasses-urea supplement. Calculations based on the concentration of P in the sampled pasture and the molasses-urea indicated that the Low P treatment breeders and weaners would have been ingesting about 39-67% and 52-84% of their respective calculated P requirements and were therefore in P deficiency. It appeared that the degree of this P deficiency was not nearly as severe as in the heifers in the Pens_A or the Pens_D experiments. During period 1 the PIP in the cows was lower than in the HighP treatment, there was a decline in PIP during late pregnancy, and the CTZX-1 concentration and the plasma Ca/P ration were also higher. There was also evidence for bone mobilization in the breeders during Period 1 from the bone biopsy samples of the *tuber coxae* at calving; there was a lower bone volume and the greater separation between trabeculae. It appeared that these mature cows in late pregnancy could accommodate substantial P deficiency during the last 2-3 months of pregnancy without overt effects on their LW at calving or on the blood or bone markers of P status. Similarly the weaners were able to tolerate a substantial deficient in P intake for about 3 months without discernible effects on the attributes measured. These results are in accord with Coates *et al.* (2018) where in steers subjected to severe P deficiency for about 12 months the treatment differences did not appear for several months when the animals were grazing dry season pastures. Similarly in breeders Dixon *et al.* (2016) and Coates *et al*. (2018) observed little or no effect of severe P deficiency on breeder cow LW through the dry season; the effects of the P deficiency on LW or PIP did not become evident until the wet season when these effects were large and where about 30% of the bone P in the animal were mobilized.

In the present experiment the effects of P deficiency during the pen phase were large in the lactating breeders but relatively minor in the weaner heifers. It was calculated that in the lactating cows the diet P intake was about 28% of the requirements, whereas in the heifers it was about 50% of their requirements. This difference was associated with the relatively high demands of lactation compared with the slow rate of growth of the heifers. In the lactating cows the P deficiency on VI and on blood PIP concentrations were observed immediately and within two weeks of the introduction of the changed diet. The VI of the P adequate cows averaged 21.1 g DM/kg LW and was decreased by 20% by the P deficiency which was comparable with the decrease observed in other experiments in the present program and with other studies. There was a similar decrease in ME intake and a LW gain of the P adequate cows was reduced to a substantial LW loss so that there was a 54 kg difference in LW after three months lactation. The P balance results depended on estimates of the milk P production derived from calf growth and were thus subject to some uncertainty. Nevertheless, these calculations indicate that the P adequate breeders were on average during lactation neither gaining nor losing P during the three months of lactation, whereas the P deficient breeders were mobilizing about 5 g P/day averaged across the lactation (Table E-14). This would be equivalent to mobilizing 500 g P or about 20% of the total skeletal reserves of P during the first three months of lactation. The differences between the two total collection intervals (TC01 and TC02) the intake and faecal excretion of P (Table E-9) also indicated that the amount of P mobilized decreased markedly as lactation progressed; at the first total collection measurement apparently about 4 g P/day were being deposited in body tissues, but by the second measurement interval this had declined to nil or a small net mobilization. The effects of the P deficient diet were also observed as

immediate decreases in PIP and Ca/P ratio (within two weeks at the first sampling after parturition). However it required about 6 weeks after parturition for CTX-1 concentrations to increase to what appeared to be a new plateaux. There was some evidence that the Ca/P ratio was increasing up to 10 weeks of lactation and that some time was required for adaptation to a severely P deficient diet.

The performance of the weaner heifers to the same low P diet was substantially different to that of the lactating breeders. PIP was not decreased to the same, or even a similar, extent(Figure E-4) and neither CTX-1 nor Ca/P ratio was increased to the values observed for the lactating breeders, and did not require the lengthy time observed in the breeders to increase. An important consideration for use of BAP as a marker of bone deposition was that the concentrations were much higher (up to about four times higher) in the heifers than in the breeders given the same P deficient diet. If BAP is to be used as a marker of bone growth caution is needed that indicator values need to be specific for various classes of animals.

The most important finding of this work was that when mature breeders calved in moderate to high P status they were able to largely maintain milk production and support calf growth over at least three months of lactation. However the P deficiency caused a large decrease in VI and thus of ME intake, and the maintenance of milk production by the cow was associated with large adverse effects on breeder cow LW and mobilization of bone P. Although this may be acceptable in the interval of early lactation it will have potentially serious consequences on the body reserves of both energy (fat) and of P by the end of lactation.

5 General Discussion

Phosphorus deficiency is widespread in cattle grazing northern Australian rangelands and has serious consequences for welfare and productivity of perhaps 6 million cattle. For a number of reasons perhaps only 10% of cattle across the northern Australian rangelands are effectively managed and/or supplemented to address a P deficiency .

A substantial body of research during the last five decades has shown that in some circumstances during the reproductive cycle in sheep, goats and dairy cattle fed P-deficient diets a large amount of P may be mobilized from the body reserves . This P is primarily from bone and is used to meet the high demands for P during late pregnancy and lactation. Research with sheep has reported that up to 40% of P body reserves could be mobilized and research with other species (including tropical cattle) that 20-30% of body P reserves could be mobilized. In a 400 kg breeder cow with about 3 kg bone a 30% mobilization of body reserves P would be equivalent to about 1000 g P. In the context of northern breeder systems the expectation is that net mobilization of P from body reserves would occur in very late pregnancy and in early to mid-lactation. This is likely to be an important physiological mechanism for breeder cows grazing P-deficient pastures in northern Australia to meet the high demands for P during late pregnancy and early lactation and thus ensure calf survival and growth. In 'normal' northern breeder management the late pregnancy interval is likely to coincide with late dry season and early-mid wet season, and early lactation with mid to late wet season.

There is evidence, particularly from southern Africa, that there are benefits of P supplementation for the breeder cow during the mid to late dry season and coinciding with late pregnancy and early-mid lactation. This occurs despite the general absence of observed responses to P supplements in growing cattle. For practicality and convenience most P supplements fed in the northern Australian industry are fed with nitrogen supplements during the mid to late dry season, and numerous anecdotal reports from producers suggest substantial benefits to inclusion of some P into dry season N supplements. There is also South African research reporting responses of breeders to P supplements fed during the dry season. An important issue is that when P supplements are fed during the dry season they should always be fed with N. There is evidence from southern Africa that P supplements fed without inclusion of N can have adverse effects. A substantial amount of P is required for conceptus growth in late pregnancy, and P supplements should have a benefit to provide P which would otherwise have to be derived from bone mobilization in the breeder during the dry season; thus P supplements fed during the late dry season can be expected to preserve bone P reserves for use during lactation.

The following discussion addresses the outcomes of the various experiment of the present project in the context of mobilisation and repletion of body reserves, and particularly of P.

A list of the publications arising from this project to date are given in Appendix 1.

5.1 The consequences of diet P deficiency or adequacy in breeders and growing cattle in various physiological states

The following commentary has been grouped in according to class of stock; mature breeders, heifers and first calf cows, or young growing animals.

5.1.1 Ability of the mature cow to accommodate P deficiency during early lactation

Key aspects of the Pens_C experiment were:

1. Mature *Bos indicus* cross (Droughtmaster) breeders were fed a high P diet (whole cotton seed, cottonseed meal, straw) for the last 2-4 months of pregnancy to parturition.

2. The diet treatments which were fed *ad libitum* comprised one P-adequate diet and three P-deficient diets where (i) the ratio of Ca/P in the diet was modified or (ii) a negative DCAD diet examined. The AdeqP diet contained 2.5 g P/kg DM and the 3 LowP diets 0.7 g P/kg DM. All diets contained 9.6-9.9 MJ ME/kg DM.

3. The AdeqP cows consumed 21.2 g DM/kg LW (*ca.* 9.8 kg DM/day), gained 18 kg LW during the 14 weeks of lactation, produced 5.8 kg milk/day. Growth of the calves was 0.70 kg/day. PIP averaged 1.5 mmol/L and was somewhat lower than expected for cows fed a P adequate diet. Calf growth was only moderate compared with that expected for the animal genotype fed a high ME content diet (as observed in other experiments).

