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Validation and demonstration of a diagnostic tool for phosphorus status of beef cattle

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Abstract

Much of northern Australia is based on soils and, hence, forages that are deficient in phosphorus (P), an essential nutrient for growing and breeder cattle. A robust test is required by industry to determine the P content of diets grazed by cattle. This project aimed to validate the use of the ratio of the concentration of P in the faeces (FecP) to the metabolisable energy (ME) content (FecP:ME; mg P/MJ ME) of the diet as a diagnostic test for the adequacy of dietary P content in relation to energy intake and to determine the likely response of cattle to P supplementation. Feed intake, liveweight gain, and the concentration of P in plasma (PiP), FecP and FecP:ME increased with increasing diet P content and P intake. Plasma inorganic P was the best indicator of P intake of steers consuming a high N, high ME diet and under grazing conditions. The responses of FecP:ME and FecP:dietary N content to increasing diet P were similar when dietary ME and N content were constant. In a field experiment auto-drafters were used to draft cattle to allocated P supplement treatments in a single paddock. Under these extensive grazing conditions the FecP:ME indicated that indicator steers and breeders were P deficient but no response to P supplementation was evident and serum inorganic P concentration suggested that the indicator steers were not P deficient. The FecP:ME may require further validation across a range of land types/pasture bases before it can be recommended for wide use by industry.

Executive summary

A large proportion of the beef industry in northern Australia is based on phosphorus (P) deficient soils and forages, resulting in low P intake by cattle. Feed intake is affected by P content of the diet, which has consequences for liveweight (LW) gain (LWG) and skeletal development of growing cattle and body condition score (BCS) and reproductive rates of mature females. For cattle grazing P deficient pastures, supplementation in the wet season is recommended, as both energy and protein are typically not limiting at this time. Despite the biological and economic advantages of P supplementation of cattle on P deficient country, implementation appears much less common than might be expected if the currently recommended practices were adopted. Potential reasons for this include the perception that stock naturally gain LW over the wet season without realising the further response that may result from P supplementation, the lack of a clear demonstration of the economic advantages of wet season P supplementation, the lack of a reliable tool to indicate if P supplementation is required, palatability issues and associated poor intakes of wet season supplements in some regions, and logistical issues around the distribution of supplements during the wet season.

The objectives of this project were to

- Evaluate in a pen feeding trial, the faecal P to dietary N (and dietary DMD) ratio as a diagnostic tool to determine P status and deficiency in weaners fed a diet representative of wet season pasture with increasing P content.
- Validate the diagnostic test for P deficiency described in (1) above, and in a field study with breeding females and steers.
- Quantify the carry-over effects of dry season P supplementation and relate any effects to the P:N observed in grazing cattle in both breeding females and steers.
- Develop recommendations for testing and interpreting P status, and associated supplementary P intake for different classes of cattle in the wet and dry seasons in northern Australia.

In Experiment 1, *Bos indicus* crossbred steers were offered treatment diets of increasing P content (0.9, 1.3, 1.8, 2.0 and 2.4 g P/kg DM) for 172 days. Dry matter (DM) intake, LW and various indicators of P intake were measured in the plasma and faeces. The key findings from Experiment 1 were

- DM intake and LWG of steers increased with increasing P intake.
- Steers fed a high P diet (2.4 g P/kg DM) gained an additional 130 kg LW than steers fed a low P diet (0.9 g P/kg DM) over 172 days.
- Reductions in DM intake and LWG of steers fed the 0.9 g P/kg DM diet were observed after 5 weeks of treatment feeding.
- The influence of dietary P content on ME intake is the key driver of the LWG response to P intake.
- Plasma inorganic P responded immediately to P intake and was relatively stable across the experimental period. PiP was a better indicator of diet P content and P intake than FecP.
- FecP and its associated ratio's with diet quality (ME and CP) did not respond to P intake as quickly or as stably as PiP.
- FecP (and its associated ratio's) provided a better indicator of diet P content (and P intake) the longer the steers had consumed their allocated treatment diets.

In Experiment 2, *Bos indicus* cows and steers (that had not received P supplement over the previous year) were offered supplements with or without P in the wet and dry seasons under extensive conditions on a commercial cattle station. The various indicators of P intake measured in Experiment 1 were measured in Experiment 2 as well as reproductive performance of cows and LW of steers. Auto-drafters were used to draft animals to allocated supplement treatments in the wet and dry seasons, resulting in four treatments

1. +P (wet season)/+P (dry season)

2. +P (wet season)/-P (dry season)
3. -P (wet season)/+P (dry season)
4. -P (wet season)/-P (dry season)

The key findings from Experiment 2 were

- The wet season LWG and serum inorganic P concentration at the end of the growing season in unsupplemented steers suggested that it was unlikely that a response to wet season P supplementation would occur in the trial paddock. Approximately two-thirds of the paddock (black soil) had a soil P concentration of approximately 2 ppm while the remaining one-third of the paddock (red soil) had a soil P concentration of approximately 9 ppm. Presumably the cattle in Stud paddock were able to compensate for the low P content of the black soil area by foraging in the red soil areas. In hindsight, the selection of this paddock was not appropriate to quantify the carry-over effects of dry season P supplementation on herd performance.
- There was no response of either cows or steers to P supplementation. Performance of the mob was excellent with weaning rates of approximately 85% across all treatments over two years and LWG of approximately 150 kg in supplemented and unsupplemented steers over the first wet season.
- Liveweight of cows was maintained throughout the year for all treatments; comparison of the measured FecP:ME with published threshold values would suggest that cows were not receiving adequate P to maintain liveweight during late pregnancy or lactation.
- FecP:ME (and serum inorganic P) indicated an increase in P intake by supplemented cows and steers in the dry season, reflecting higher supplement intakes over that period of time. Incorporation of a source of P into dry season supplements resulted in higher P intakes than were achieved by offering a P supplement in the wet season.
- A positive outcome from Experiment 2 was the improvements in the remote livestock management system (RLMS).

The project demonstrated the importance of P on DM intake and LWG of cattle and the time taken before steers consuming a low P diet will display a reduction in LWG. The project confirmed previous recommendations that PiP is the most appropriate indicator of P intake of cattle. The project demonstrated that FecP was related to diet P content but raised some issues around timing of collection, the number of samples required and the reliability of threshold values for different land types/pasture mixes. Further validation of FecP:ME is required under a much wider range of land types/pasture mixes and across different seasonal conditions before its use can be recommended to industry.

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1 Background

Phosphorus is the second most abundant mineral in the body of cattle, with 80-85% of body P stored in the skeleton (ARC, 1980). Phosphorus is required for skeletal growth (and strength), energy metabolism, DNA and protein synthesis, lactation and microbe function in the rumen. Soils and, hence, forages across large areas of northern Australia are acutely P deficient (<5 ppm P in soil), and are often associated with low CP content of forages in the dry season. Forage P content increases during the wet season but a P deficiency still occurs as CP and ME intake are much higher in the wet season and P becomes the first limiting nutrient and is unable to meet the requirements for growth of young cattle at this time. Young cattle grazing these areas have low LWG, and show a positive LWG response to wet season P supplementation. As such, wet season P supplementation of growing cattle is recommended in areas across northern Australia where acute P deficiencies occur. This recommendation has also being carried through to include breeder cattle, although P requirements and mobilisation/deposition are very different in breeders compared to growing cattle.

For cattle grazing low P diets, a P deficiency is likely to occur when P requirements increase during periods of LWG over the wet season, late pregnancy and lactation unless additional P is supplied, either through fertilizer application to forages or, more commonly in extensive grazing systems, through supplementation with an inorganic source of P. Over the short-term ruminants can cope with a dietary P deficiency, with P requirements met from P recycling and absorption from bone, however once these reserves are depleted a deficiency will occur. The initial response of ruminants to a P deficiency is a reduction in feed intake (reviewed by Ternouth, 1990). A reduction in feed intake due to a P deficiency is not associated with any changes in rumen microbial digestibility but is more likely to be a function of a change in cellular metabolism in the soft-tissue pool (Milton and Ternouth, 1985). A P deficiency induced reduction in feed intake reduced LWG of sheep (Ternouth and Sevilla, 1990) and cattle in individual pens (Bortolussi *et al*., 1996), finishing in feedlots (Geisert *et al*., 2010) and grazing tropical forages on P deficient soils across northern Australia (Winter *et al*., 1990).

Despite the fact that large areas of land used for cattle production in northern Australia are based on P deficient soils, the uptake of P supplementation remains low. One potential reason for the low uptake of P supplementation is the lack of a reliable diagnostic tool to determine the P content of the diet consumed by cattle, particularly in determining if a marginal P deficiency occurs and at what level of P deficiency would an economic response to P supplementation occur. Plasma inorganic P (PiP) concentration reflects P intake, particularly at low P intakes and at the end of the growing season (Bravo *et al*., 2003), and has a good relationship with LWG (Wadsworth *et al*., 1990). While PiP has been advocated by some as a useful indicator of P intake, results can be confounded by mobilisation of P from body reserves, especially in lactating cows, and the timing of sampling relative to other variables, for example dietary N content and growth of the animal. Measurements of bone thickness (Little, 1984), specific gravity (SG) and P content (Little, 1972) of cortical bone biopsies have also been used to describe P status of ruminants. However, the technique lacks standardisation, is invasive and not practical under commercial conditions. The major route of excretion of P is via the faeces and FecP typically reflects dietary P content (Holechek *et al*., 1985) and has been associated with LWG in response to P supplementation or fertilizer application of forages during the wet season in cattle (Wadsworth *et al*., 1990). The majority of the P in the faeces is endogenous in origin, mainly from saliva. The concentration of P in the faeces on its own has generally been rejected as a diagnostic tool for P deficiency in cattle (Read *et al*., 1986; Wadsworth *et al*., 1990). The FecP:N or its associated dry matter digestibility (DMD):FecP have been suggested to provide a better indicator of P intake of cattle relative to requirements than FecP alone because it provides a means of identifying if P is the first limiting nutrient (Dixon *et al*., 2011). It is known that only weaners getting adequate ME or CP will exhibit a response to P in line with the first limiting nutrient concept. Phosphorus requirements are usually expressed relative to diet DM content or g/day but neither expression takes into account the P required/unit available ME or CP which is a

better physiological expression. Very little P is excreted in the urine except in unusual circumstances, such as when grain based rations or when diets high in P are fed. Hence the FecP:dietary N (CP) or FecP:ME (or DMD:FecP) has a physiological rationale in unsupplemented animals. Faecal P content can be measured chemically and dietary N and DMD can be predicted by faecal near-infrared spectroscopy (NIRS) on the same sample, with the ratio of FecP to dietary ME (FecP:ME) and FecP to dietary CP (FecP:CP) calculated.

A second potential constraint to the adoption of wet season P supplementation by the northern Australian cattle industry is around accessibility of paddocks during the wet season to distribute P supplements. Earlier studies (Barnes and Jephcott, 1955; Hart and Mitchell, 1965) reported the prevalence, clinical signs and impacts on reproduction of a P deficiency on breeder cattle on the Barkly Tableland, Northern Territory. These reports indicated that the effects of a P deficiency on health and reproductive performance were more evident in cows that lactated during the dry season. This is supported by subsequent studies in northern Australia (Ternouth and Coates, 1997; Miller *et al*., 1996) where cows in early lactation were in negative P balance and dry cows were generally in positive P balance, particularly after weaning in the early dry season. Regardless of the timing of lactation, P reserves will need to be replenished within an annual cycle to meet subsequent lactation requirements. Cows that are not lactating in the dry season will have minimal P requirements and any P surplus to these low requirements may be deposited for future use. Cows that are lactating in the dry season will have high P requirements at this time and are likely to require P supplementation in the dry season to meet lactation P demands. Therefore, it is possible that dry season P supplementation may be a more effective P management strategy for breeders in P deficient areas, regardless of the physiological state they are in during the dry season. Such a strategy would overcome issues associated with the distribution of supplements in the wet season and may also facilitate a more uniform P supplement intake by combining P with standard dry season N supplements which may be more palatable than a wet season P based supplement.

2 Project objectives

1. Evaluate in a pen feeding trial, the faecal P to dietary N (and dietary DMD) ratio as a diagnostic tool to determine P status and deficiency in weaners fed a diet representative of wet season pasture with increasing P content.

2. Validate the diagnostic test for P deficiency described in (1) above, in a field study with breeding females and steers.

3. Quantify the carryover effects of dry season P supplementation and relate any effects to the P:N observed in grazing cattle in both breeding females and steers.

4. Develop recommendations for testing and interpreting P status, and associated supplementary P intake for different classes of cattle in the wet and dry seasons in northern Australia.

3 Methodology

3.1 Experiment 1. Pen study

Feed intake, growth and faecal and plasma phosphorus concentration in steers fed diets with increasing P content.

The experiment was conducted at the Centre for Advanced Animal Science at the University of Queensland (Gatton, QLD) in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and was approved by the University of Queensland Animal Ethics Committee.

Experimental design, animals, diets and feeding
Thirty *Bos indicus* crossbred steers were fed a pangola grass (*Digitaria eriantha*)/lucerne (*Medicago sativa*) hay diet [924 g organic matter (OM), 118 g CP, 570 g ash-free neutral detergent fibre (NDF), 405 g ash-free acid detergent fibre (ADF), 2.8 g P/kg DM) at approximately 20 g DM/kg LW.day in individual pens for approximately 90 days prior to the commencement of the experiment. This diet did not deplete the P reserves of the steers used in the experiment.

At the commencement of the experiment the steers [227.8 ± 1.9 kg LW; mean ± standard error of the mean (s.e.m.)] were blocked on LW and randomly allocated to pens and one of five dietary P treatments in a completely randomized block design. During the experiment, steers were fed pelleted diets that provided approximately 0.9 (P-1), 1.3 (P-2), 1.9 (P-3), 2.0 (P-4) and 2.4 g P/kg DM (P-5) P, and were formulated to provide 100 to 110 g CP/kg DM and 60 to 65% DMD (i.e. typical of early wet season tropical pastures) (Tables 1 and 2). The pellets contained approximately 59% barley straw, 23% sugar, 8% gluten, 8% wheat starch, 0.8% urea, 0.5% KCl, 0.4% $Ca(OH)_2$ with 0.2% MgO and 0.1% premix containing monensin (TMV mono 20) (on an as is basis) with Biofos (Rumevite, Ridley Agri-products) added as needed to meet the required P content of the pellets. No biofos was added to the low P pellet (P-1). The pellets were manufactured by Johnson's Stockfeed (Kapunda, SA). The calculated P content of the pellets were 0.7 (P-1), 1.0 (P-2), 1.5 (P-3), 2.0 (P-4) and 2.5 g P/kg DM (P-5) based on analysis of all raw materials prior to manufacture of the pellets. The chop length of the barley straw was approximately 15 mm and the pellets were, on average, 20 mm in diameter and 40 mm in length. A second batch of the P-1 pellet was required and was manufactured using the same ingredients in the same amounts as described for the original batch of P-1 pellets.

Steers were adapted to the treatment pellet diets over 16 days (adaptation period; days -16 to 0) and were then fed treatment pellets *ad libitum* for a further 172 days (experimental period; days 1 to 172). From day 43 of the experiment all steers were offered a fixed amount (0.5 kg/head.day) of Mitchell grass (*Astrebla* spp.) hay chopped to between 50 to 100 mm in length to increase the effective NDF content of the overall diet, with treatment pellets offered *ad libitum*. This combination of pellets and hay resulted in five treatment diets with increasing P content of 0.9 g P/kg DM (0.9P), 1.3 g P/kg DM (1.3P), 1.8 g P/kg DM (1.8P), 2.0 g P/kg DM (2.0P) and 2.4 g P/kg DM (2.4P) averaged across the entire experiment. Drinking water was available at all times.

Table 1. Organic matter (OM), crude protein (CP), ash-free neutral detergent fibre (NDF) and ash-free acid detergent fibre (ADF) and P, Ca, Mg, K, Na and S (g/kg DM) and Fe, Mn and Zn (mg/kg DM) content of the five pelleted diets and Mitchell grass hay (Hay) offered to steers during Experiment 1[1]

Parameter	P-1 pellet[2]	P-1 pellet	P-2 pellet	P-3 pellet	P-4 pellet	P-5 pellet	Hay
OM	.	926 ± 1.7	937 ± 0.3	895 ± 1.1	936 ± 0.4	937 ± 0.5	901 ± 3.3
CP	104	111 ± 0.5	109 ± 1.0	107 ± 1.8	104 ± 2.1	107 ± 0.5	39 ± 1.0
NDF	.	359	426	379	449	441	666
ADF	.	242	242	235	262	287	407
P	0.6	0.91 ± 0.04	1.29 ± 0.06	1.88 ± 0.09	2.00 ± 0.07	2.42 ± 0.09	1.05 ± 0.09
Ca	3.6	7.7 ± 1.6	4.43 ± 0.1	20.91 ± 0.5	4.17 ± 0.1	4.70 ± 0.1	3.26 ± 0.1
Mg	1.7	1.80 ± 0.11	1.71 ± 0.03	1.97 ± 0.04	1.62 ± 0.04	1.48 ± 0.03	1.14 ± 0.08
K	.	9.53 ± 0.3	7.79 ± 0.11	9.04 ± 0.06	7.69 ± 0.06	6.24 ± 0.08	7.68 ± 0.54
Na	5.7	1.87 ± 0.11	1.73 ± 0.02	1.66 ± 0.02	1.34 ± 0.02	1.50 ± 0.02	0.42 ± 0.11
S	1.9	1.33 ± 0.03	1.34 ± 0.02	1.29 ± 0.02	1.30 ± 0.01	1.42 ± 0.01	2.12 ± 0.22
Fe	.	219 ± 14	210 ± 10	393 ± 10	310 ± 12	341 ± 10	239 ± 12
Mn	.	33.6 ± 1.4	36.7 ± 1.8	48.5 ± 1.4	28.9 ± 0.5	48.8 ± 2.2	39.3 ± 1.7
Zn	.	36.9 ± 0.7	29.1 ± 0.8	41.1 ± 0.8	5.22 ± 0.5	40.9 ± 1.5	26.9 ± 0.94
Ca:P	6:1	8.4:1	3.4:1	11.6:1	2.1:1	1.9:1	3.1:1

[1]Samples of each feed offered were collected weekly. Sub-samples of weekly feed offered were bulked every three weeks and each bulked sample was analysed for the above parameters, with the exception of NDF and ADF which were analysed on a single sample bulked across the entire experiment for each feed offered. The above results are the mean and s.e.m. of eight measurements for each feed offered across the entire experiment.
[2]Predicted composition of the P-1 pellet when formulated using the ingredients in the percentages listed in the methodology; the mineral composition of the ingredients was tested prior to formulation.

Table 2. Digestibility of dry matter (DMD), organic matter (OMD), digestible organic matter in dry matter (DOMD) (%), estimated metabolisable energy (ME) content (MJ/kg DM) and rumination time (h/day) of the combined pellet plus Mitchell grass hay treatment diets (0.9P, 1.3P, 1.8P, 2.0P, 2.4P) offered to steers during Experiment 1[1,2,3]

Parameter	Treatment diet				
	0.9P	1.3P	1.8P	2.0P	2.4P
DMD	62.7 ± 0.7[b]	66.2 ± 0.7[c]	62.5 ± 0.7[b]	58.9 ± 0.7[a]	60.8 ± 0.7[ab]
OMD	66.7 ± 0.7[b]	69.4 ± 0.7[c]	67.7 ± 0.7b[c]	61.8 ± 0.7[a]	63.7 ± 0.7[a]
DOMD	61.3 ± 0. 7[b]	65.1 ± 0.7[c]	60.8 ± 0.6[b]	57.8 ± 0.6[a]	59.6 ± 0.6[b]
ME[4]	9.3 ± 0.1[b]	10.1 ± 0.1[c]	9.2 ± 0.1[b]	8.6 ± 0.1[a]	9.0 ± 0.1[ab]
Rumination time[5]	5.2 ± 0.9	6.9 ± 1.1	6.5 ± 0.9	5.4 ± 0.9	6.1 ± 0.9

[1]Digestibility of DM and OM were determined by total faecal collection over seven consecutive days as described in the measurements and sample collection section of the methodology. Values are mean and s.e.m. of six animal replicates per treatment, with the exception of 0.9P and 1.3P, where the number of replicates was only five.
[2]0.9P (0.9 g P/kg DM), 1.3P (1.3 g P/kg DM), 1.8P (1.8 g P/kg DM), 2.0P (2.0 g P/kg DM) and 2.4P (2.4 g P/kg DM).
[3]Data are least-squares means with standard error of the mean. Different alphabetical superscripts across each row indicate a significant difference between previous P diets (P<0.05).
[4]Calculated from Freer *et al*. (2007) where ME = 0.194 x DOMD% - 2.577.
[5]Rumination time was determined by observing n=3 steers/treatment over a 24 hour period as described in the measurements and sample collection section of the methodology; one steer from the 1.3P treatment was excluded from the analysis.

Steers were fed at 0800 and 1630 h each day, with approximately 2 kg of pellet residue maintained in the feed trough over a 7 day period. Residues were collected and weighed every 7 days and total intake and average daily intake over a 7 day period calculated. Duplicate sub-samples of hay and pellets offered and refused for each steer were dried to a constant weight at 65^{o}C each week and stored for subsequent analysis. Sub-samples of feed offered, collected each week, were bulked over 21 day periods to align with faecal and blood sampling with OM, CP, NDF, ADF and mineral content determined on these bulked samples, giving a diet composition analysis for each diet every 21 days across the experiment (i.e. n=8 measurements/diet across the entire experiment). These aligned with the 21 day sampling schedule for plasma and faeces, also analysed for P concentration. Sub-samples of feed residues collected each week were bulked over the entire experiment, with OM, CP, NDF ADF and mineral content determined on these bulked samples, giving a single residue composition analysis for each pen across the experiment. In addition, sub-samples of feed offered and residues over the digestibility period were analysed for the above parameters for individual steers.

Measurements and sample collection

An unfasted steer LW was measured at the same time every week and hip height (HH) of steers was measured every 21 days at the same time as LW measurements. Blood samples were collected from the jugular vein of steers into lithium heparin vacutainers every 21 days at the same time as LW measurements; samples were inverted 6 to 8 times and placed on ice until centrifugation at 1300 *g* for 10 min with plasma samples stored at -80^{o}C for subsequent analysis. Faecal grab samples were collected from the rectum of steers every 21 days at the same time as blood sample collection; faecal grab samples were dried to a constant weight at 65^{o}C and stored at room temperature prior to subsequent analysis.

Between days 131 and 154 of the experiment each steer was placed in metabolism crates for 9 consecutive days, consisting of 2 days for adaptation to the metabolism crates and 7 days for collection of total urine and faecal output. This was conducted in two cohorts with 15 steers/cohort, with 5 days between cohorts; steers entered the metabolism crates in the same sequence in which they were randomly allocated to pens at the commencement of the experiment (i.e. Cohort 1 = Pens 1 to 15; Cohort 2 = Pens 16 to 30). One 0.9P steer had very low feed intake in the metabolism crates and was returned to his individual pen on day 2 of the collection period; the mean estimated dietary ME content for the 0.9P treatment group is used in subsequent calculations for this steer in the absence of any digestibility data. Steer urine was collected into a volume of 5% H_2SO_4 sufficient to maintain a urine pH of less than 3. Total urine output was weighed and a 5% sub-sample collected each day, stored at 4^oC and bulked over the 7 day collection period. Total faecal output was weighed and a 10% sub-sample collected each day, stored at 4^oC and bulked over the 7 day collection period. On day 6 of the collection period clean un-acidified urine samples (spot samples) were collected from each steer and immediately frozen in dry ice for mineral concentration analysis and possible metabolomic studies using nuclear magnetic resonance (NMR). Upon completion of each collection period bulked acidified urine samples were mixed well and aliquots were stored at -20^oC for subsequent analysis, and bulked faecal samples were mixed well with triplicate sub-samples dried to a constant weight at 65^oC. At the end of each collection period, steers were removed from the metabolism crates, LW was measured, blood samples collected (as described above) and rumen fluid samples were collected by stomach tube. The pH of the rumen sample was measured and duplicate sub-samples of rumen fluid were stored in 1N H_2SO_4, 20% metaphosphoric acid with internal standard (4 methyl n-valeric acid) or un-acidified. All samples were placed on ice prior to storage at -20^oC until analysis was conducted.

On day 158 of the experiment three blocks of steers (n=3/treatment) were observed for 24 hour, with time spent eating, ruminating or doing neither recorded every 5 minutes for each steer.
Radiographs of the left (near-side) cannon (metacarpal) bone of each steer were taken at the commencement and conclusion of the experiment using a portable X-ray machine (Atomscope) and digital X-ray plates (Fuji). Metacarpal length, dorsal cortical bone thickness (at 50% of the length), physeal width and epiphyseal height of the cannon bone of each steer were determined using Fuji PACS image storage/analysis software. An aluminium step wedge was included in each image to check for any magnification.

Bone biopsies were collected from the 12th rib and tuber coxae bones of each steer prior to commencement (near-side) and at the completion (off-side) of the experiment. Approximately 20 and 10 days prior to biopsies, steers were administered with 10 mg/kg LW of tetracycline (Engemycin, 100 mg tetracycline HCl/mL) intravenously to label the dynamic bone growth over that period in time. At both sites the area was clipped and scrubbed with Chlorhex surgical scrub and the incision sites were infiltrated with lignocaine (Illium Lignocaine 20; 20 mg/mL lignocaine hydrochloride). Bone cores, 16 mm in diameter, were collected from each site. The tuber coxae biopsy sample was quartered longitudinally, with two samples snap frozen in liquid N and the remaining samples fixed in 70% ethanol or 10% normal buffered formalin. The rib bone biopsies were rinsed and stored in 0.9% saline prior to storage at -20^oC.

At the end of the experiment, biopsies were collected from *m. semitendinosus* and overlying subcutaneous fat. The mid-belly region of the *m. semitendinosus* was clipped and scrubbed with Chlorhex surgical scrub and infiltrated with Lignocaine. A small 3 cm incision was made, with subcutaneous adipose tissue removed by scalpel and a 1 g muscle biopsy collected using a 10 mm punch biopsy. The biopsy site was closed with absorbable suture and surgical staples. Samples were placed in cryo-tubes and frozen in liquid N prior to storage at -80^oC for potential gene expression analysis.

Sample analysis
Organic matter content of feeds offered, residues and faeces were determined after combustion of samples at 550^{o}C for 8 hours (Modutemp Pty. Ltd.; Perth, WA, Australia) (AOAC, 1990). The N content was determined by the Kjeldahl method using an N analyser (Kjeltec, 8400 FOSS; Hillerod, North Zealand, Denmark). Ash-free NDF and ash-free ADF were determined using an Ankom fibre digestion unit (Ankom Technology; Macedon, NY, USA) following procedures described by the manufacturer. Mineral content of all samples was determined on an inductively coupled plasma spectrometer (ICPS; Optima 7300 DV, Perkin Elmer; Wellesley, MA, USA). Approximately 0.3 g of dried feed and faeces, 1 mL of rumen fluid and 4 mL of urine were digested overnight in nitric:perchloric (3:1) acid prior to ICPS analysis. Metabolisable energy content of the treatment diets was calculated using Equation 1.12 C in Freer *et al*. (2007; ME = 0.194 x DOMD% - 2.577).

The inorganic phosphorus concentration in plasma and serum was determined on an Olympus AU400 auto-analyser (Beckman Coulter Diagnostic Systems Division) using Beckman Coulter inorganic phosphorus reagents. Plasma glucose and plasma urea N concentrations were determined on samples collected at the end of the digestibility period an Olympus AU400 auto-analyser using Beckman Coulter reagents. The concentration of total, undercarboxylated (Glu-) and carboxylated (Gla-) forms of osteocalcin (OCN) were determined on plasma samples collected at the commencement and conclusion of the experiment by enzyme immune-assay (EIA) according to the manufacturer's instructions with kits supplied by Quidel (total) and Takara (Glu- and Gla-). Circulating insulin-like growth factor-1 (IGF1) concentration was determined on plasma samples collected at the commencement and conclusion of the experiment by radioimmunoassay (RIA) according to the manufacturer's instructions (Bioclone).

The concentration of NH_3N in rumen fluid was determined by distillation after the pH was adjusted by sodium tetraborate (Buchi-321 distillation unit; Flawil) followed by titration against 0.01 M HCl (TIM840 Titration Manager Workstation; Radiometer Analytical SAS). The concentration of volatile fatty acids present in rumen fluid were determined by gas-liquid chromatography (GC17 Schimadzu) using a polar capillary column (ZB-FFAP; Phenomenex).

Rib bone biopsies were processed by removing trabecular bone by scalpel. The cortical bone thickness (CBT) was measured using vernier callipers, with six measurements conducted on each biopsy and the average of the three thickest measurements used in the analysis. Specific gravity was determined by weighing the biopsies in air and then suspended in water.

Statistical analysis
One steer (Pen 7) from the 1.3P treatment group was not included in the statistical analysis as it spent significantly less time ruminating than the other steers throughout the experiment; this has important consequences for P recycling. Liveweight change, HH change, DM intake, P intake, PiP, FecP, FecP:ME and FecP:CP were statistically analysed as repeated measures using the MIXED procedure in SAS (SAS Inst. Inc., Cary, NC), with the model including treatment (dietary P content), stage (day of measurement/sample collection), treatment by stage interaction and block (allocated animal/pen block). Data generated during the digestibility period were analysed using the GLM procedure in SAS, with the model including treatment, cohort (rotation group through metabolism crates) and block. Data describing bone biopsies, bone x-rays and the concentration of osteocalcin and IGF1 in the plasma were analysed using the GLM procedure in SAS, with the model including treatment, stage of experiment, treatment by stage interaction and block. Interaction terms that were not significantly different ($P>0.05$) were removed from the model.

Linear and quadratic effects of dietary P intake (g P/day and mg P/kg LW.day) and dietary P content (g P/kg DM) were analysed using the GLM procedure in SAS or orthogonal contrasts for linear, quadratic and cubic effects; the data used to test these effects were derived from a repeated measures analysis (PROC MIXED) of each variable for individual animals across the

entire experimental period of 172 days, or for specific periods of time as indicated. Quadratic and cubic relationships that were not significant (P>0.05) were removed from the model and the linear relationship only was tested.