4. In cows fed the severely P deficient diets milk output and calf growth were maintained to be similar to the cows fed the P adequate diet.

5. Voluntary intake was reduced in the severely P deficient diets but breeders maintained milk output and calf growth by extensive mobilization of body reserves. This was particularly evident for soft tissues with LW loss of about 20 kg (in contrast to the LW gain of 20 kg in the adequate P diet). Body reserves as bone P were reduced due to these P deficient diets.

6. Cows fed the LowP – negative DCAD diet were better able to accommodate the diet P deficiency with a greater capacity to maintain voluntary intake and lesser loss of LW. This was associated with greater mobilization of bone P to alleviate the diet P deficiency.

Key aspects of the Pens_E experiment were:

1. The voluntary intake during lactation was reduced substantially (by *ca.* 20%) by a severely P deficient diet providing only 28% of the calculated P requirements. Breeders were able to largely maintain milk output and calf growth during this severe P deficiency, but at the expense of LW. The P deficient breeders lost 0.37 kg LW/day during lactation. The P-adequate breeders gained almost 0.26 kg LW/day.

2. Calf growth of 0.92 kg/day in the cows fed the P-adequate diet was reduced significantly to 0.80 kg/day in cows fed the LowP diet. This difference in calf growth alone would not justify P supplementation.

3. There was little discernible effect on the breeders of a sub-optimal P intake (*ca.* 60-70% of animal requirements) during the last four months of pregnancy. Although there was a decrease

in PIP during late pregnancy associated with this sub-optimal P intake the breeders were able to accommodate for this presumably from body P reserves without any discernible effects of their performance during lactation.

These two experiment showed that mature breeders calving in good body condition have considerable capacity to use body reserves for calf growth and could do so despite moderate P deficiency in late pregnancy. A constraint of these experiments was that they did not examine the consequences of severe P deficiency during pregnancy.

5.1.2 Ability of the first-calf cow (FCC) to accommodate P deficiency during early lactation.

Key aspects of the Pens_A experiment were:

1. The main effect of LowP diets in lactation was to cause a major decrease in diet DM intake, to cause a large LW loss during lactation, to reduce milk output, and to reduce calf growth. These adverse effects during lactation were greater in these FCC than in mature breeders.

2. There were positive effects of the P adequate diet during pregnancy (HP) on FCC performance during lactation. A lower FCC LW at calving had carryover effects to seriously reduce DM intake and calf growth during lactation.

3. In heifers fed the HP-LP diet treatment (HP in pregnancy and LP in lactation) the feed intake during lactation was reduced by 42% by the LowP diet. Heifers also fed the AdeqP diet in lactation gained 25 kg during three months of lactation while cows fed the LowP diet in lactation (HL) lost 65 kg during lactation. Milk output was reduced from 8.7 to 6.5 kg/day (25% reduction) and calf growth was reduced from 0.93 kg/day to 0.69 kg/day

4. In the heifers fed the P deficient diet in pregnancy the intake during lactation of the AdeqP heifers approached that of the heifers fed adequate P in pregnancy (9.4 versus 10.9 kg DM/day). The intake of the heifers fed the P deficient diet was 4.9 kg/day and hence was only 52% of the intake of the LH heifers. These LH heifers gained 49 kg LW during lactation compared with a LW loss of 73 kg in the LL heifers. Calf growth of the LL heifers and the HL heifers were 0.57 and 0.69 kg/day indicating that the AdeqP diet in pregnancy allowed heifers to maintain a substantially higher milk output and calf growth post-calving.

5. The HL heifers had a higher intake than the LL heifers (6.3 and 4.9 kg DM/day) indicating that the AdeqP diet in pregnancy allowed heifers to maintain a substantially higher DM intake during lactation.

Key aspects of the Pens_D experiment were:

1. In lactation all the FCC were fed a P deficient diet and lost liveweight. The effects of diet during pregnancy on calf growth was explained by body condition score at calving rather than by diet treatment during pregnancy.

These two experiments showed that lactating FCC lost both liveweight and bone P, but despite rapid mobilization of body reserves calf growth rate was adversely affected. Also FCC were more adversely affected than mature cows by diet P deficiency in lactation.

5.1.3 Ability of the heifer / cow to accommodate P deficiency during late pregnancy.

Key aspects of the Pens_A experiment were:

1. Diet quality was sufficient for the Adeq-P heifers fed a P adequate diet to gain ca. 50 kg CF-LW during late pregnancy. The LowP heifers almost exactly maintained CF-LW and this was associated with a 23% lower DM intake.

Key aspects of the Pens_D experiment were:

1. Restricted amounts of diet were fed so that the heifers lost about 50, 28 or nil kg CF-LW during the last three months of pregnancy. Heifers were expected to be initially in a high P status.
2. There was no effect of diet P on intake of DM or ME in the moderate and severe LW loss treatments. The heifers must have been able to obtain sufficient P from the diet + tissue mobilisation to maintain the intake of the diet.
3. In the NIL CF-LW change treatments the P adequate AdeqP heifers ingested all the diet offered, but the LowP heifers ingested less DM, had a ME intake similar to the moderate LW loss treatments, and had CF-LW change similar to the moderate CG-LW loss treatments. The PIP in the LowP heifers fed at energy maintenance was ca. 0.8 mmol/L i.e. not severely deficient.
4. The diet ME was apparently used with the same efficiency for CF-LW maintenance regardless of whether the diet was adequate or deficient in P.

Key aspects of the Pens_E experiment were:

Mature cows recently weaned, in good body condition and likely in moderate P status. Grazed as two herds on senesced pasture and fed molasses-urea supplement. The P deficient cows were ingesting 60-70% of the expected P requirements, and the P adequate cows at 120% of P requirements. Both herds of cows lost ca. 10 kg (0.1 kg CF-LW per day) in pregnancy and this was not different between the two P treatments.

These experiments showed that in FCC there were major adverse effects with lower LW at calving. If heifers were gaining or maintaining CF-LW the severe P deficiency reduce voluntary intake and reduced bone P at calving. Mature cows were less affected by P deficiency in late pregnancy.

5.1.4 Ability of the young growing cattle or mature cows in mid-pregnancy and post-weaning to respond to high diet P versus low diet P intakes

1. In mature cows given the moderate or high ME content diets the provision of adequate diet P increased LW change and accretion of bone P. Effects were much larger in the high ME content diet.

2. Young (180-250 kg) growing cattle were much less affected by P deficient diets than were lactating cows. This may largely be due to the lower requirements of the growing than the lactating animal (in mg P per kg animal LW, or mg P per kg diet DM) but also to the capacity of this class of animal to continue moderate LW gain while mobilizing bone minerals including P.

5.2 Physiological mechanisms in pregnancy and lactation

A primary objective of the project was to improve our current understanding of the physiological mechanisms controlling mobilisation and deposition of body P reserves in breeder cattle. The following key results and conclusions can be drawn from the series of experiments in this project.

Diet P deficiency (compared to diet P adequacy) in breeder cows can in summary be characterised by the following changes in biochemical and hormonal markers in plasma:

- decreased inorganic P
- increased total Ca
- unchanged or slightly increase Mg
- increased Ca to P ratio
- decreased PTH
- increased active 1,25 diOH vitamin D3
- no change in precursor 25 OH vitamin D3
- increased CTX-1, as a marker of bone mobilisation
- no change in OCN, as a marker of bone formation
- increased BAP, as a marker of bone formation/mineralisation

These changes need to be considered in the context that some of these will also be modified by other nutritional deficiencies such as metabolisable energy, protein and/or Ca.

5.2.1 Pregnancy

During pregnancy the requirements for P and Ca increase markedly in the last trimester, and in cattle escalate in the last few weeks before parturition. This was apparent in cows fed high P diets in the present study with both plasma PIP and Ca concentrations often decreasing slightly in the last month before calving (Pens A, D and E). Coincident with this decrease in plasma mineral concentrations there was a small increase in plasma CTX-1, indicative of increased bone mobilisation (Pens A, D and E). Throughout most of pregnancy the P and Ca demand for foetal skeletal growth is small and with high P diets there is no need for mobilisation of bone P stores to provide P and Ca for this need. This was illustrated by the small changes in CTX-1 concentrations in cows fed high P diets from mid to late pregnancy (Pens B).