3.2 Experiment 2. Field study

Reproductive performance of cows and growth of steers that were offered dry and/or wet season phosphorus supplements and the validity of FecP:ME to indicate a P deficient diet under a commercial cattle production system

The experiment was conducted in Stud paddock at Brunchilly outstation of Helen Springs station, Tennant Creek, Northern Territory (hereafter called Brunchilly) in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and was approved by the Charles Darwin University Animal Ethics Committee.

In the process of selecting a site to conduct the P project it became apparent that it was going to be very difficult to find a site that satisfied all the requirements for the project. There were a number of logistical requirements that excluded most properties in the NT. These requirements were,

- That the site has good paddock security with no creeks, waterholes, or other forms of long lasting surface water (since the project relied on drafting cattle through an auto-drafter as they came in to drink).
- That the site would have the ability to run 600-700 breeders in a single paddock with only 1 or 2 water points.
- That the property was willing to supply approximately 600 pregnant non-lactating multiparous females at the start of the project in WR1 2011. In many commercial operations, this would have been quite a considerable undertaking, involving pregnancy testing cattle and moving identified trial cattle to the selected paddock. Also, the collaborating property had to be willing to not supplement the trial cattle over the 2010/11 wet season to deplete skeletal P reserves prior to the commencement of the experiment.
- That the collaborating property was willing to have auto-drafters and associated infrastructure installed at the watering points in the trial paddock and have cattle trained to use these devices. The training would also involve some help from the participating property, which was initially estimated to be approximately 6 weeks.
- That the manager of the property was keen to be involved in the research, had a good understanding of what being involved in research meant and was considered to be likely to be good to work with and reliable over the course of the research project.
- The other major criterion in choosing the site was that it had to be P deficient.

After extensive consultation it was considered at the time that Stud paddock would be P deficient. The information that was used to make this decision is summarised below,

- Brunchilly is located in a region (which includes most of the Barkly Tableland) that is generally considered to be P deficient and where it is generally considered that cattle respond to P supplementation.
- At the time recent FecP:ME data collected in Stud paddock and other paddocks on Brunchilly (in the Cash Cow project) indicated that Stud paddock was less than adequate for P (e.g. Figure 8.2.1).
- Anecdotal evidence from the property suggested that it was likely to be P deficient. This included the opinion of the manager of Brunchilly at the time who thought that the paddock was likely to be P deficient and that cattle in that paddock had responded to P supplementation previously. The opinion of other people with experience and/or knowledge of managing cattle in the Barkly was sought and the general consensus was that it was likely that Stud paddock would be P deficient.

Stud paddock is 66 km^2 and according to NT Department of Primary Fisheries Land Systems and Infrastructure mapping was 34% Wonorah (red soil) and 66% Barkly1 (black soil) land types (Figure 1). Surface soil cores (0-10 cm) were collected from 10 random sites within each land type. A full analysis of soil properties was conducted on a bulk sample from each land type, including Colwell P, while Colwell P was also analysed on each of the individual soil cores. The Colwell P was 3 and 9 ppm on the black and red soils respectively. Results of the soil analysis are presented in Appendix 8.2. There were two water points within Stud paddock, No 19 and Stud bores.

Throughout the experiment the cattle grazed as a single mob in Stud paddock with bulls present at all times throughout the experimental period. All cattle had NLIS electronic identification (EID) tags in their ears (Alflex). At the start of the project, enclosures were built around each water point so that in order to access the water, cattle had to enter the enclosures through an auto-drafter with walk-over-weighing facility and exit through spear gates (see Appendix 8.2). Each water point enclosure consisted of two supplement yards, with a common water trough between the yards. The cattle were drafted two-ways into one of two supplement yards on entry into each water point (+P or -P). The auto-drafters were used to draft animals into their correct yard using the draft list of EIDs of animals allocated to each treatment. The EID tag was scanned as the animal passed through the walk-over weighing platform and then a computer controlled drafting gate drafted the animal into an enclosure according to the allocation draft list. All cattle received supplement, although only those allocated to the +P supplement received supplementary P. Access to supplements was *ad libitum* and animal EID was read on exit from the +P supplement yard to confirm accuracy of draft.

It took a period of approximately six weeks to train animals to use the auto-drafters. Initially they were accustomed to passing alongside the auto-drafters and then through the auto-drafters with the gates wide open and with the drafting function turned off. The spear gates were gradually narrowed over time and finally the auto-drafting gate was turned on. The cattle were checked daily during this period by station staff and some gentle coaching was used to try to get reluctant animals to use the auto-drafters. Some animals refused to use the auto-drafters and 32 had to be removed from the experiment for this reason.

The trial commenced at the end of the 2011 dry season (October 2011). A mob of 544 (after 32 cows were removed that would not use the auto-drafters) pregnant (due to calve between October 2011 and April 2012), non-lactating cows and 80 No. 0 steers (327 ± 3 kg), none of which had access to P supplements during the 2010/2011 wet season or 2011 dry season, were allocated to either +P or -P supplement treatment groups on the basis of fetal aging and LW (determined in August 2011) for cows or LW for steers. Baseline bone biopsies, serum samples collected from the coccygeal vein and faecal samples were collected from a cohort of ‘sample cattle’ (n=87 cows and n=40 steers for blood and faeces; n=40 cows for bone biopsies) prior to commencement of the wet season treatments.

Originally the experiment was scheduled to start in June 2011 but it was not possible at this time to source enough cows at the right stage of pregnancy that had been trained to use the auto-drafters. Therefore the start of the experiment was delayed until October 2011 to allow more time to source suitable cows and train them to use the auto drafters. The experiment was scheduled to finish at the end of the 2012 dry season but it was decided to extend it over the 2012/13 wet season due to the late start and the fact that seasonal conditions had been unusually good up until this point and it was felt that there would be value in extending the experiment through a more normal wet season. A further 40 No. 2 (weaned in 2012) steers were added at the muster in October 2012 with 20 being allocated to each of the +P and -P treatments. Blood and faecal samples were collected from these animals at the musters in October 2012 and May 2013.

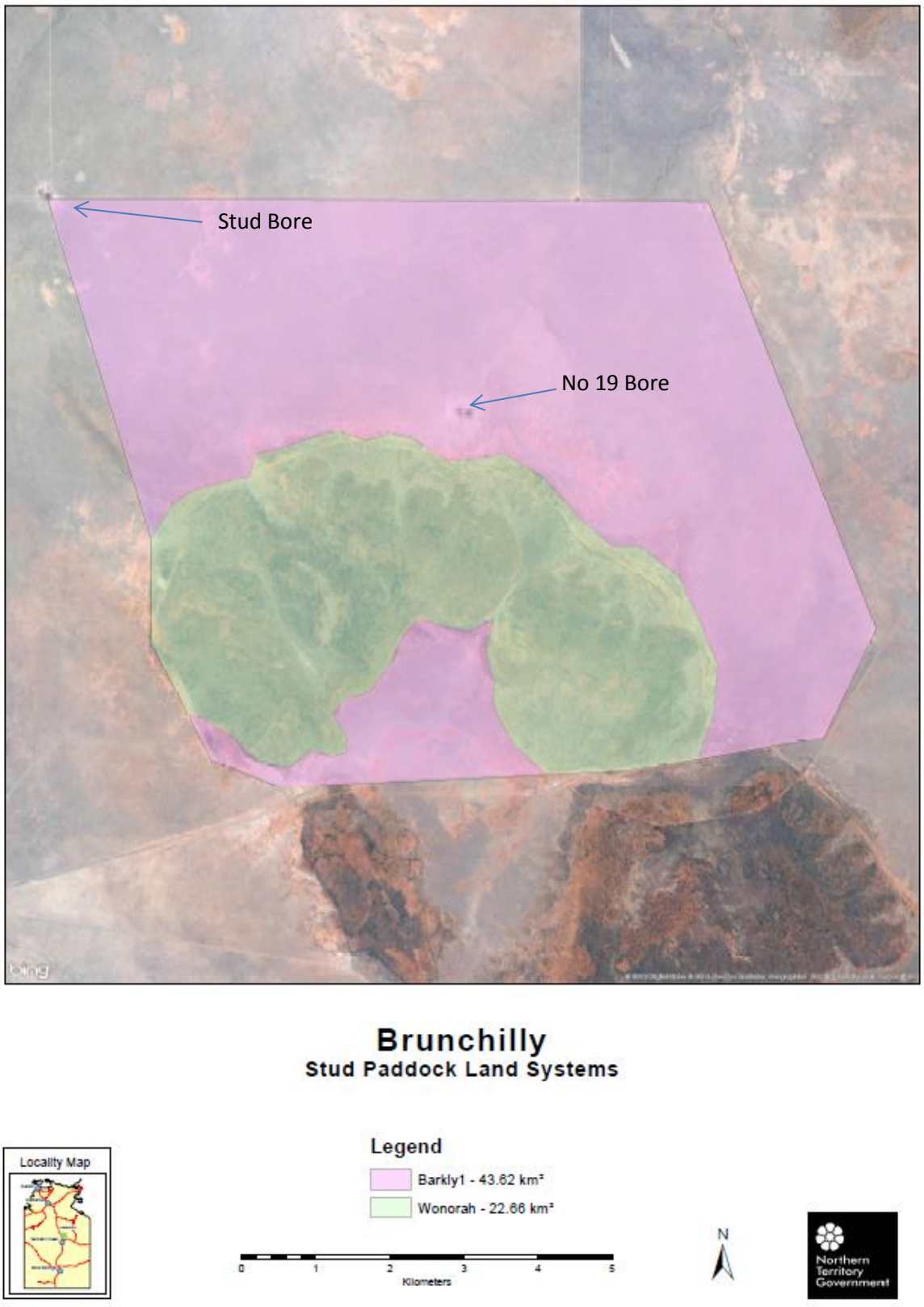


Figure 1. Land systems of Stud paddock, Brunchilly (Wonorah land system is based on deep red earths and was 34% of the area within Stud paddock).

After the 2011/2012 wet season (in May 2012) the allocation was changed and the cattle were allocated to +P and -P dry season treatments on the basis of pregnancy status, fetal aging and LW. The changed allocation was achieved through uploading a new draft allocation list to the auto-drafters. To examine the carryover effects of dry season P supplementation the cattle were reintroduced to their original allocated wet season treatments over the 2012/2013 wet season (by uploading the original draft allocation list to the auto-drafters. This established four experimental treatments for the entire experiment,

1. +P in wet season 1, followed by +P in dry season 1, followed by +P in wet season 2
2. +P in wet season 1, followed by -P in dry season 1, followed by +P in wet season 2
3. -P in wet season 1, followed by +P in dry season 1, followed by -P in wet season 2
4. -P in wet season 1, followed by -P in dry season 1, followed by -P in wet season 2

The duration of these treatment periods was,

1. Wet season 1 – 12-Oct-2011 to 26-May-2012 (227 days)
2. Dry season 1 – 30-May-2012 to 10-December-2012 (194 days)
3. Wet season 2 – 11-Dec-2012 to 28-May-2013 (169 days)

The +P and -P supplements were supplied as a loose lick (Rumevite, Ridley Agri-products) and the formulations are presented in Table 3. The formulation of the supplement was based on the following

- Comparable in composition to the 'Brunchilly Breeder' mix that was reportedly used for breeders on Brunchilly
- Biofos was the source of P
- A target supplement P intake of approximately 10 g P/head.day
- A target supplement intake of approximately 100 and 150 g/head.day, in the wet and dry seasons respectively

The composition of the wet season supplement was modified for the 2012/2013 wet season to include 10% cottonseed meal (CSM). This was due to the low supplement intakes observed over the 2011/2012 wet season and was an attempt to stimulate higher supplement intake over the wet season. Preliminary results would suggest that the inclusion of CSM in the 2012/2013 wet season had little effect on supplement intake.

Table 3. Formulation of supplements either with (+P) or without (-P) phosphorus to be offered during the 2011/2012 wet and 2012 dry seasons at Helen Springs

Raw material	Wet season		Dry season	
	+P (%)	-P (%)	+P (%)	-P (%)
Cottonseed meal (43)	0	0	10	10
Limestone fine	0	0	10	10
MDCP Biofos	50	0	30	0
Urea prills	0	0	25	25
Gran-Am	0	0	10	10
Salt	45	95	14.5	44.5
Premix	0	0	0.5	0.5
EC Feed	5	5	0	0

Throughout the experiment LW was attempted to be measured regularly by walk-over-weighing. In theory each animal should have been weighed by walk-over-weighing each time it entered the enclosure for a drink, however weights were not recorded on all occasions due to equipment failures. All animals were mustered to the yards for data and sample collection on 26 May 2012,

19 October 2012, and 28 May 2013. At these musters BCS and LW were measured and samples were collected for serum P, faecal P and bone thickness, density and P content. Blood and faecal samples were collected from the same cattle on each occasion (n=87 cows and n=40 steers). Bone biospies were collected from the same 32 cows on each occasion. The cows selected for sampling were all expected to calve in November/December 2011 in the first year of the experiment. Bone biopsies were collected in the following sequence near-side 12th rib (June-2011), near-side 11th rib (May-2012), off-side 12th rib (October-2012) and off-side 11th rib (May-2013), this meant that full year comparisons of treatment effects were conducted on the same rib on opposite sides of the body, whereas seasonal comparisons were conducted on adjacent ribs on the same side of the body. Pregnancy status and fetal aging were determined in May and October 2012 and May and August 2013; the final measurement was conducted on those cows for which a pregnancy was not detected in May 2013. Individual faecal samples were collected for faecal NIRS from the ground after random cows and steers were observed to defecate in each supplement yard, each month throughout the study (n=10 cows and n=10 steers/month/+P and -P treatments). Almost all samples were collected from No. 19 bore due to the infrequent use of Stud bore by cattle during the experiment. Supplement intake (of treatment groups) was estimated at regular intervals during the experiment by measuring the amount of supplement offered and refused over a period of time (usually every one or two weeks). Faecal NIR and faecal P (wet chemistry) were conducted by Symbio Alliance (Eight Mile Plains, Queensland) on faecal samples that were collected on site, frozen at -20^{o}C and then thawed and dried to a constant weight at 65^{o}C prior to submission to Symbio, with NIR and FecP results reported on an as received basis. Dietary ME content was estimated from faecal NIRS prediction of DMD using Equation 1.12A in Freer et al. 2007 (ME = 0.172 x DMD – 1.707).

There was no withdrawal of P supplements prior to sample collection in Experiment 2 but samples were collected from both +/- P supplement groups. The FecP:ME of -P cattle would indicate the likely response to P supplementation of animals in Stud paddock during the period of the experiment. As faecal samples were to be collected once/month from the lick yards and this often occurred over several weeks each month, withdrawal of supplements one to two weeks prior to sampling would have meant that cattle would not have had access to supplements for between 25 to 50% (one to two weeks/month) of the duration of the experiment. While supplements could have been withdrawn prior to sampling conducted at the yards this would have been inconsistent with monthly sampling protocols. In addition it was considered that sampling in relation to P intake be consistent between Experiments 1 and 2. In Experiment 1 the inorganic supplement was included in the pellet so could not be withdrawn from the diet; withdrawal of the P supplement for a period of one to two weeks prior to sampling, which was occurring every three weeks, would significantly reduce the amount of dietary P available to the steers over the duration of the experiment. The non-removal of supplements prior to sampling would not have influenced FecP in cattle allocated to the -P supplement. The difference between the two treatment groups would reflect the effect of supplement intake on faecal P.

A fire burnt out a proportion of Stud paddock in the late dry season, including much of the red soil areas of the paddock. This burnt area of the paddock would have been preferentially grazed by cattle after the commencement of the wet season.

3.3 Additional activities

In addition to the two main experiments within this project a series of related activities were undertaken. The background, materials and methods and results of these activities can be located in the following appendices

Appendix 8.3. Development of remote technologies during Experiment 2 at Brunchilly

Appendix 8.4. Comparison of blood components and sampling site on circulating P concentration

Appendix 8.5. *In vitro* solubility of various P supplements

Appendix 8.6. Validation of laboratory results

Appendix 8.7. Re-alimentation of P deficient cattle

Appendix 8.8. Bone histology

Appendix 8.9. Glucose homeostasis in steers with different P status

4 Results and discussion

4.1 Experiment 1. Pen study

Feed intake, growth and faecal and plasma phosphorus concentration in steers fed diets with increasing P content.

The results presented here are specific to the class of animal, diets and experimental methodologies as described in the materials and methods section. It is not possible to make conclusions regarding the response of other classes of animals to these diets, or the response of this class of animal to diets with different P, ME or CP content or ingredients to those which were tested in this experiment.

Diets and formulation
Despite the pellets being formulated with the same ingredients in the same proportions, with the exception of the additional P supplied in the form of Biofos, there was variability in the chemical composition of the pellets. The main issues relate to the high Ca content in the P-3 pellet, and to a lesser extent in the P-1 pellet, and the low Zn content of the P-4 pellet. As the formulation of ingredients supplied to the manufacturer was identical for each pellet we can only assume that the ingredients were incorporated into some batches of pellets at a rate different to that recommended. It appears likely that wrong amounts of $Ca(OH)_2$ were added to the P-3 pellet. Interestingly, the slightly elevated mean Ca concentration in the P-1 pellet, compared to the P-2, P-4 and P-5 pellets, was due to a higher Ca concentration in the second batch of P-1 pellets only; the original batch of P-1 pellets had comparable Ca concentration to the P-2, P-4 and P-5 pellets. The low Zn concentration in the P-4 pellet compared to the other pellets also appears to be an anomaly in the pellet manufacture process, although this is less easily explained as the P-4 pellet has comparable levels of all other minerals and the primary source of Zn was the premix which contained the other minerals, which are all balanced. The actual P content of the pellets is different to that predicted based on the chemical composition of the ingredients. If the ingredients used were simply different in P content to the initial test results a proportional shift in P content of pellets would have been expected. This did not occur and further suggests that there were issues related with the manufacture of specific pellet batches. In hindsight a better approach would have been to manufacture a single batch of low P (0.9 g P/kg DM) pellet and separately offer the steers increasing amounts of supplementary P in proportion to pellet intake, although this approach may have introduced issues around achieving the targeted intake of P supplements.

The Ca:P content of the diets in the current experiment were 7.1:1 (0.9P), 3.5:1 (1.3P), 11.2:1 (1.8P), 2.1:1 (2.0P) and 2.0:1 (2.4P). While the metabolism of Ca and P are linked the importance of the ratio in the diet is not clear for grazing ruminants where Ca:P is usually high, certainly greater than 2:1. Wise *et al*. (1963) suggested there were no deleterious effects when young calves were fed diets with Ca:P of 7.2:1 (comparable to 0.9P in the current experiment). There was a small negative impact on intake and LWG when the Ca:P was 14.3:1 but this was unlikely to be biologically significant and was not as marked as when the Ca:P was below 1:1. Wise *et al*. (1963) did not test the Ca:P of diets between 7.2:1 and 14.3:1, so it is unknown at exactly what ratio a negative response commenced. Field *et al*. (1975) demonstrated that a P mediated depression in intake was alleviated when a diet also low in Ca was fed but the effects of a high Ca/low P diet were not examined. Under most grazing situations in northern Australia, the Ca concentration of the diet is likely to be adequate rather than deficient for growing and non-lactating animals. There was no difference in DM intake when P deficient diets were fed to sheep with either a low or high Ca concentration (Ternouth and Savilla, 1990) with intakes of these two diets being lower than when a high P/high Ca diet was fed, suggesting that dietary P content is more important than Ca content, or the Ca:P, in respect to DM intake and, hence, LWG. In contrast, Coates (1994) demonstrated that in stylosanthes based pastures grown on P deficient soils the Ca:P may be as high as 20:1 [or 50:1 for verano stylosanthes in the dry season, Winter (1988)], and that the response of cattle grazing these pastures to P supplements may be higher than the response of animals grazing pastures with similar P content but lower Ca content. A diet with a high Ca:P is probably only an issue when the diet is deficient in P, with P incorporated/retained in bone in association with Ca, resulting in depletion of soft tissue P reserves leading to a subsequent depression in intake and LWG. In Experiment 1, data pertaining to steers fed the 1.8P treatment diet generally sat within the expected linear and quadratic responses for various parameters to P intake with little indication that the high Ca:P of the diet influenced the results. In summary, we do not believe the high Ca:P of the 1.8P treatment in Experiment 1 had an impact on the results obtained for that treatment group of steers, with the results reflecting P intake rather than the Ca:P or Ca content of the diet.

Despite statistically significant differences in DMD and OMD and estimated ME content between the treatment diets these differences were small and unlikely to be biologically important and suggest that dietary P content does not affect rumen metabolism. These differences in digestibility appear to be related to a metabolic regulation of DM intake itself rather than any issues with the composition of the diet, where digestibility was highest for steers that had the lowest DM intake. The important point is that the estimated ME and CP content of all five treatment diets was not limiting growth. It was expected that a LWG of 1.0 kg/day could potentially be achieved for this class of cattle fed these diets provided all other nutrients, including P, were adequate and this was achieved for steers consuming the 2.0P and 2.4P treatment diets.

Rumination time (h/day) was similar between treatments (approximately 6 h/day) and was within the expected range (6 to 10 h/day) for grazing cattle described by Church (1969), albeit at the lower end of the range.

Time related changes in dry matter intake, phosphorus intake, liveweight, hip height, plasma phosphorus, faecal phosphorus, faecal phosphorus:diet metabolisable energy content and faecal phosphorus:diet crude protein content

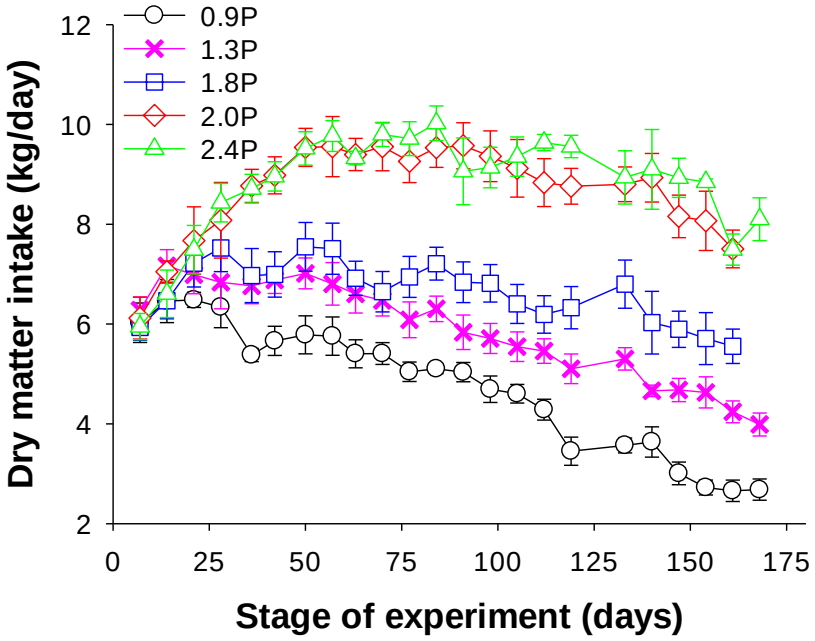
Phosphorus (Figure 2) and DM (Figure 3) intake, LW and HH (Figure 4), PiP and FecP (Figure 5) and FecP:ME and FecP:CP (Figure 6) all changed over time during the experimental period. Phosphorus intake was lower for steers offered the 0.9P treatment compared to the 2.0P and 2.4P treatments from week 1 of the experimental period. With the exception of PiP, all parameters demonstrated a significant treatment effect by day 36 of the experimental period, at which time steers offered the 0.9P treatment had significantly different values to steers offered the 2.0P and 2.4P treatments. This suggests that the negative impacts of a low P diet on DM intake and growth of steers occurs after approximately 5 weeks of exposure to a low P diet. This

is comparable to the time taken (7 weeks) for a 14% reduction in feed intake to present itself in P adequate cattle fed P deficient diets by Bortolussi *et al*. (1996) but shorter than other experiments (19 weeks; Gartner *et al*., 1982). The effect of P on intake is widely reported and is in line with the principle of the first limiting nutrient and this effect was evident in Experiment 1, where steers fed low P content diets had low DM and ME intake, resulting in low LWG.

The concentration of PiP and FecP were not significantly different for steers allocated to the five treatment diets at the commencement of the experiment (day -16), indicating that P intake of all steers was similar prior to commencement of the experiment. The concentration of PiP was significantly lower in steers offered the 0.9P treatment compared to steers offered the 2.0P and 2.4P treatments on day 1 of, and throughout, the experimental period, demonstrating that PiP is sensitive to a gradual introduction to diets divergent in P content over 16 days and these differences persist while steers continue to consume diets divergent in P content. Significant treatment by stage of experiment interactions were observed for PiP, FecP, FecP:ME and FecP:CP throughout the experiment. In general, steers offered the 0.9P and 1.3P treatments had decreasing levels of all four indices as the experiment progressed while levels remained relatively constant and significantly higher for steers offered the 2.0P and 2.4P treatments. Phosphorus indices for steers offered the 1.8P treatment were typically intermediate between the two treatments with the lowest P content (0.9P and 1.3P) and the two treatments with the highest P content (2.0P and 2.4P). The PiP concentration remained relatively constant throughout the experimental period for each of the five treatments compared with faecal P indices which were more variable from measurement to measurement within treatments and continued to diverge throughout the experimental period. The FecP and its ratio's with dietary ME and CP content will only provide an indication of P intake approximately 36 days after commencement of consumption of a low P diet by which time negative impacts on production may have started to occur.

Differences in size and body condition of steers at the end of the experiment are presented in Figure 7. The steers in Figure 7 a. were fed 0.9P and 2.4P treatments for 172 days and were in the same experimental block, so were of comparable LW at the commencement of the experiment. Similarly the steers in Figure 7 b. (0.9P) and 7 c. (2.4P) were in the same experimental block.

a.



b.

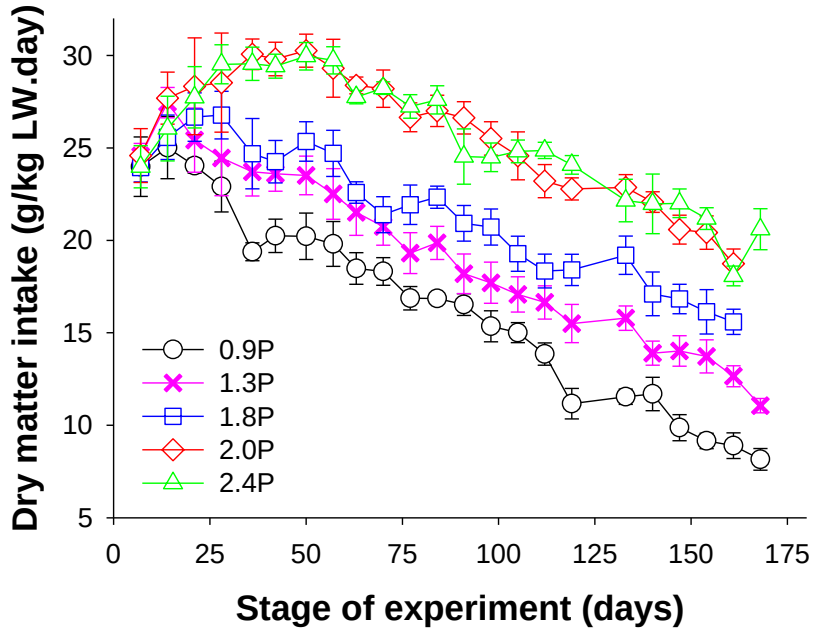
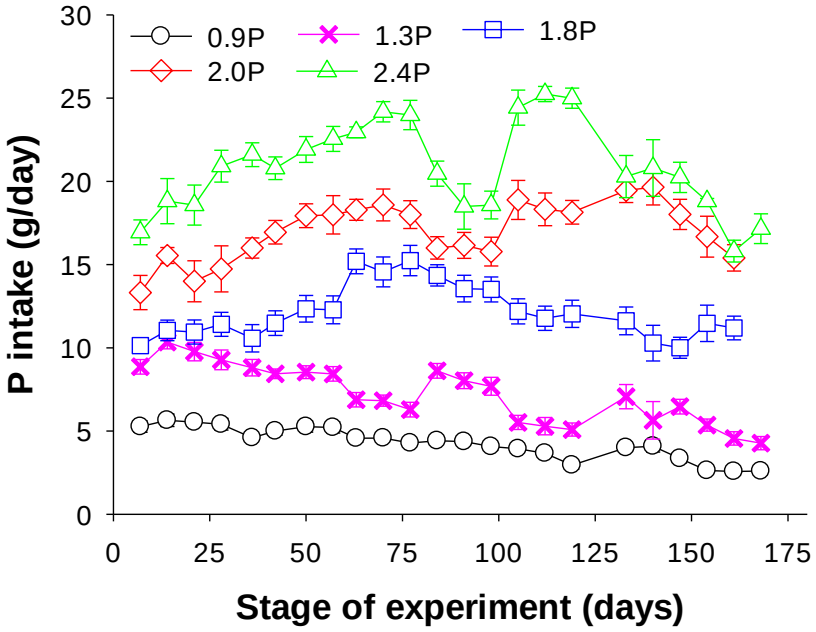


Figure 2. Dry matter intake (a. kg/day; b. g/kg LW.day) of steers offered diets with increasing phosphorus (P) (0.9, 1.3, 1.8, 2.0 and 2.4 g P/kg DM) content over 172 days.

a.