In contrast cows fed low P diets in pregnancy had markedly increased bone mobilisation in an attempt to maintain P homeostasis (Pens B, D and E). This increase on low P diets appeared substantial, as CTX-1 concentrations in P deficient pregnant cows were equivalent to concentrations usually observed only in lactation. Further the amount of bone mobilisation, as indicated by CTX-1 concentrations, was quite similar in cows on different ME diets (Pens B and D). It appears that the degree of mobilisation of soft tissue P reserves via LW loss did not influence the degree of bone mobilisation. The bone marker results suggest the relative contribution of soft tissue P reserves to maintain homeostasis in P deficiency during pregnancy is minor.

An important finding was that plasma total Ca concentrations increased over time in pregnant cows given low P diets (Pens B and D) although the increase in P deficient cows was within the normal homeostatic range for Ca. Such an increase in plasma Ca concentrations most likely indicates mobilisation of bone mineral, since 99% of body Ca reserves occur in bone as hydroxyapatite. However plasma Ca concentrations represent the net flux between various body compartments and an increased uptake of Ca from the gastrointestinal tract would also contribute to plasma Ca. We observed an increase in active 1,25 diOH Vitamin D in pregnancy as a result of low P diets. The increase in 1,25 diOH Vit D concentrations is likely a direct effect of low PIP on 1 alpha-hydroxylase expression and production of active 1,25diOH Vit D in the kidneys. The other major regulatory hormone that stimulates active Vitamin D3 production is PTH, but plasma concentrations of PTH were suppressed in cows on low P diets during pregnancy (Pens A, B). It should be noted that the increase in active 1,25 diOH Vitamin D in cows as a direct effect of low plasma PIP concentrations, as shown in the current experiments, challenges the perception that low P does not increase active Vitamin D in ruminants (unlike monogastrics). In our experiments there are clear increases in 1,25 diOH vitamin D3, and not the precursor 25 OH Vitamin D3. The results suggest that low P diets fed to pregnant cows results in increased plasma Ca concentrations within the normal homeostatic range, and this increase is likely a result of increased bone mobilisation together with increased gut uptake of Ca mediated by increased 1,25 diOH Vitamin D3 (not PTH).

During pregnancy plasma OCN concentrations were in general relatively stable in mid-pregnancy in mature cows (Pens B), and decreased in late pregnancy in young cows (Pens A and D). The overall plasma OCN concentration was observed to be much higher in young than in mature cows, indicative of continued high bone formation in young first calf cows (Pens A and D). In regards to diet P effects it was surprisingly that there were no major effects of low P diets on plasma OCN concentrations over time in cows fed low P diets (Pens A, B and D), suggesting no change in the rate of bone formation in such cows. Only in young cows were significant increases in plasma OCN observed as a result of feeding high P diets (Pens A). Altogether, from examining the ratio of CTX-1 to OCN concentrations as an indicator of bone turnover, it is apparent that low P diets are characterised by increased bone turnover. The main effect of P supplementation (high P diets) in reducing bone turnover in pregnancy is to decrease bone mobilisation in mature cows (Pens B). However in young growing first-calf cows (Pens A) there is a combined effect of P supplementation on reducing the rate of bone mobilisation together with an increase in degree of bone formation. These results suggest that P supplementation of young first-calf cows in late pregnancy favours net bone deposition.

The BAP results during pregnancy (Pens A and B) were quite different from the OCN results. Consistently across all experiments plasma BAP concentrations significantly increased in cows fed

low P diets. In contrast plasma BAP concentrations in pregnancy did not change markedly in cows fed high P diets. Also plasma BAP concentrations were quite similar in the young first-calf cows (Pens A) and mature cows (Pens B and E). A plausible explanation relates to the biological function of BAP. BAP is an enzyme that is expressed on the external surface of the osteoblast cell membrane, where it is thought to act in removing (hydrolyse) phosphate from various molecules, making the P available locally for hydroxyapatite formation. It is a marker of bone formation as circulating levels of BAP reflect the activity of osteoblasts, but more specifically this marker reflects the process of bone mineralisation. In cows on low P diets, there is low availability of PIP for bone mineralisation (low plasma PIP concentrations), so a consequence is that BAP levels are upregulated in bone to counteract whole body P deficiency.

5.2.2 Lactation

Overall in lactation many of the changes in blood minerals, hormones and bone markers due to low diet P were more pronounced as a result of increased mineral demand for milk production. In early lactation increased bone mobilisation to meet the mineral demand for milk production is a mandatory process in all animals, as dietary intake generally cannot meet demands.

During lactation first calf cows fed high P diets had plasma PIP concentrations well within the normal range, but in mature cows (Pens C and E) the PIP concentrations were marginal to low, likely reflecting higher milk production in older cows. Additionally plasma Ca concentrations in mature cows fed high P diets were at the lower end of normal range and were associated with higher PTH concentrations (Pens C and E). Together these results highlight that the physiological control of mineral homeostasis of cows in adequate P nutrient status in part reflects the interplay between PTH and Ca. Plasma CTX-1 concentrations, as a marker of bone mobilisation, show small increases in lactation in cows fed high P diets (Pens A, C, and E).

In cows fed low P diets during lactation plasma PIP concentrations were markedly and rapidly reduced (e.g. at the first blood sampling and within two weeks) in early lactation, even in animals previously fed high P diets during late pregnancy (Pens A, C, and D). This suggests plasma PIP concentrations as a marker primarily reflect current P intake in relation to the current demands for P by the animal, and are of limited value as an indicator of body P reserves. A steady increase in plasma CTX-1 was observed over the first month of lactation, and with ongoing low P diets CTX-1 remained high throughout lactation (Pens A, C, D, and E). Similarly there was an increase in the Ca/P ratio as lactation progressed in cows fed low P diets, which was reflecting an increase in Ca relative to decrease in PIP as a result of low P diets. Remarkably a plateau in CTX-1 concentrations and Ca/P ratios was observed in later lactation across all low P diets (Pens A, C, D and E). This suggests there is an upper limit to the rate of bone mobilisation that can occur in lactating cows on low P diets. Further evidence to support this concept was observed in the Pens C cows fed a negative DCAD diet; such diets are well-known to improve mineral homeostasis in dairy cows by increasing bone mobilisation. Breeder cows fed the negative DCAD in the Pens_C experiment had consistently higher CTX-1 concentrations in later lactation which was indicative of greater bone mobilisation. The mean CTX-1 concentrations in these cows also exhibited a plateau from six weeks to 14 weeks post-calving. There must be limits to the amount of bone P which can be mobilized. It seems possible that negative DCAD diets fed for long intervals could have adverse effects on the animal.

In P deficient cows the mechanisms of physiological control of mineral homeostasis in lactation appear quite different to cows fed high P diets. The substantial bone mobilisation observed in P deficient lactating cows was not associated with any increase in the main regulatory hormone PTH. Instead PTH concentrations were reduced in low P diets and in many cows were too low to be detected. This suppression of PTH secretion to plasma is most likely a consequence of increased plasma Ca concentrations. The physiological control of mineral homeostasis in P deficient cows is independent of the usual regulatory interplay between Ca and PTH. One candidate for physiological control is 1,25 diOH Vitamin D3, which beside increasing gut uptake of Ca and P is well-known to also increase bone mobilisation. It would appear that although 1,25 diOH Vitamin D3 concentrations were increased by low P diets in lactation, the timing of changes in active Vitamin D and CTX-1 were not the same in young and mature cows (Pens A and C). Therefore the signal(s) that drive substantial bone mobilisation in P deficient cows during pregnancy and lactation remain to be determined.

In lactation, as well as in pregnancy, the results for OCN and BAP were dissimilar. At calving plasma OCN concentrations are low and at a nadir, suggesting bone formation is suppressed by the physiological events at parturition. OCN concentrations then steadily increased over the first two months of lactation (Pens A and C). In this early lactation period a substantial increase in plasma OCN concentrations was observed in first-calf cows given P supplementation following low P diets in pregnancy (LP-HP cows, Pens A). Furthermore during this interval there was a decrease in plasma CTX-1 concentrations indicating a net bone deposition in these cows. Conversely plasma BAP concentrations were reduced in the first two weeks of lactation in all cows. This together with OCN results suggest that bone formation and mineralisation are reduced at a time of peak demand for P and Ca for milk synthesis. In later lactation there was a consistent increase in plasma BAP concentrations in cows fed the low P diets (Pens A, C, D and E).

5.3 Measurement of the amounts and changes in bone P reserves of the animal

It would be desirable to be able to measure the total bone and thus P stores of an animal and be able to predict how much of this could be mobilised without significantly affecting production, animal health or long-term productivity. Prediction of total body bone is difficult in live animals. Recognised methods of measuring total body composition *in vivo* include DEXA, bioelectrical impedance and deuterium oxide but each of these approaches has constraints which make their routine use on-farm difficult or impossible. In the present project bone biopsies of rib bone and hip bone were used to monitor changes in bone volume (amount of bone tissue) lost or gained over time in a representative sample of bone (Appendix 3). This approach has been widely used in research to monitor changes in bone tissue in P deficiency studies.

Biopsy measurements were valuable in conjunction with the blood minerals, hormones and markers to understand the physiological events and changes with the cattle across the variety of states and circumstances, and in particular in relation to changes in rates and extent of bone mobilisation and deposition. Cows showing the greatest increases in plasma CTX-1 during pregnancy tended to have the largest bone loss over the corresponding period. There were some differences seen in the patterns of bone loss and gain between the rib and tuber coxae samples. As well as differences in the surface area of bone on which mobilisation occurs, results identified patterns of cortical and trabecular bone growth as cattle age. Young cattle have greater trabecular bone volume at the tuber

coxae site than older animals and this bone volume appears to decrease as the animal matures. This is in contrast to the rib CBT and overall size of the rib which continues to increase as the animal matures. Maximum rib CBT appears to occur only in the mature animal. It was important to take these factors into account when interpreting results from bone biopsies. These site-specific differences help to explain the different patterns of bone mobilisation and deposition seen in these samples during physiological challenges and P deficiency. In first-calf cows (Pens A and D) CBT increased during pregnancy, albeit less in cows fed low P diets, while trabecular bone decreased over the same period. In P deficiency, cortical bone mobilisation may only reduce the amount of growth rather than decrease the actual size of a bone. As trabecular bone is decreasing in volume naturally, bone mobilisation can be seen as rapid losses of bone volume because of mobilisation of P.

In human medicine there is a concept of “peak bone mass” where the aim of health interventions is to ensure individuals attain their maximal potential bone mass while they are growing. If growth in total body bone mass is slowed during development, individuals may have a lower total bone mass at the time when bone starts to be lost again in adulthood. We do not have a good understanding of if or when cattle start to decline in total bone mass during ageing. The concept of peak bone mass may be an important and valuable approach for management of heifers and young cows. If young animals are required to mobilise bone for pregnancy and lactation they may not be able to achieve full bone development and have a lesser capacity to mobilise total bone P stores during diet P deficiencies during subsequent years.

This project has identified a number of important features of P metabolism that will allow improved management of breeder cows over their production lifecycle in P deficient regions. These include: (i) better understanding of the P homeostasis and the physiological response of cattle to P deficiency, (ii) discovery of plasma metabolites, hormones and bone biomarkers that specifically identify increased bone turnover caused by P deficiency, (iii) greater knowledge of the response of PIP to P intake and refining the sensitivity of PIP as a diagnostic tool by using plasma Ca/P as an improved marker of reduced P intake, (iv) associations between markers of bone turnover (mobilisation and deposition) with actual changes in bone volume and mass seen in samples of bone obtained using bone biopsy sites, (v) confirmation that changes in bone mass and P reserves will be closely related to changes in liveweight,mobilisation of bone P will be associated with tissue losses and loss of LW, and (vi) identification of the differences between young growing animals, young breeders and mature breeders in their capacity to accommodate and adapt to intervals of diet P deficiency.

5.4 Improving established markers and developing new markers of P status

A secondary objective of this project was to use the new knowledge of the physiology of breeder cows to identify new potential markers of P adequacy and P status, with the long-term goal of better diagnostic tools. The most promising candidates have been identified.

This project confirmed that PIP in breeder cows is somewhat an indicator of P status. However as noted in young growing cattle, PIP appears to predominantly reflect the diet P intake around the time of sampling in relation to the current demands for P, and gives little if any indication of body P reserves in soft tissues or bone. Such issues limit the potential usefulness of PIP, particularly as a marker in breeder cows capable of mobilising substantial skeletal P reserves. However PIP may be a

valuable indicator if it is used in conjunction with other measurements that reflect other attributes of P intake, P requirements and P status.

A consistent increase in total plasma Ca concentrations in both pregnancy and lactation was observed in cows fed low P diets. Such an increase in Ca is most likely due to movement of Ca from bone to plasma, coincident with mobilisation of bone P reserves. However the changes in plasma Ca are small, within the normal homeostatic range for plasma Ca. Results suggest using total Ca in combination with PIP as the ratio Ca/P appears to be a simple and valid indicator of the degree of bone mobilisation in breeder cows during P deficiency.

A more specific marker of bone mobilisation in breeder cows is the biochemical marker of bone mobilisation CTX-1. This marker is used in human clinical testing, in particular for osteoporosis where it is used to determine the effectiveness of treatments in reducing bone mobilisation. In the current experiments large and significant differences in plasma CTX-1 concentrations were observed between breeder cows consuming P deficient and P adequate diets. Further P adequate diets over time decreased plasma CTX-1 to low levels. Therefore CTX-1 would seem a specific and valid biochemical marker to diagnose P deficiency or P adequacy, and moreover responses to P supplementation over time. The bone formation marker bone alkaline phosphatase (BAP) is another potential marker of P deficiency. Whilst this marker is best known as a marker of increased bone growth in young growing cattle, in mature cows BAP concentrations were relatively lower and more stable. A major finding of the current project is that cows fed low P diets plasma BAP levels increase, indicative of the functional role of this bone enzyme in providing P locally for bone mineral formation. Reproducing cows fed high P diets exhibit low, stable BAP concentrations.

The use of biochemical markers of bone metabolism to diagnose P deficiency in cattle is not new. Increasing identification of a wider variety and associated tests for biochemical markers of bone metabolism offers new opportunities for the determination of P status in breeder cows. A distinct advantage using bone metabolism markers such as CTX-1 and BAP is they provide a measure of whole body bone metabolism, rather than specific information about bone metabolism at a specific site. Also bone metabolism markers indicate current, or near real-time, bone processes.

A number of issues should be addressed in future work on markers of P status in cattle. One key consideration is to examine potential markers over a wide range of diet P, Ca and ME intakes directly relevant to northern Australia. The studies described here examined quite distinct diet P contents, but there is some evidence that the potential markers change appropriately with a change in diet P, and are not affected by diet ME.

A further consideration in bone marker work in clinical medicine is that the biological variability (within and between animal) needs to be examined for each potential marker. Such variability in humans has been attributed to circadian (diurnal and seasonal) rhythms, stress, exercise, food intake around the time of sampling and age differences. It should be noted many of these issues are known to affect PIP concentrations in growing cattle. With regard to breeder cattle the effects of issues such as age of cow, parity, and current reproductive status (pregnancy, lactation) need to be understood for the development of appropriate reference values for each marker. There are important technical considerations for markers around sampling protocols, specimen type (plasma, serum, urine), marker stability during transport, appropriate storage of samples, through to choice of diagnostic test.