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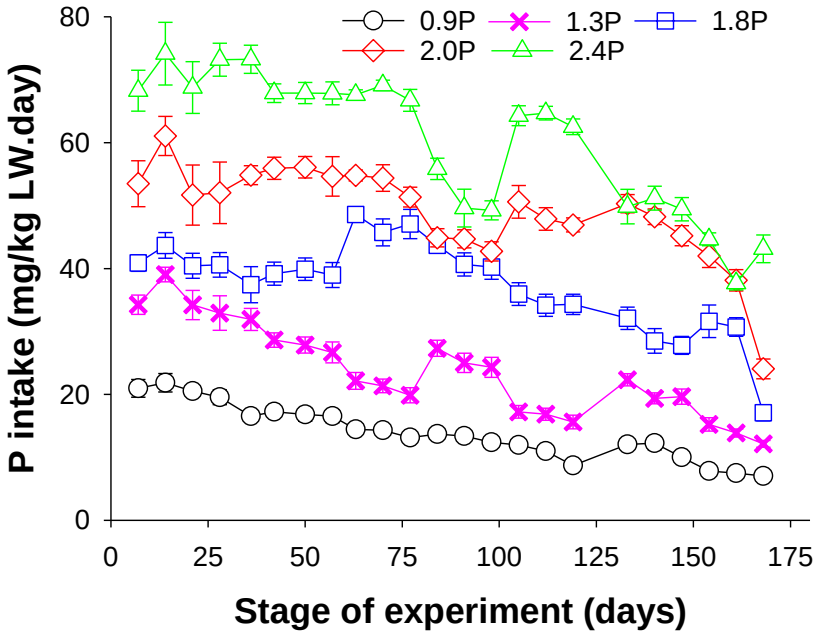
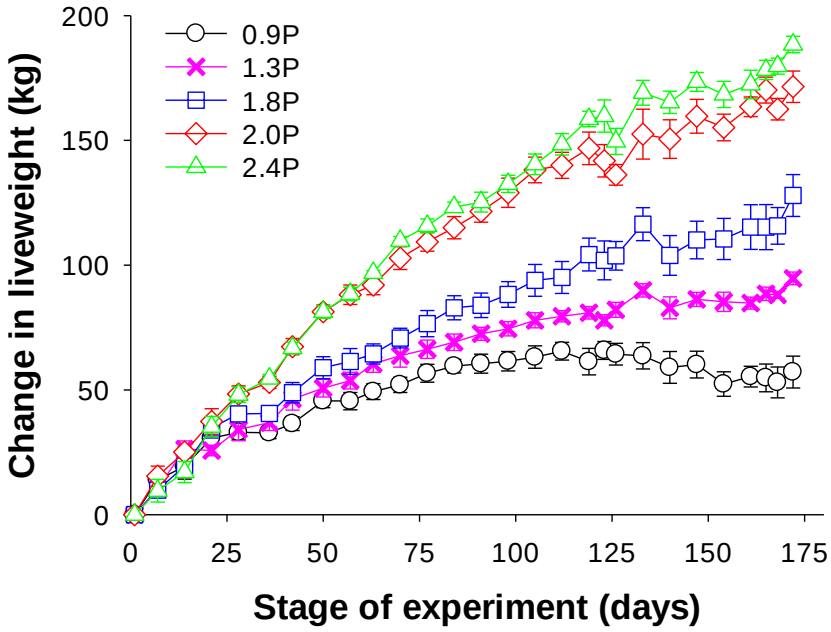


Figure 3. Phosphorus (P) intake (a. g/day; b. mg/kg LW.day) of steers offered diets with increasing P content (0.9, 1.3, 1.8, 2.0 and 2.4 g P/kg DM) over 172 days.

a.



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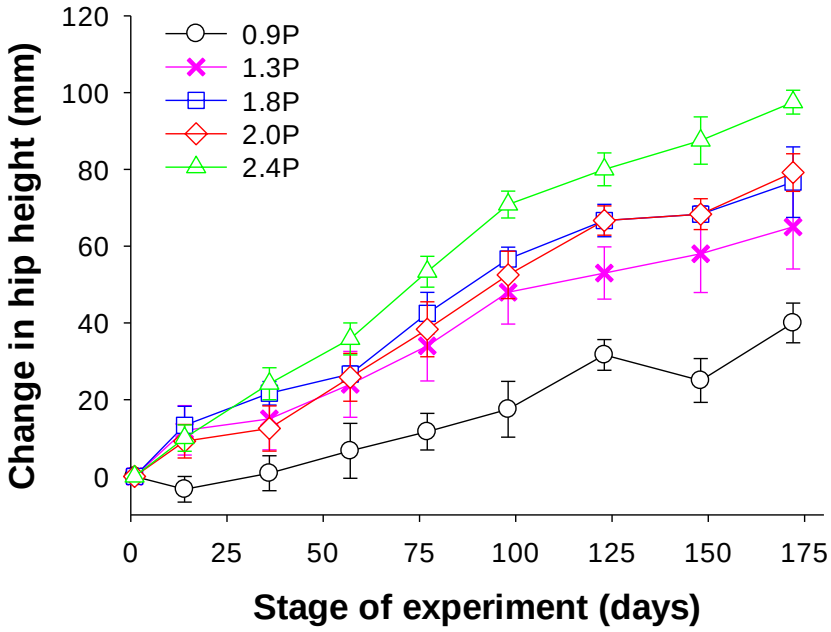
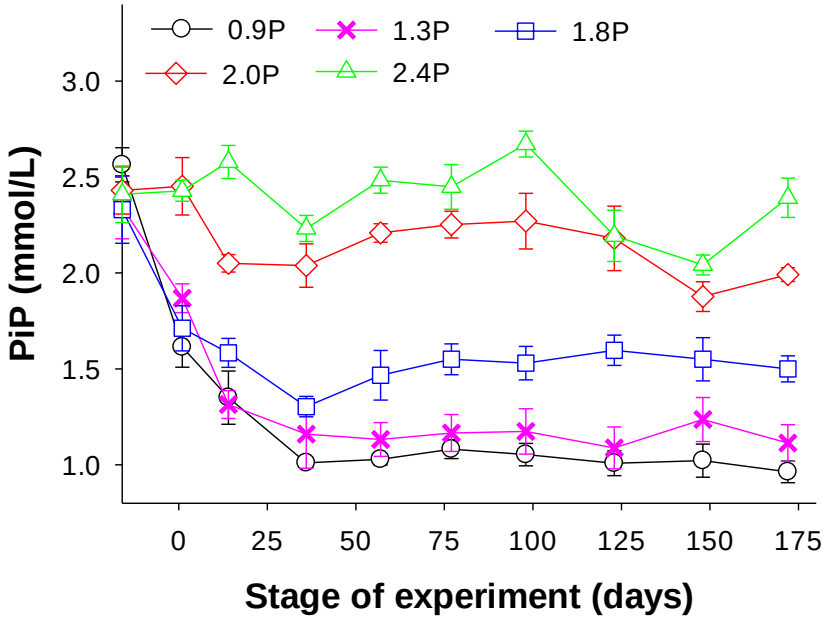


Figure 4. Cumulative change in liveweight (a.) and hip height (b.) of steers offered diets with increasing phosphorus (P) content (0.9, 1.3, 1.8, 2.0 and 2.4 g P/kg DM) over 172 days.

a.



b.

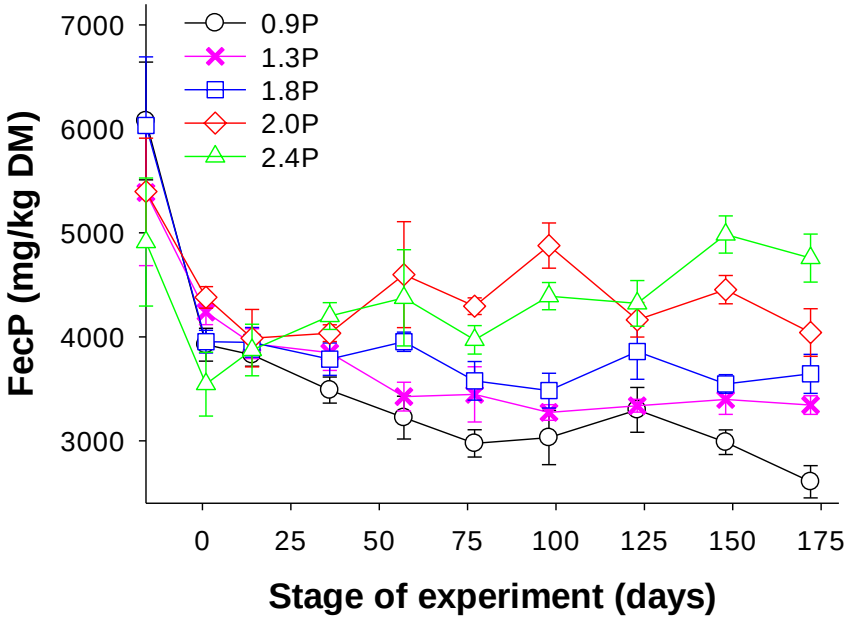
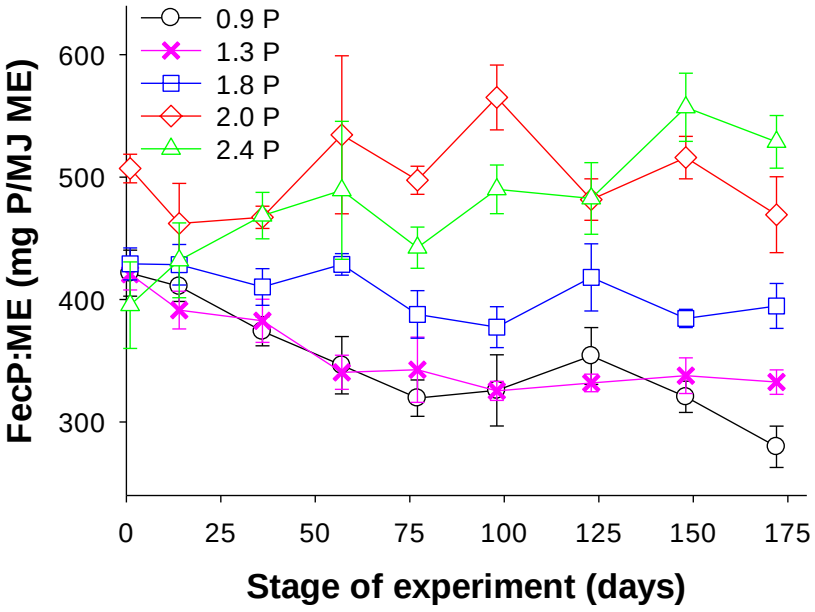


Figure 5. Change in the concentration of phosphorus (P) in the plasma (PiP) (a.) and faeces (FecP) (b.) of steers offered diets with increasing P content (0.9, 1.3, 1.8, 2.0 and 2.4 g P/kg DM) over 172 days.

a.



b.

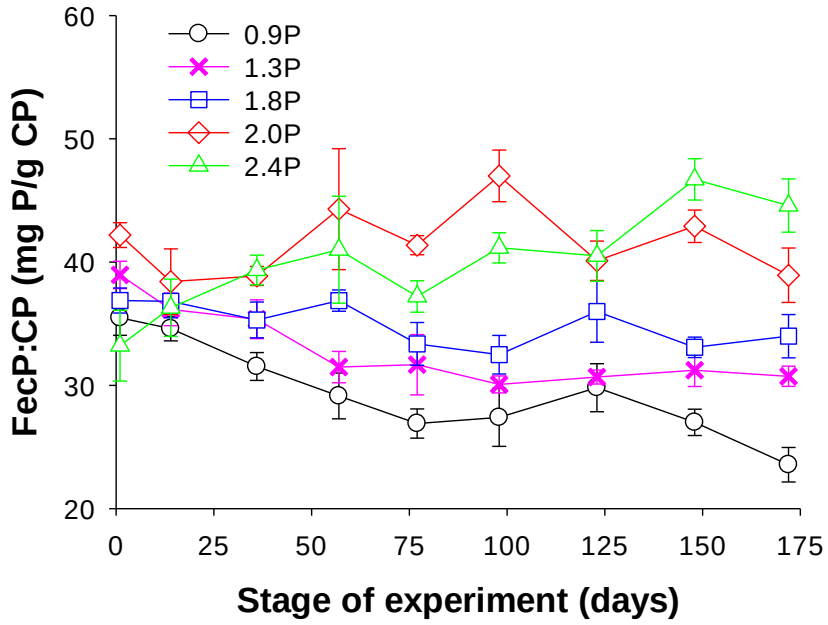


Figure 6. Change in the concentration of phosphorus (P) in the faeces (FecP):diet metabolisable energy content (ME) (a.) and FecP:diet crude protein (CP) content (b.) of steers offered diets with increasing P content (0.9, 1.3, 1.8, 2.0 and 2.4 g P/kg DM) over 172 days.

a.

b. **c.**

Figure 7. Steers that were offered 0.9P and 2.4P diets for 172 days (photos taken at end of Experiment 1; a. 2.4P steer left and 0.9P steer right; b. 0.9P steer; c. 2.4P steer). (Source: L Jarvis and S Quigley)

The effect of dietary phosphorus content on dry matter and phosphorus intake, plasma and faecal phosphorus concentration and liveweight and hip height of steers

Results below are described on both a g P/kg DM basis, which is applicable to industry, and an mg P/kg LW.day basis, which is important in understanding P metabolism within the animal. Results in terms of g P intake/day are presented in Appendix 8.1, as this is also of use at a practical level by industry and aligns with recommendations provided in '*Phosphorus management of beef cattle in northern Australia*' (Jackson *et al*., 2012).

Phosphorus intake was significantly different between all treatment groups on both a g P/kg DM and mg P/kg LW.day basis (Table 4) over the entire experimental period. Phosphorus intake

ranged from approximately 4 to 21 g/day or 14 to 60 mg P/kg LW.day over the experiment, providing a wide range of P intakes over which responses of variables could be determined. Dry matter intake increased with dietary P content and P intake in a linear fashion over the entire experimental period (Table 5; Figure 8). The decreased DM intake of the low P diets further exasperated the already low P content of the diet, resulting in further decreasing P and ME intake throughout the experimental period. The initial effect of a P deficient diet reducing DM (and hence ME) intake measured in Experiment 1 is widely reported and is in line with P being the first limiting nutrient in the wet season in P deficient areas.

The FecP was significantly higher in steers offered the 2.0P and 2.4P diets compared with the 0.9P diet and increased in response to DM and P intake, as expected. The basis for the use of FecP and associated ratio's (FecP:ME and FecP:CP) as an indicator of dietary P content (or more specifically P intake) is that the amount of P in the faeces is directly related to the P content of the diet (Holechek *et al*., 1985). Faeces are the primary route of P excretion from the grazing ruminant, with the majority of faecal P being endogenous in origin. In Experiment 1, there was a linear relationship between FecP and dietary P content and P intake (Figure 9) when using the mean FecP, dietary P content and P intake data over the entire experimental period. However, this linear relationship was not observed at earlier stages of the experiment and was strongest at the end of the experiment (day 172 sample collection) (Figure 10). This suggests that the timing of sample collection in relation to time spent grazing P deficient diets may be important if FecP and the associated ratio's are used to determine the P content of the diet during the wet season when both ME and CP are not likely to be limiting LWG. It is possible that the lack of a relationship between FecP and P intake in the early stages of Experiment 1 was related to mobilisation of P from soft tissue and skeletal pools and as the experiment progressed these became depleted resulting in a decrease in endogenous P levels and hence FecP. However, if mobilisation of P from body stores was occurring in steers fed the 0.9P treatment it would have been expected that PiP would also have been maintained at a higher concentration at the start of the experiment reflecting maintenance of endogenous P levels. This did not occur in the current experiment, where PiP displayed an immediate response to P intake which was maintained throughout the experiment. Nevertheless the results clearly demonstrate that the period of time an animal has been consuming a P deficient diet will influence FecP and its relationship with P intake.