6 Conclusions/recommendations

6.1 Conclusions

The project has developed a greatly improved understanding of phosphorus nutrition and associated physiology in *Bos indicus* breeder cattle in a seasonally dry tropical environment. It has improved the understanding of the consequences of deficient or surplus dietary P across seasons , the impact on breeder herd production through P deficient periods on fertility, breeder body reserves and weaner output in the year of deficiency and in subsequent years.

We have demonstrated that mature breeders calving in good body condition and with good P reserves have substantial capacity to mobilize body reserves of energy (as liveweight and fat) and P to maintain milk production in early lactation and largely maintain early calf growth to at least three months of age. However maintenance of calf growth is at the expense of bone P reserves and major liveweight loss is common. The main detrimental consequences will be reduced capacity to reconceive while lactating and achieve annual calving, and reduced body reserves (liveweight) entering the next dry season. Feeding P adequate diets (by P supplementation) during the dry season will allow some replenishment of body P reserves and this replenishment will be more rapid and extensive on higher quality pastures.

The first-calf cow also has some capacity to mobilize body reserves to maintain milk production in early lactation, but to a much lesser extent than the mature cow. Following extensive and prolonged mobilization of bone P the replenishment process in first-calf cows may be relatively slow. It was shown that provision of additional P (as P supplements) to heifers grazing late dry season pasture was beneficial. When the dry season pasture is of sufficient quality to maintain or increase conceptus-free LW gain, voluntary intake was substantially reduced during P deficiency with consequences for the liveweight and body reserves at calving. There was no effect of P deficiency of intake of heifers losing conceptus free liveweight due to very poor quality or lack of dry season pasture. There is a benefit of providing additional diet P to pregnant heifers regardless of conceptus-free liveweight change through increased bone P reserves at calving.

The general recommendation to industry that P supplements should be fed in the wet season has not changed. In circumstances where P supplements cannot be fed at this time, then the next best alternative is to commence P supplementation as early as possible in the mid or late wet season, and/or to feed low amounts of supplementary P during the dry season particularly when breeders are in their third trimester or are still lactating. This strategy will allow slow replenishment of bone P reserves. There are likely to be biological inefficiencies in mobilization and subsequent replenishment of bone P and for this reason it is still desirable to feed P supplements from the early wet season when this is possible.

The project allowed the development of a greatly improved understanding of the physiological mechanisms controlling P metabolism in breeder cows. Key findings are that severely P deficient cows mobilise substantial P reserves to maintain homeostasis and ensure calf growth. Breeders do mobilise soft tissue P reserves, by losing bodyweight, but these reserves only make limited contribution to homeostasis. Instead breeders rely predominantly on bone P reserves and there is significant mobilisation of bone mineral in breeders on low P diets. This mobilisation of skeletal P

reserves occurs independent of the main regulatory hormone PTH, which is suppressed in P deficient cows. Low P diets increase active Vitamin D3, which is known to increase gut uptake of both P and Ca and stimulate bone mobilisation. Measurements of bone obtained from biopsy samples has allowed understanding and explanation of animal responses across the three levels of (i) nutrient intake and responses of the animals as liveweight change and milk, (ii) physiological mechanisms and control, and (iii) actual changes in the amounts and histology of bone.

Whilst the above physiological mechanisms occur in both first-calf and mature cows, there were some key differences in young growing cows. Continued growth of first-calf cows during late pregnancy and lactation was noted, with P supplementation during both periods having positive effects on bone deposition. Also because young cows are continuing to grow bone the partitioning of P and other nutrients between maternal tissues (particularly bone) and milk synthesis apparently changes with the animal's capacity to mobilize and replenish bone P throughout periods of deficient and adequate P.

This project represents the first attempt to identify new markers of P status in breeder cattle. By measuring hormones and bone metabolism markers in cows fed distinct P deficient versus P adequate diets we have identified prospective markers of P status. We propose that using the existing measure of PIP concentrations together with plasma Ca concentrations (as a ratio) is a simple diagnostic test and may provide an indication of bone mobilisation in breeders in diet P-deficiency. A more specific and sensitive measure of bone mobilisation is plasma CTX-1 concentrations. An interesting additional marker in breeders is the bone enzyme BAP, which likely reflects defective bone mineralisation observed in P deficiency. Other markers and hormones, like OCN, PTH, and active Vitamin D, may also be useful as measures of P status. Commercial analytical equipment designed for veterinary clinics could be suitable for crush-side diagnosis from plasma PIP and plasma Ca, and also of some other potential blood markers.

6.2 Recommendations

This project has developed a new perspective for the management of breeder herds grazing P deficient northern pastures. It showed that in at least some circumstances the natural physiological mechanisms of reproducing beef cattle can be used to advantage to more effectively manage the P nutrition of grazing cattle.

There are a number of areas where more information is needed:

- the concepts developed in the project need to be validated in breeder herds grazing P deficient pasture systems in industry circumstances
- there is need for dose-response measurements of P intake to understand the optimal amounts and intervals of diet P intake, and therefore in best practice for P supplementation
- there is little understanding of the consequences of the duration of P deficient and of P replenishment and the inefficiencies which are likely to occur due to mobilization / replenishment mechanisms.

A way of assessing skeletal P reserves on farm is critically required to further improve our understanding of phosphorus nutrition in breeder cattle. Although a number of approaches have been developed these need to be refined and implemented, and/or new approaches developed.

Further work identifying useful biomarkers of P status in breeder cows is required. Testing new markers over a range of nutritional intakes and across different categories of breeder cows is essential to determine appropriate reference values for P-deficiency. Testing new markers in field conditions is also necessary to confirm the current results that were obtained in controlled experiments. Following identification of suitable markers the development of supporting technologies that enable sampling, offer crush-side testing, and easy interpretation of results is essential to ensure uptake of research by beef producers.

7 Key messages

The current recommendations for the P supplementation of breeders in lactation/wet season and low amounts (or in marginal P circumstances no P) remain the optimal approach to addressing P nutrition of breeder herds.

Where P supplementation during the entire wet season is not possible then P supplementation should be fed in the early wet season and then later commenced as early as possible after the mid or late wet season.

It is possible to use P supplementation during the dry season and in late pregnancy to alleviate the effects of P deficiency in breeder herds. It is likely to be difficult, or not possible, to achieve the same increases in animal productivity as achieved by P supplementing through the wet season. Also the P supplement fed is likely to be used with lower efficiency for increased breeder productivity.

Phosphorus supplementation during the dry season should have beneficial effects on productivity of breeders in the wet season if it is not possible to feed P supplements during the wet season.

Breeders in late pregnancy or early lactation grazing senesced dry season pasture need P supplementation in addition to during the wet season.

Supplementation of heifers in P deficient regions is essential as these animals still growing and it is possible to increase bone deposition (P reserves). Supplementation in either late pregnancy and/or early lactation is beneficial.

With low P diets breeders mobilise substantial bone P reserves that must be replaced within annual cycle to maintain appropriate bone health.

Repletion of P reserves post-weaning requires high P diets (usually P supplementation) with a low amount of additional P but for a prolonged period. For breeders which are severely P deficient replenishment may not be complete even if some P supplement is fed for the entire dry season.

Plasma inorganic phosphorus alone is inadequate as a marker of P status in breeders, as it predominantly reflects dietary P intake. Blood markers that reflect bone mobilisation or mineralisation can provide additional information and improve diagnosis of P deficiency in breeders.

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9 Appendices

9.1 Appendix 1. Publications from the project and in associated areas of phosphorus nutrition by the project team

9.1.1 Bones_A experiment

Dixon RM, Fletcher MT, Goodwin K, McNeill DM, Yong KWL, Petherick JC (2015) Attraction of phosphorus deficient cattle to bones. *Recent Advances in Animal Nutrition 2015* Armidale, Aust. *49-50.*

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9.1.2 Pens_A experiment

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9.1.1 Pens_B experiment

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9.1.3 Pens_D experiment

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9.1.4 Pens_E experiment

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9.2 Appendix 2. Summary of hormones and bone biomarkers

The following describes the hormones and marker of bone metabolism measured in the experiments.