FecP:ME and FecP:CP increased with increasing dietary P content and P intake (Figures 11 and 12) in a cubic and linear fashion respectively. Linear responses in FecP:ME and FecP:CP with P intake were observed for the samples collected at the end of the experiment (day 172 of the experimental period) but these were different (lower) to the mean values observed across the experimental period. The strong relationships between FecP, FecP:ME and FecP:CP in the current experiment were expected given that ME and CP were similar between treatment diets and suggests that there is no advantage in using either the FecP:ME or FecP:CP over FecP alone to indicate P intake in growing steers fed a diet with a high and constant ME and CP content (i.e. consuming a diet similar in composition to that of early wet season pasture). It is unknown if these strong relationships persist when the ME and CP content of the diet changes as ME and CP were constant both over time and between treatments in Experiment 1.

Table 4. Phosphorus (P) and dry matter (DM) intake and the concentration of inorganic P in plasma (PiP) and faeces (FecP) and the ratio of FecP to diet metabolisable energy (ME) and crude protein (CP) content of steers offered diets with increasing P content[1,2]

	Treatment					
Parameter	0.9P	1.3P	1.8P	2.0P	2.4P	SEM
P intake - day 1 to 172 (g/day)[3]	4.3[a]	7.5[b]	12.1[c]	17.0[d]	21.0[e]	0.4
P intake - day 36 to 172 (g/day)[3]	4.1[a]	7.0[b]	12.5[c]	17.7[d]	21.4[e]	0.5
P intake - day 1 to 172 (mg/kg LW.day)[3]	14.2[a]	24.1[b]	38.3[c]	50.1[d]	61.1[e]	1.0
P intake - day 36 to 172 (mg/kg LW.day)[3]	12.5[a]	21.2[b]	37.6[c]	48.8[d]	58.0[e]	1.0
DM intake - day 1 to 172 (kg/day)[3]	4.84[a]	5.93[b]	6.66[b]	8.66[c]	8.83[c]	0.23
DM intake - day 36 to 172 (kg/day)[3]	4.46[a]	5.68[b]	6.61[b]	9.00[c]	9.24[c]	0.27
DM intake - day 1 to 172 (g/kg LW.day)[3]	16.8[a]	19.5[b]	21.5[b]	25.7[c]	25.7[c]	0.6
DM intake - day 36 to 172 (g/kg LW.day)[3]	15.0[a]	17.9[b]	20.3[b]	25.1[c]	25.2[c]	0.6
PiP - day 1 to 172 (mmol/L)[3]	1.13[a]	1.26[a]	1.53[b]	2.15[c]	2.39[d]	0.06
PiP - day 36 to 172 (mmol/L)[3]	1.02[a]	1.17[b]	1.50[c]	2.12[d]	2.35[e]	0.03
PiP (mmol/L)[4]	0.96[a]	1.11[a]	1.50[b]	1.99[c]	2.39[d]	0.08
FecP - day 1 to 172 (mg/kg DM)[3]	3262[a]	3784[b]	3750[b]	4315[c]	4269[c]	111
FecP - day 36 to 172 (mg/kg DM)[3]	3087[a]	3497[b]	3692[b]	4352[c]	4429[c]	74
FecP (mg/kg DM)[4]	2606[a]	3396[ab]	3644[b]	4041[bc]	4758[c]	197
FecP:ME - day 1 to 172 (mg P/MJ ME)[3]	350[a]	355[ab]	407[b]	500[c]	476[c]	13
FecP:ME - day 36 to 172 (mg P/MJ ME)[3]	331[a]	342[ab]	400[b]	505[c]	494[c]	14
FecP:ME (mg P/MJ ME)[4]	280[a]	333[ab]	395[bc]	469[cd]	529[d]	21
FecP:CP - day 1 to 172 (mg P/g CP)[3]	29.5[a]	34.8[b]	35.0[b]	41.6[c]	40.0[c]	0.8
FecP:CP - day 36 to 172 (mg P/g CP)[3]	27.9[a]	32.2[b]	34.4[b]	41.9[c]	41.5[c]	0.7
FecP:CP (mg P/g CP)[4]	23.6[a]	31.2[ab]	34.0[b]	38.9[bc]	44.6[c]	1.9

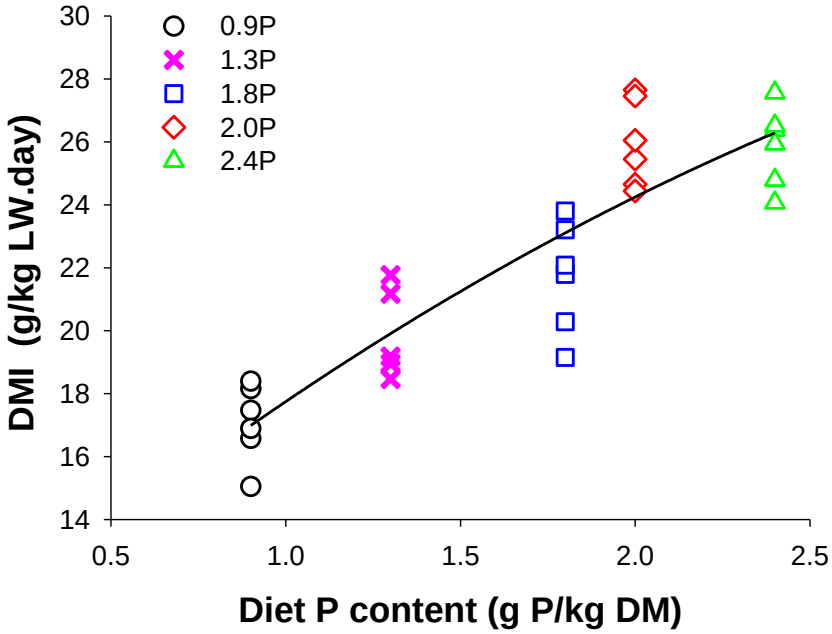
[1]0.9P (0.9 g P/kg DM), 1.3P (1.3 g P/kg DM), 1.8P (1.8 g P/kg DM), 2.0P (2.0 g P/kg DM) and 2.4P (2.4 g P/kg DM).
[2]Data are least-squares means with pooled standard error of the mean (SEM). Different alphabetical superscripts across each row indicate a significant difference between treatments (P<0.05) from the repeated measures analysis.
[3]Repeated measures over the experimental period as indicated.
[4]Day 172 of the experiment only.

Table 5. Response of dry matter intake (DMI), liveweight (LW), hip height (HH), plasma inorganic phosphorus (PiP), faecal P (FecP) and associated ratio's with diet metabolisable energy (ME) and crude protein (CP) content to increasing P intake over the entire experimental period[1,2]

Parameter (*Y*)	Equation	R^2	Root MSE[3]	*P-value*
	P content (g/kg DM) (*X*)			
DMI (kg/day)	$Y = 2.74x + 2.42$	0.80	0.74	<0.001
DMIW (g/kg LW.day)	$Y = 6.23x + 11.65$	0.80	1.73	<0.001
LWG (kg/day)	$Y = 0.472x – 0.11$	0.89	0.09	<0.001
LW change (kg)	$Y = 90.34x – 23.86$	0.90	16.2	<0.001
HH change (mm/100 d)	$Y = 20.9x + 8.37$	0.70	7.5	<0.001
PiP (mmol/L)	$Y = 0.395x^2 – 0.41x + 1.15$	0.87	0.19	0.015
FecP (mg/kg DM)	$Y = 723.3x + 2617.1$	0.68	272.5	<0.001
FecP:ME (mg P/MJ ME)	$Y = -232.9x^3 + 173.5x^2 – 1754.4x + 1151.3$	0.73	37.9	0.007
FecP:CP (mg P/MJ ME)	$Y = 7.79x + 22.68$	0.69	2.84	<0.001
	P intake (mg/kg LW.day) (*X*)			
DMI (kg/day)	$Y = 0.09x + 3.66$	0.89	0.56	<0.001
DMIW (g/kg LW.day)	$Y = 0.203x + 14.42$	0.89	1.26	<0.001
LWG (kg/day)	$Y = 0.02x + 0.123$	0.92	0.08	<0.001
LW change (kg)	$Y = 2.81x + 20.97$	0.93	13.99	<0.001
HH change (mm/100 d)	$Y = 0.73x + 14.0$	0.72	8.2	<0.001
PiP (mmol/L)	$Y = 0.03x + 0.64$	0.88	0.20	<0.001
FecP (mg/kg DM)	$Y = 22.4x + 2980.2$	0.69	267.4	<0.001
FecP:ME (mg P/MJ ME)	$Y = -0.005x^3 + 0.61x^2 – 17.5x + 492.4$	0.75	36.5	0.020
FecP:CP (mg P/g CP)	$Y = 0.24x + 26.57$	0.71	2.77	<0.001
	FecP:ME (mg P/MJ ME) (*X*)			
LWG (kg/day)	$Y = -0.000014x^2 + 0.0155x - 3.26$	0.72	0.15	0.035
	PiP (mmol/L) (*X*)			
LWG (kg/day)	$Y = 0.48x – 0.109$	0.82	0.12	<0.001

[1]0.9P (0.9 g P/kg DM), 1.3P (1.3 g P/kg DM), 1.8P (1.8 g P/kg DM), 2.0P (2.0 g P/kg DM) and 2.4P (2.4 g P/kg DM)
[2]Quadratic and cubic terms were removed from the model if not significant (P>0.05)
[3]Root mean squared error

a.



b.

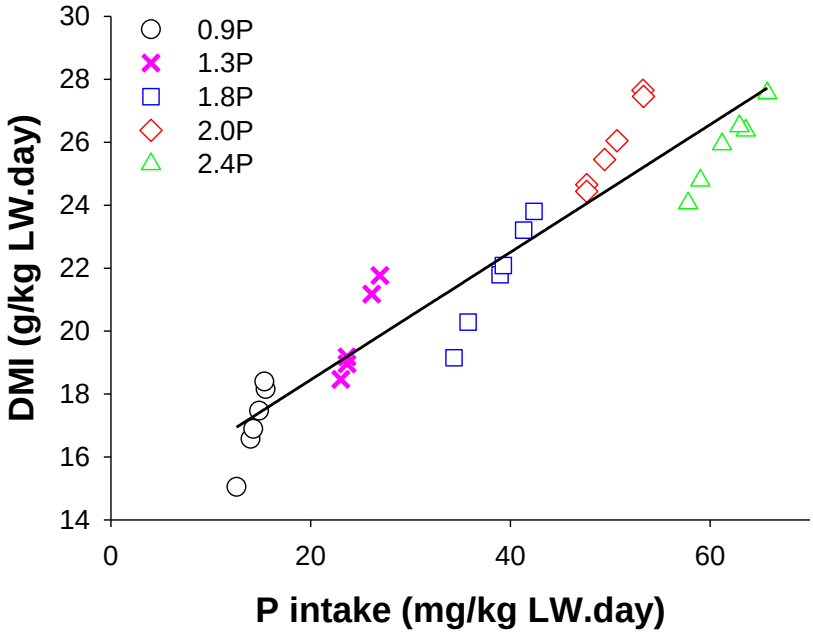
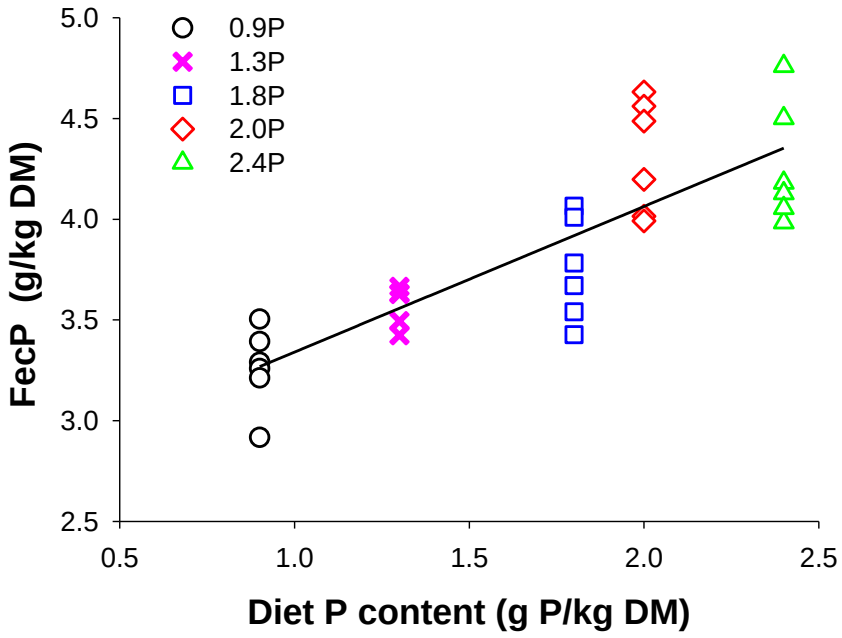


Figure 8. Relationship between dry matter intake (DMI) and the phosphorus (P) content of the diet (a.) and P intake (b.) of steers fed diets containing increasing amounts of P (0.9, 1.3, 1.8, 2.0 and 2.4 g P/kg DM). *Individual symbols represent individual steers. Relationships are based on repeated measures data over the entire experimental period; equations are presented in Table 5.*

a.