Parathyroid Hormone (PTH): is a key hormone that regulates Ca levels in the body. Low blood Ca concentrations stimulate the release of PTH from the parathyroid gland, and overall PTH acts to increase blood Ca. PTH simulates the mobilization of bone mineral reserves by increasing the activity of osteoclast cells in bone. PTH also acts in the kidneys to reabsorb Ca, so there is decreases Ca loss in urine. Lastly, PTH indirectly stimulates increase uptake of both Ca and P in the gut, by increasing the formation of active 1,25 dihydroxy Vitamin D. It should be noted that whilst PTH stringently controls blood Ca, it can also affect blood P levels.

Vitamin D3: is a hormone that this converted from an inactive form 25 hydroxy Vitamin D to the bioactive 1,25-dihydroxy Vitamin D3 by the enzyme 1 alpha hydroxylase found in the kidney. 1,25 dihydroxy Vitamin D3 increase uptake of both Ca and P in the small intestine. Further, there is evidence that 1,25 dihydroxy Vitamin D3 acts to promote bone mobilisation, in conjunction with PTH (above). The formation of bioactive 1,25-dihydroxy Vitamin D3 is stimulated by PTH and therefore low Ca, and directly by low P.

Osteocalcin (OCN): a bone formation marker. OCN is a small protein that is part of the bone matrix secreted by osteoblast cells. Osteocalcin is thought to be the site of bone mineral (hydroxyapatite) deposition, although this has never been fully proven. The protein structure of OCN includes a number of amino acids that when carboxylated are able to act as Ca binding sites. OCN is normally increased in young growing animals. OCN is therefore a specific marker of bone formation reflecting the activity of osteoblasts that secrete the bone matrix.

Carboxy-terminal telopeptides of type I collagen (CTX-1): a bone mobilisation marker. Type I collagen is the most abundant protein in the bone matrix and is the framework upon which bone mineral is deposited. Like OCN, type I collagen is secreted by osteoblasts in the formation of bone. During bone mobilisation the bone collagen matrix is degraded by the action of osteoclast cells, and in this process bone mineral is released into circulation. At the same time peptide fragments of type 1 collagen are released into circulation and one such product is CTX-1. Therefore plasma CTX-1 concentrations reflect activity of osteoclasts in mobilising (resorbing) bone.

“Total” Alkaline Phosphatase (ALP): Alkaline phosphatase is a family of enzymes that act to remove phosphate residues from various molecules, including nucleic acids and proteins. In circulation there are two main ALP forms; produced either by the liver or bone. In clinical biochemistry an ALP activity test measures the “total” ALP enzyme activity in blood and is routinely used to screen for either liver disease or bone disorders. If the total ALP activity is increased other tests are used to determine whether the increase is derived from liver or bones.

Aspartate Aminotransferase (AST): AST is an enzyme involved in amino acid metabolism and found in various cells in the body. Circulating concentrations of AST are used clinically to determine abnormal liver function. In healthy animals circulating AST levels are usually low. Increased AST

levels together with increased ALP concentrations indicate liver disease. In bone disease AST is not increased.

Bone alkaline phosphatase (BAP): a bone formation marker. BAP is the ALP enzyme produced by bone. It is produced by osteoblast bone cells during bone formation and anchored to the outer surface of the osteoblast cell membrane. In this location it is thought to act to increase local concentrations of phosphorus by splitting phosphate from various molecules and thereby making phosphorus available for bone mineral (hydroxyapatite) deposition. Although most BAP is anchored to the osteoblast cell it can be split away from the membrane and appears in circulation. Plasma BAP concentrations reflect the amount of BAP produced by osteoblasts, and therefore BAP is a marker of bone formation. BAP concentrations are increased in healthy, young growing animals. In mature animals plasma BAP concentrations are more stable, but increased BAP concentrations are indicative of bone disorders particularly those associated with abnormal bone mineralisation, like osteomalacia.

9.3 Appendix 3. Bone biopsies protocols

9.3.1 Tuber coxae biopsies

Surgical technique
Bone biopsies were collected from the tuber coxae on the left and right side of each animal at two times as indicated in the protocol for each experiment.

The biopsy site was clipped and scrubbed with chlorhexidine surgical scrub and wiped clean with 4% Chlorhexidine concentrate in methylated spirits. The skin and deeper tissue over the tuber coxae were infiltrated with 35 to 40 mL of lignocaine hydrochloride (20 mg lignocaine hydrochloride/mL; Troy laboratories; Glendenning, NSW, Australia) and left for 5 min for effect. An incision approximately 80 mm in length was made and skin and any overlying muscle were retracted. A single biopsy 15-25 mm deep was obtained from the most central part of the tuber coxae of the ilium. A 16 mm bone hand-trephine (Sontec Instruments Centennial, CO, USA) was used to start the biopsy and then a 16 mm metal hole saw was used to obtain a deeper sample. One side of a long pair of scissors was used to loosen and separate the sample from the parent bone. This involved breaking the deep attachment of the bone core from the parent bone. The full core could then be pulled from the hole easily. The skin incision was closed with size 0 or 1 non-absorbable monofilament nylon or polypropylene suture, the surgical site was then wiped clean of blood and sprayed with Chloromide antiseptic spray (Troy laboratories; Glendenning, NSW, Australia). The bone cores were divided lengthwise using a scalpel blade and sub-samples were fixed with 10% normal buffered formalin (NBF) and 70% ethanol and placed on ice prior to transfer to the laboratory. The samples remained in fixative for 4 to 12 weeks.

Laboratory procedures
Bone samples fixed with 10% NBF were decalcified in 10% EDTA (pH 7.0) for 6-12 weeks with solution changed every two weeks. Adequate decalcification was determined when weekly weighing showed no further loss of weight of the sample. A small cut with a scalpel blade was also used to assess decalcification. After decalcification the samples were embedded in paraffin in a routine fashion and multiple 5 µm thick sections were taken parallel to the longitudinal direction of the bone axis. The sections were stained with toluidine blue and Masson's trichrome and photographed twice with a 10 x objective using a Olympus BX41 microscope (Olympus America Inc.; Melville, NY, USA) equipped with a digital camera Q-Imaging camera (Qimaging Corporation; Surrey, BC, Canada).

Histomorphometry measurements
One representative section for each animal was selected for measures of trabecular bone parameters. The pictures were taken at a standardised distance from the growth plate (300 µm). The images were then analysed using the software ImageJ (Schneider *et al.*, 2012) and the plugin BoneJ (Doube *et al*., 2010) to obtain values for bone volume (BV.TV), trabecular separation (Tb.Sp) and trabecular thickness (Tb.Th).

9.3.2 Rib biopsies

Surgical technique

Bone biopsies were collected from the middle 1/3rd of the left and right 11th rib or 12th rib on days indicated in the protocols of individual experiments. Thus up to four biopsies were obtained from each animal. The rib biopsy site was selected at the region of the rib where it interested by a virtual line between the tuber coxae and the point of the shoulder

The biopsy site was clipped using electrical clippers with a number 40 clipper blade. The skin was scrubbed with chlorhexidine surgical scrub and wiped clean with 4% Chlorhexidine concentrate in methylated spirits. The skin, muscle and deeper tissue over the rib were infiltrated with 35 to 40 mL of lignocaine hydrochloride (20 mg lignocaine hydrochloride/mL; Troy laboratories; Glendenning, NSW, Australia) and left for 5 min for effect. An incision approximately 80 mm in length was made and skin and any overlying muscle were retracted. A single biopsy was obtained from the most central part of the outer cortex of the rib. A 16 mm bone hand-trephine (Sontec Instruments Centennial, CO, USA) attached to a cordless drill was used to obtain the biopsy. The skin incision was closed with size 0 or 1 non-absorbable monofilament nylon or polypropylene suture, the surgical site was then wiped clean of blood and sprayed with Chloromide antiseptic spray (Troy laboratories; Glendenning, NSW, Australia). Surgical instruments were cleaned with detergent in fresh water and then soaked for at least 10 minutes in chlorhexidine/methylated spirits solution. New scalpel blade, suture needle and gloves were used for each animal. Previously sterilised, clean gauze swabs were used during surgery to control haemorrhage.