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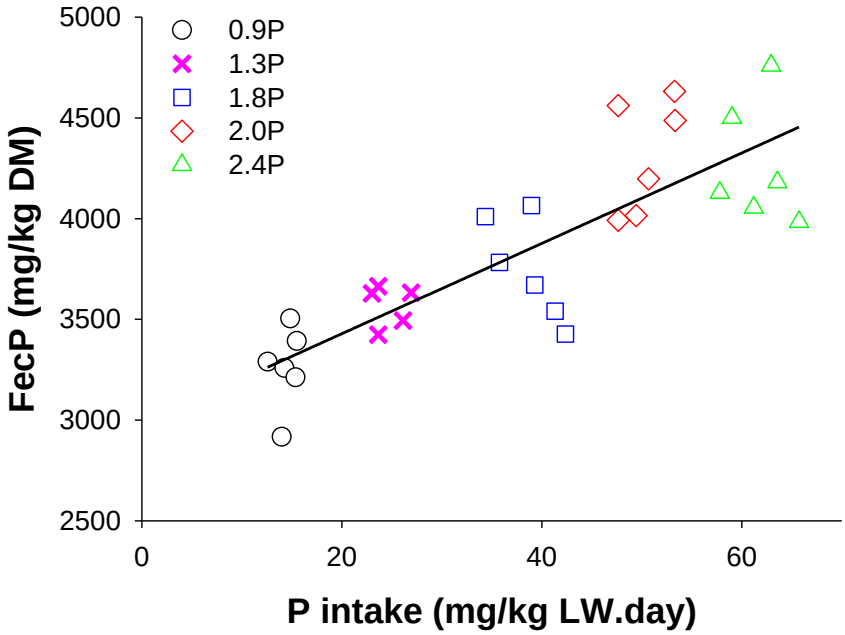


Figure 9. Relationship between the the concentration of phosphorus (P) in the faeces (FecP) and the P content of the diet (a.) and P intake (b.) of steers fed diets containing increasing amounts of P (0.9, 1.3, 1.8, 2.0 and 2.4 g P/kg DM). *Individual symbols represent individual steers. Relationships are based on repeated measures data over the entire experimental period; equations are presented in Table 5.*

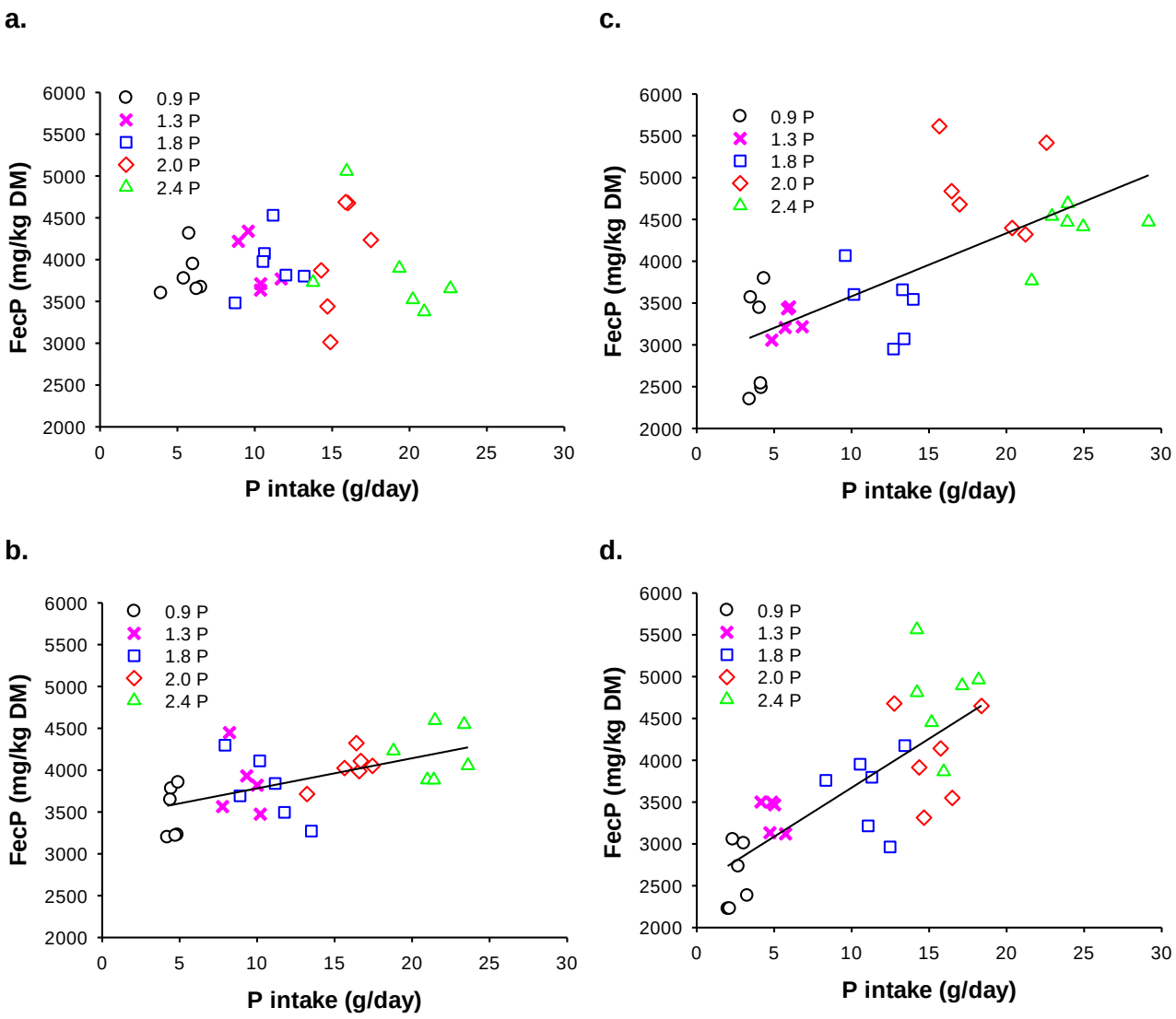
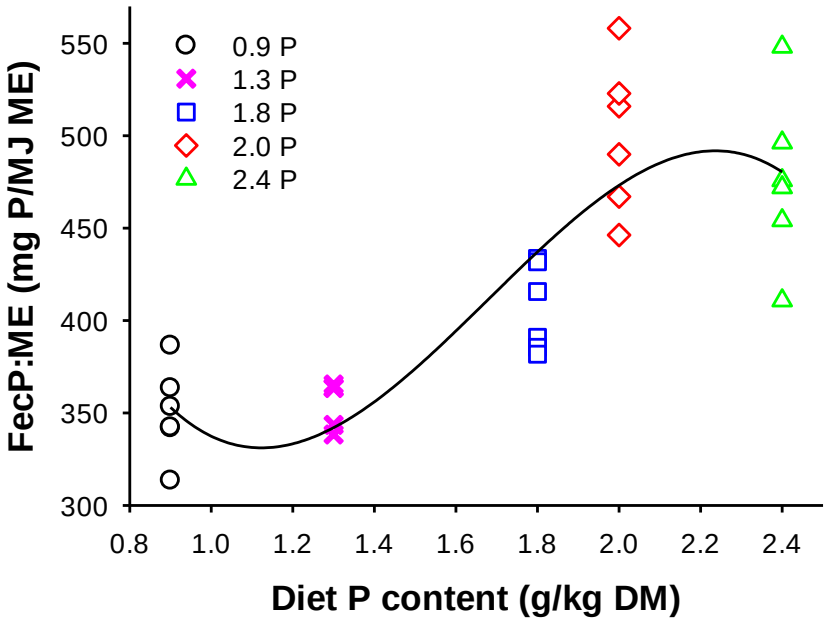


Figure 10. Relationship between the concentration of phosphorus (P) in the faeces (FecP) and dietary P intake of steers offered diets with increasing P content (0.9, 1.3, 1.8, 2.0 and 2.4 g P/kg DM) at 14 (a.), 35 (b.; R^2=0.33, P<0.01), 98 (c.; R^2=0.54, P<0.001) and 172 (d.; R^2=0.60, P<0.001) days after the commencement of treatment feeding. *Individual symbols represent individual steers.*

a.



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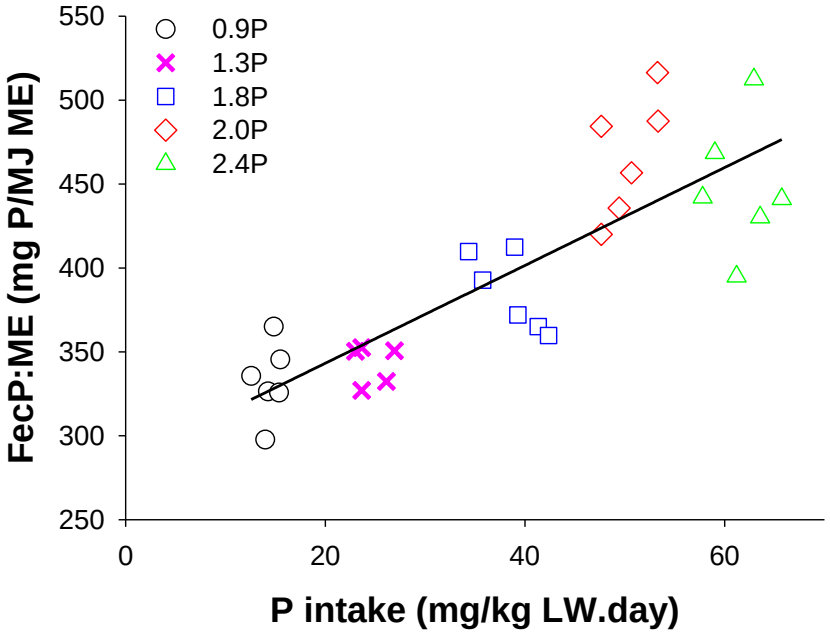
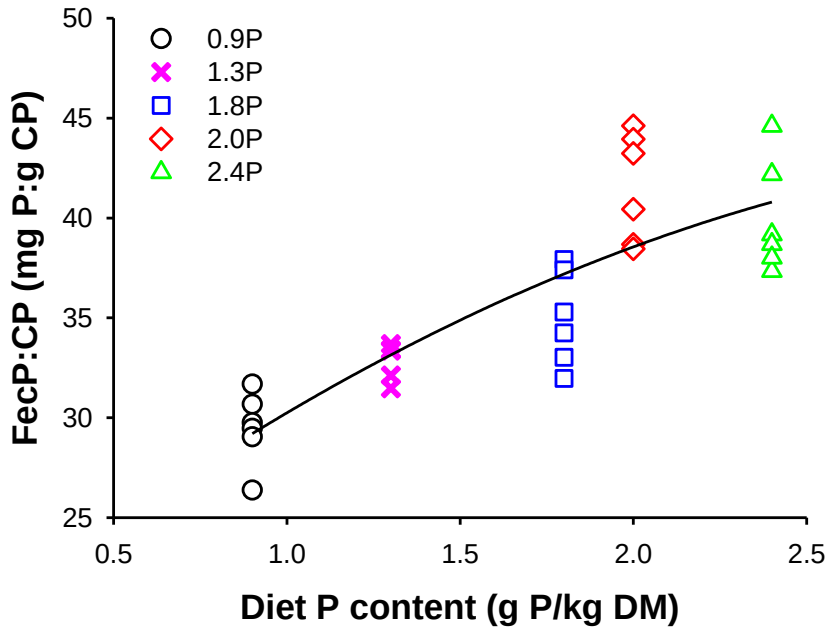


Figure 11. Relationship between the ratio of the concentration of phosphorus (P) in the faeces to dietary metabolisable energy (ME) content (FecP:ME) and the P content of the diet (a.) and P intake (b.) of steers fed diets containing increasing amounts of P (0.9, 1.3, 1.8, 2.0 and 2.4 g P/kg DM). *Individual symbols represent individual steers. Relationships are based on repeated measures data over the experimental period; equations are presented in Table 5.*

a.



b.

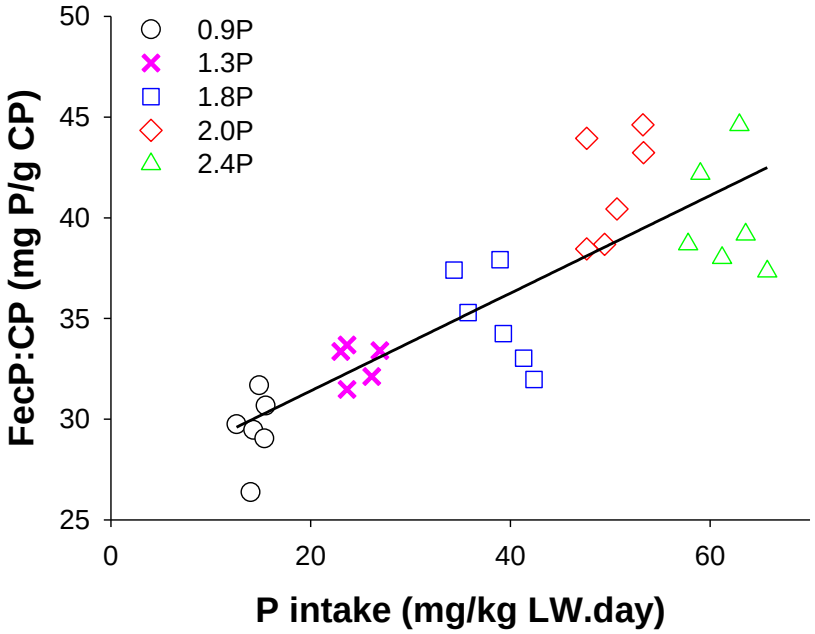


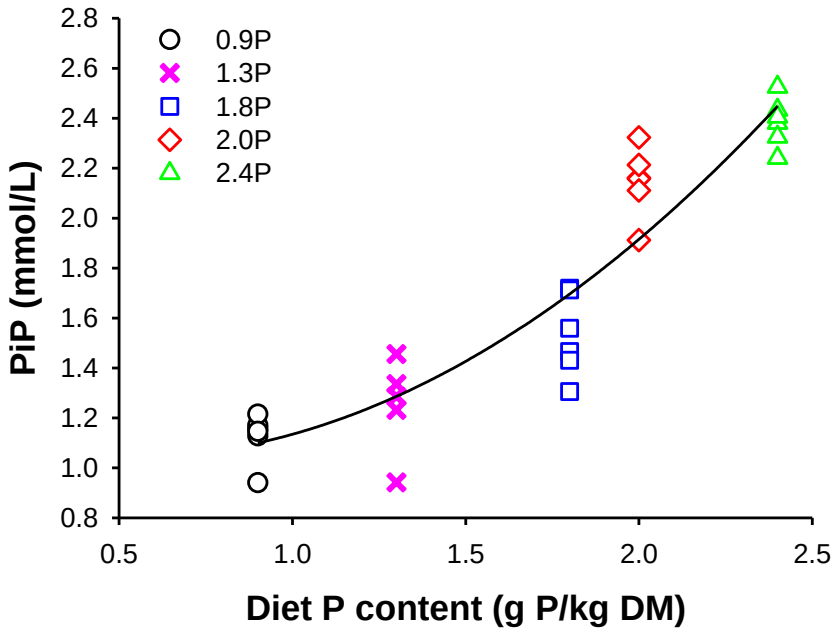
Figure 12. Relationship between the ratio of the concentration of phosphorus (P) in the faeces to dietary crude protein content (FecP:CP) and the P content of the diet (a.) and P intake (b.) of steers fed diets containing increasing amounts of P (0.9, 1.3, 1.8, 2.0 and 2.4 g P/kg DM). *Individual symbols represent individual steers. Relationships are based on repeated measures data over the entire experimental period; equations are presented in Table 5.*

The concentration of PiP was a more immediate indicator of P intake and was more stable than FecP and its associated ratio's with diet quality (FecP:ME and FecP:CP) over the experimental period. Plasma inorganic P can be used to indicate P intake (or more specifically P absorption) regardless of the length of time a growing steer has consumed a low P, high ME and CP diet. In Experiment 1, PiP was significantly different between all treatment groups from day 36 to 172 of the experiment and increased in a linear fashion with P intake (Figure 13). The concentration of PiP has previously been reported to be associated with P intake, with Coates (1994) suggesting a response to P supplementation will occur when PiP is <50 mg/L (1.60 mmol/L) but not when it is above 60 mg/L (1.90 mmol/L). The results of Experiment 1 were different to Coates (1994) in that increased ME intake and LWG occurred in response to increasing P intake when PiP was above 1.6 mmol/L. This raises questions regarding recommendations on what factors influence PiP concentration and at what PiP concentration is a response to P supplementation likely to occur.