Laboratory procedures

The bone cores were divided using a small hack saw, perpendicular to the orientation of the trabeculae such that samples would be the top and the bottom of the rib sample. Sub-samples were either fixed with 10% normal buffered formalin (NBF) or placed in saline prior to transfer to the laboratory. Samples in saline were frozen for future CBT and specific gravity measurements. The fixed samples remained in fixative for 4 to 12 weeks.

Bone samples fixed with 10% NBF were decalcified in 10% EDTA (pH 7.0) for 12-18 weeks with solution changed every 2 weeks. Adequate decalcification was determined when weekly weighing showed no further loss of weight of the sample. A small cut with a scalpel blade was also used to assess decalcification. After decalcification the samples were embedded in paraffin in a routine fashion and multiple 5 µm thick sections were taken transverse to the longitudinal direction of the bone axis. The sections were stained with toluidine blue and Masson's trichrome and photographed twice with a 10 x objective using a Olympus BX41 microscope (Olympus America Inc.; Melville, NY, USA) equipped with a digital camera Q-Imaging camera (Qimaging Corporation; Surrey, BC, Canada).

Histomorphometry measurements

One representative section for each animal was selected for measures of cortical bone thickness (CBT) and bone porosity. The thickest region of the rib was selected for measurement to avoid underestimating the true thickness.

9.3.3 Use of bone biopsies to evaluate bone mobilisation and deposition

Any study examining bone mineral storage and mobilisation needs to try to identify the total amount of mineral stored in the skeleton and the amount gained or lost over time. This is difficult to do without total body composition studies so proxy methods of determining skeletal mineral stores need to be used.

The total amount of mineral stored in the skeleton depends on 2 major variables, the total volume of bone tissue and the concentration of mineral within that bone tissue. The actual mineral concentration within bone remains reasonably consistent and in most situations bone mineral is lost by changes to the structure of bone so that bone tissue is lost. For example, osteoporosis is a loss of bone volume (thinning of trabecular and cortical thinning) that causes reduction in bone strength. Major changes in bone mineralization occur less commonly. One example is severe osteomalacia where bone containing a greater proportion of un-mineralized new bone (osteoid) becomes “softer” and vulnerable to damage. Severe Vitamin D or P deficiency are possible causes of osteomalacia.

In humans DXA imaging is widely used to estimate how much the total body and site-specific bone mass (amount of mineralised bone) varies in comparison to population-based normal ranges. Although the measurement obtained by DXA is described as “bone mineral density”, this is actually a calculated measure that combines aspects of bone volume and mineralisation rather than measuring the actual concentration of mineral within bone tissue. Changes in DXA bone mineral density mostly reflect changes in bone volume due to cortical and trabecular bone structural changes.

Because bone has such a complex and variable geometry throughout the body it is difficult to determine the total bone volume by measuring one site alone. However the magnitude of bone gain or loss can be estimated by measuring changes in bone volume at standard sites. Changes in the bone volume over time or compared to control animals can indicate if there has been a net loss of bone due to resorption (“mobilisation”) or a net gain in bone due to formation (“deposition”) in response to P deficiency or supplementation.

Cortical bone volume changes occur through changes in the thickness of the cortex or through resorption within the cortex (Figure X). Trabecular bone volume loss occurs by resorption along the surface of the trabecular bone framework. This leads to thinning and loss of trabeculae and greater space between the trabecular components (Figure XX).

Measures of bone volume (amount of bone tissue)

Cortical:

- Cortical bone thickness (CBT, mm).
- Cortical bone volume or porosity. Volume (%) + porosity (%) will essentially be 100 %

Trabecular:

- Trabecular bone volume (%)
- Trabecular thickness (um)
- Trabecular separation (um)

Measures of bone mineralization ie. The concentration of mineral within bone tissue (mg/mm^3)

- Ash per known volume of bone tissue
- Imaging a known volume with calibration for mineral optical density (ie uCT)

Estimating changes in bone mineralization

- Osteoid is un-mineralised bone and if it is excessive it will lead to under-mineralized bone. The proportion of bone tissue that is osteoid can be measured using histology.

Specific gravity

- Measuring specific gravity of rib biopsies gives an estimate of how much mineral is within a volume of bone but will measure changes in mineral composition due to both reduced tissue mineralization AND porosity (loss of cortical volume).

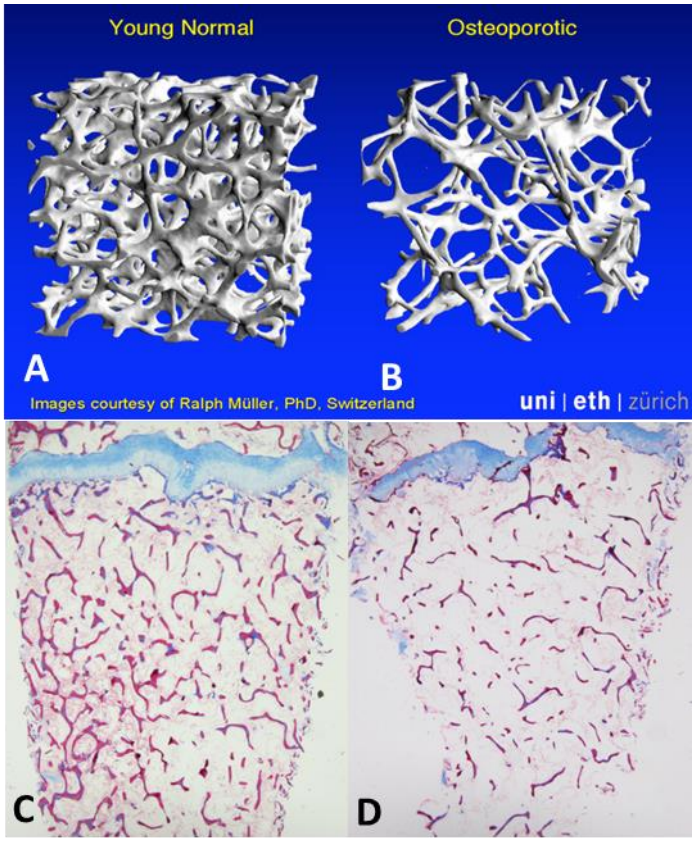

Figure Appendix 3-1. This demonstrates the 3 dimensional structure of typical trabecular bone that comprises multiple, interweaving trabecular components. B demonstrates the loss of trabecular bone seen as thinner individual trabeculae, less trabeculae and greater separation of the trabecular components. These changes lead to a reduction in the total bone volume within this space. C and D are 2 dimensional histological sections of trabecular bone in tuber coxae biopsies from mature cows. The bone sample in A is from a cow in adequate P status showing normal trabecular bone volume. The section in D is from a P deficient cow demonstrating loss of trabeculae, trabecular thinning and less area occupied by bone tissue. Measurements of this 2 dimensional bone area are used to estimate bone volume in this sample.

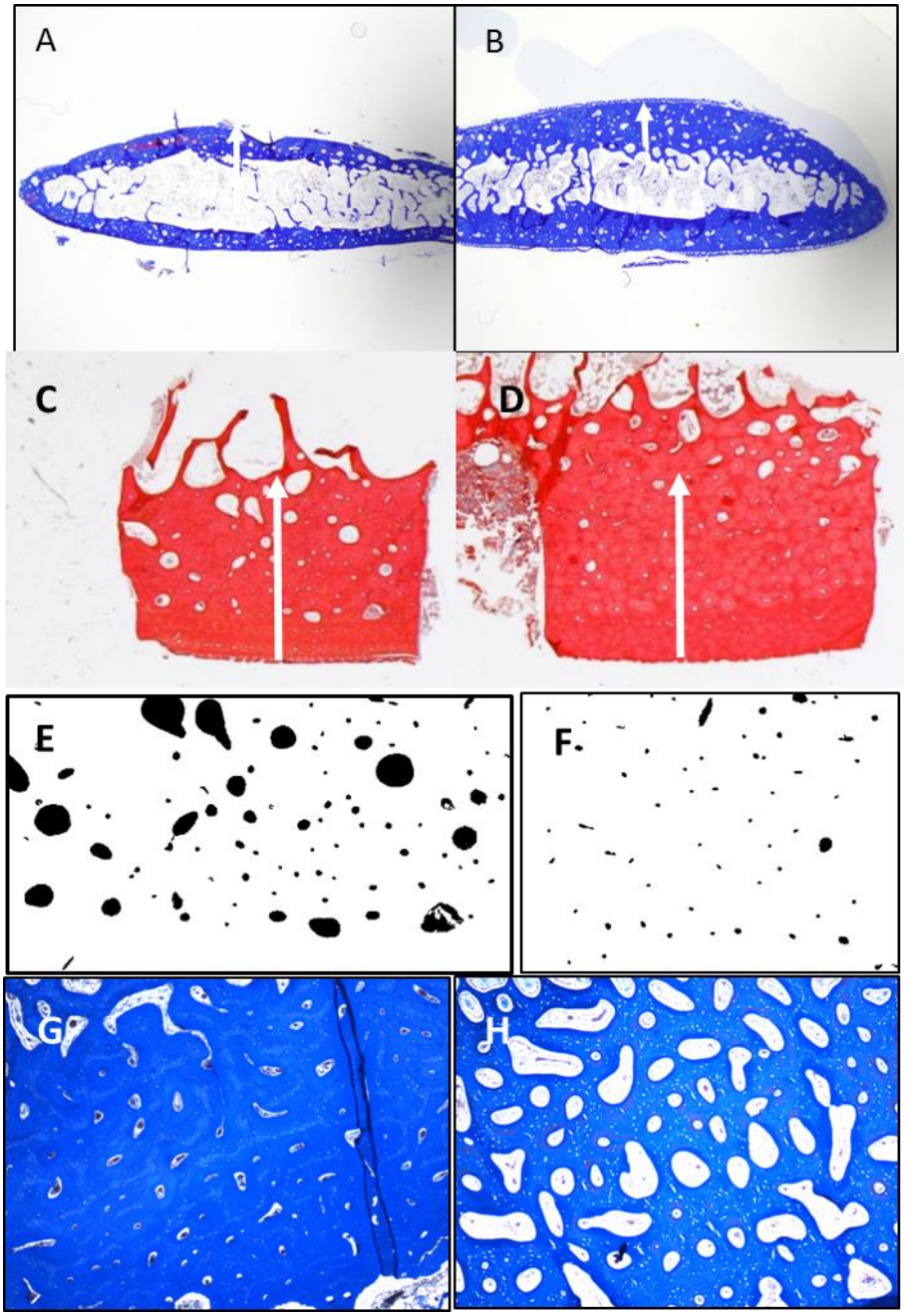

Figure Appendix 3-2. Examples of rib histology to demonstrate aspects of cortical bone volume. A and B are cross sections of rib 12. In A the rib has a thin cortex with a much smaller cross sectional area than seen in B. Typical site of measurement of CBT shown as white arrow. C and D show in higher magnification, typical segments of the outer cortex of a rib obtained by bone biopsy. The rib in C has less CBT than the one in D. E and F are diagrams of the porosity of the rib samples in C and D. The black areas are the "holes" in the bone formed by bone resorption/mobilisation. The white is the remaining cortical bone area that can be used to estimate bone volume in this region. The rib sample in E has greater porosity and thus less bone volume than the rib in F. G and H are greater magnification and again show segments of rib cortical biopsy. The rib in H has extensive bone resorption and thus much greater porosity and reduced bone volume compared to the rib in G.

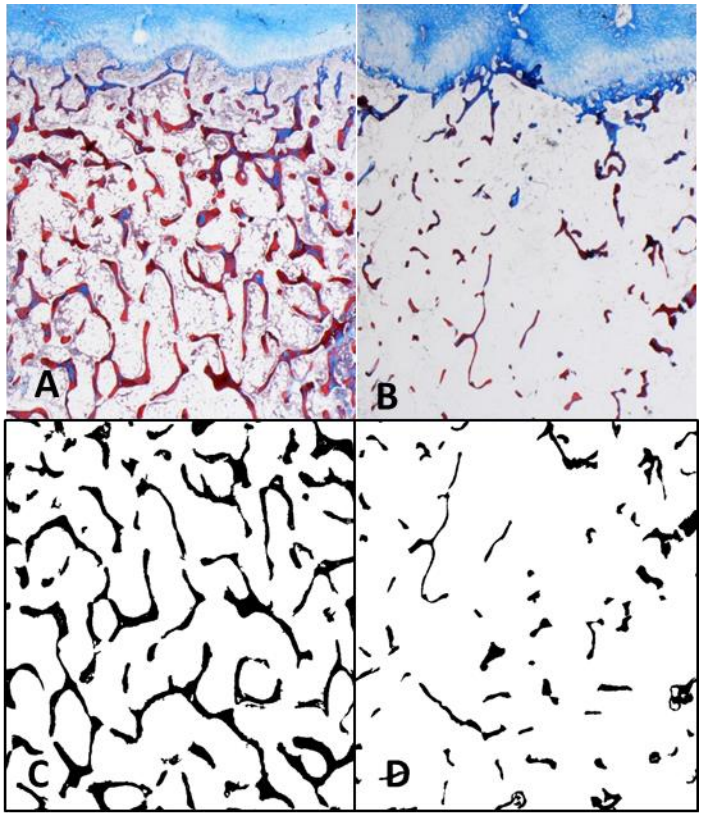

Figure Appendix 3-3. A and B are images of histology sections of tuber coxae bone from young cows. In image A the trabecular bone is normal while in image B there is marked loss of bone tissue. C and D show binary images created in ImageJ that can be used to obtain values for bone volume (BV/TV, %), trabecular thickness (Tb.Th um) and trabecular separation (Tb.Sp, um) using the pluggin BoneJ.

9.4 Appendix 4. Experiment Bones_A. Full paper available on-line, Animal Production Science

Factors involved in the attraction of phosphorus-deficient cattle to ingest bones (2018)

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Summary for Table of Contents

Severely phosphorus deficient cattle demonstrate pica, a behaviour where materials such as sticks, soil and old bones are investigated and chewed. It has been considered innate. An experiment demonstrated that bone chewing in phosphorus deficient cattle is primarily a learned positive post-ingestive feedback response and allows severely deficient grazing animals to obtain additional diet phosphorus from one of the few concentrated sources of phosphorus in natural rangelands.

Abstract. Grazing cattle deficient in phosphorus (P) often seek out and chew bones, apparently to obtain dietary P. To investigate this phenomenon, heifers naïve to P deficiency were either fed a P-deficient diet (LowP) or grazed P-adequate pasture (AdeqP), and preference tests examined their attraction to weathered bones or a control of wood. During phase 1 (d 1-145) the LowP heifers developed severe P deficiency and pica, but demonstrated little attraction to weathered bones. During phase 2 (d 146-155) heifers were allowed to interact with and to chew a variety of weathered bones. After this experience LowP heifers were more attracted to bones during phase 3 (d 156-166) than during phase 1 (P<0.05), and more attracted than AdeqP heifers during either phase. Subsequently, in phase 4 (d 167-171) LowP heifers were more attracted than AdeqP heifers (P<0.01) to weathered bones than a control of wood, and in phase 5 (d 172-176) to bones with more extended weathering. During phase 6 (d 177-182) attraction was reduced when bones were placed inside a cloth bag. The olfactory constituents from weathered bones were dominated by aliphatic aldehydes and ketones, consistent with long chain fatty acid breakdown. It was concluded that attraction of P-deficient cattle to seek and ingest bones is primarily a learned response. Smell, taste and visual appearance all appear to be important cues for attraction. Pica is likely important in causing P-deficient cattle to investigate unusual materials, including bones, resulting in cattle learning an association between bone chewing and P ingestion.