The results of Experiment 1 demonstrate linear responses of LWG (kg/day; Figure 14) and cumulative LW (kg; Figure 15) to increasing dietary P content and P intake over the experimental period. Average daily LWG of steers was higher between days 1 and 36 compared to between days 36 and 172 for all treatment groups. Steers offered the 2.0P and 2.4P treatments gained approximately 120 kg more LW than steers offered the 0.9P treatment over the experimental period (Tables 5 and 6). The main effect of P on LWG was through DM intake, with a linear relationship between increasing DM intake and LWG (Figure 16). Average daily LWG increased in a quadratic fashion to FecP:ME and a linear fashion to PiP (Figure 17). The FecP:ME and PiP at which no further increase in LWG was likely to occur (i.e. indicates no further response to supplementation is likely) were approximately 460 mg FecP/MJ of diet ME and 2.1 mmol P/L plasma collected from the jugular.

Rate of change in HH was higher in steers offered the 2.0P and 2.4P diets compared to the 0.9P diet, and increased in a linear fashion with P intake reflecting increased skeletal growth in response to dietary P content and ME intake. Steers fed the 0.9P treatment continue to grow in HH, albeit at much lower rates that steers fed the 2.4P treatments. This was supported by histomorphometric data of the tuber coxae bone growth plates at the end of the experiment (Appendix 8.8) where, after 172 days on a low P diet, bone formation was still occurring in steers offered the 0.9P treatment albeit at much lower rates than steers offered the 2.4P treatment. This would suggest that even after approximately 6 months on a low P diet, not only were those steers not mobilizing minerals (P) from bone, they were continuing to mineralise and form new bone, albeit at a much lower rate than those on the higher P content diets.

a.



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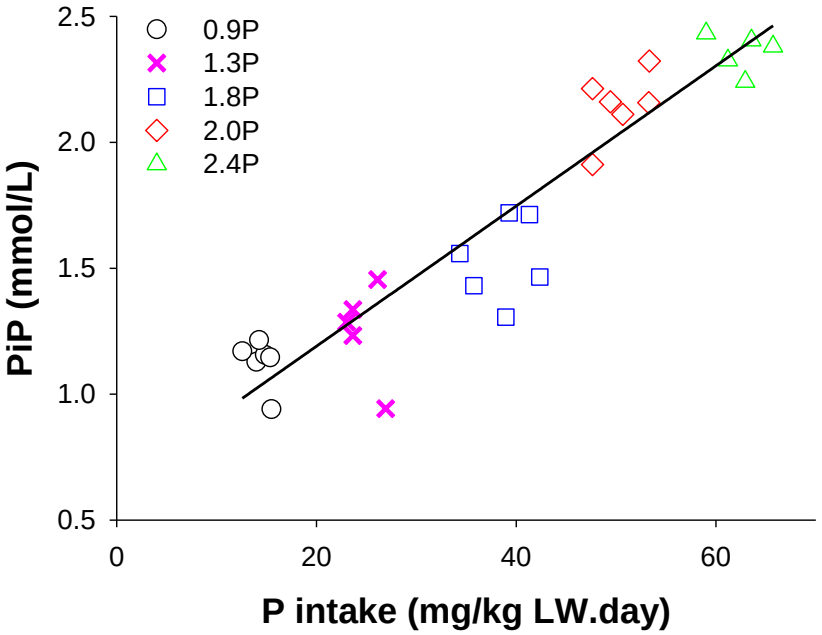
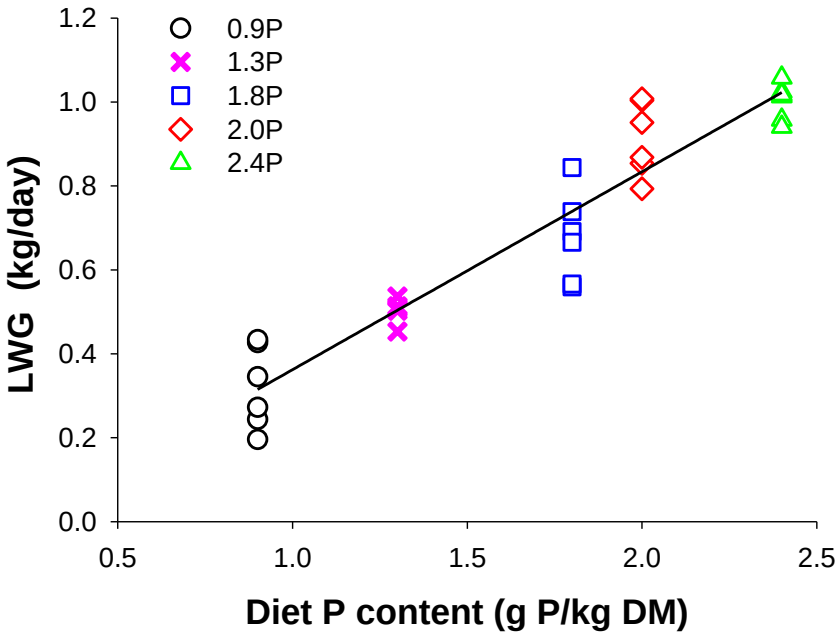


Figure 13. Relationship between the concentration of phosphorus (P) in the plasma (PiP) and the P content of the diet (a.) and P intake (b.) of steers fed diets containing increasing amounts of P (0.9, 1.3, 1.8, 2.0 and 2.4 g P/kg DM). *Individual symbols represent individual steers. Relationships are based on repeated measures data over the experimental period; equations are presented in Table 5.*

a.



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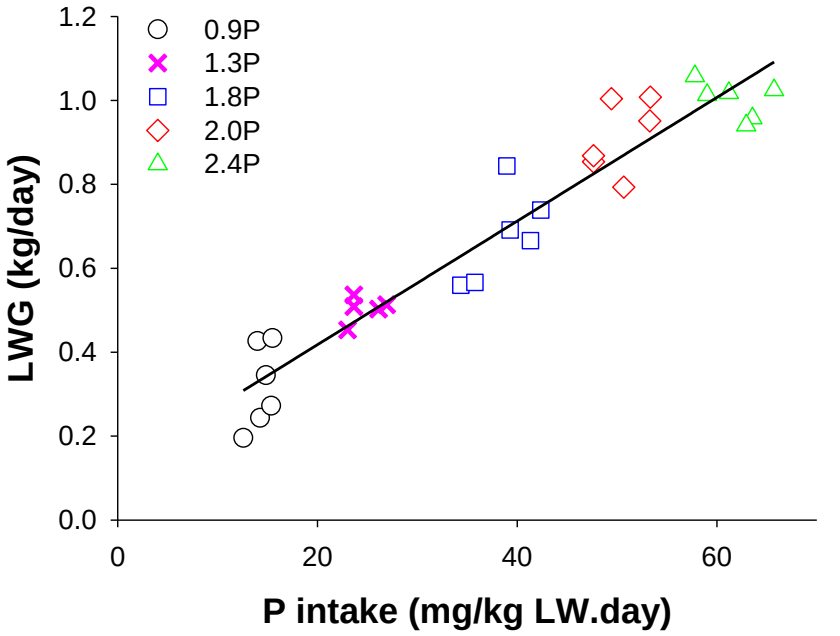
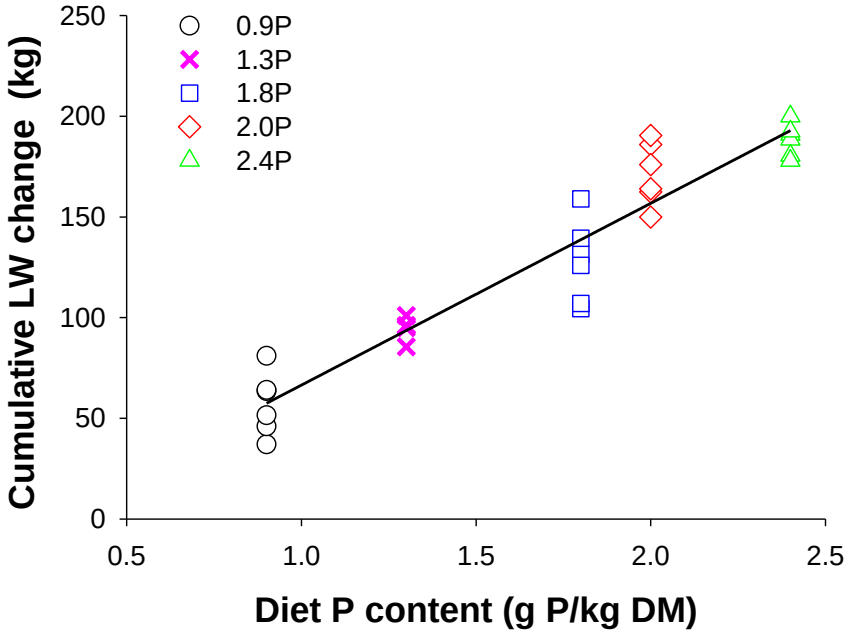


Figure 14. Relationship between the average liveweight (LW) gain (LWG) and the phosphorus (P) content of the diet (a.) and P intake (b.) of steers fed diets containing increasing amounts of P (0.9, 1.3, 1.8, 2.0 and 2.4 g P/kg DM). *Individual symbols represent individual steers. Relationships are based on repeated measures data over the experimental period; equations are presented in Table 5.*

a.



b.

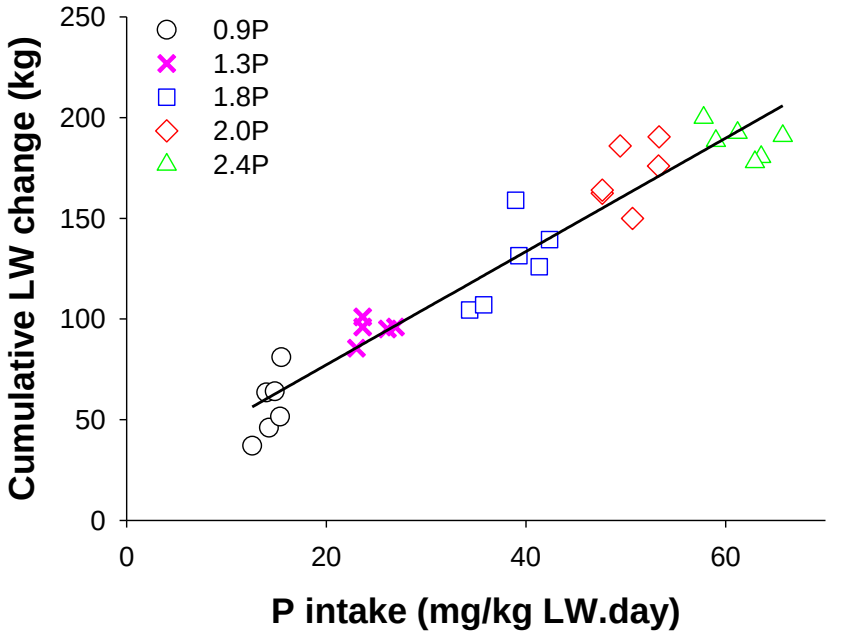


Figure 15. Relationship between the cumulative liveweight (LW) change and the phosphorus (P) content of the diet (a.) and P intake (b.) of steers fed diets containing increasing amounts of P (0.9, 1.3, 1.8, 2.0 and 2.4 g P/kg DM). *Individual symbols represent individual steers. Relationships are based on repeated measures data over the experimental period; equations are presented in Table 5.*

Table 6. Liveweight (LW), LW gain (LWG), hip height (HH) and LW:HH of steers offered diets with increasing phosphorus (P) content[1,2]

	Treatment					
Parameter	0.9P	1.3P	1.8P	2.0P	2.4P	SEM
LW - day 1 (kg)	244.7	249.3	242.6	240.7	243.6	3.2
LW - day 172 (kg)	301.8^{a}	343.4^{b}	370.5^{b}	412.2^{c}	432.0^{c}	7.3
LW change - day 1 to 172 (kg)	57.2^{a}	94.1^{b}	127.9^{c}	171.5^{d}	188.4^{d}	6.2
LW change - day 36 to 172 (kg)	24.3^{a}	57.3^{b}	87.3^{c}	118.5^{d}	133.8^{d}	5.0
LWG - day 1 to 172 (kg/day)[3]	0.29^{a}	0.52^{b}	0.72^{c}	0.98^{d}	1.07^{d}	0.03
LWG - day 36 to 172 (kg/day)[3]	0.16^{a}	0.40^{b}	0.63^{c}	0.86^{d}	0.97^{d}	0.04
HH change – day 1 to 172 (mm/100 day)[3]	22.6^{a}	31.4^{a}	47.0^{b}	47.4^{b}	59.0^{b}	3.6
HH change (mm/100 day)[4]	25.9^{a}	33.5^{ab}	45.3^{b}	48.3^{bc}	58.8^{c}	3.7
LW:HH (kg/mm)[5]	0.226^{a}	0.240^{a}	0.240^{a}	0.257^{b}	0.255^{b}	0.003

[1]0.9P (0.9 g P/kg DM), 1.3P (1.3 g P/kg DM), 1.8P (1.8 g P/kg DM), 2.0P (2.0 g P/kg DM) and 2.4P (2.4 g P/kg DM).
[2]Data are least-squares means with pooled standard error of the mean (SEM). Different alphabetical superscripts across each row indicate a significant difference between treatments (P<0.05).
[3]Repeated measures over the experimental period as indicated.
[4]Regression (change in HH over number of days) from day 1 to 172 of the experimental period.
[5]Based on LW and HH at day 172 of the experiment.

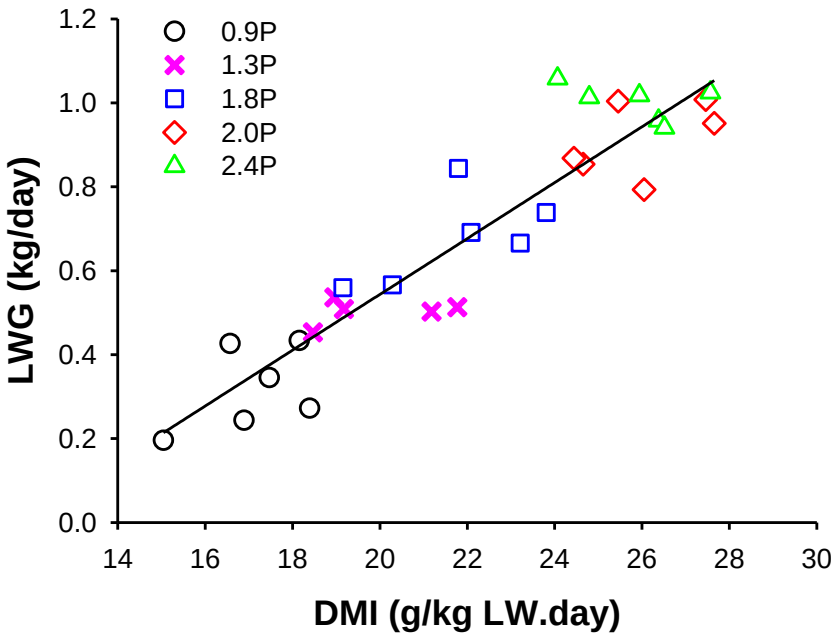


Figure 16. Relationship between liveweight (LW) gain (LWG) and dry matter intake (DMI). *Individual symbols represent individual steers. Relationships are based on repeated measures data over the experimental period. [LWG (kg/day) = 0.067 DMI (g/kg LW.day) – 0.788; R^2=0.87, MSE = 0.10, P<0.001).*

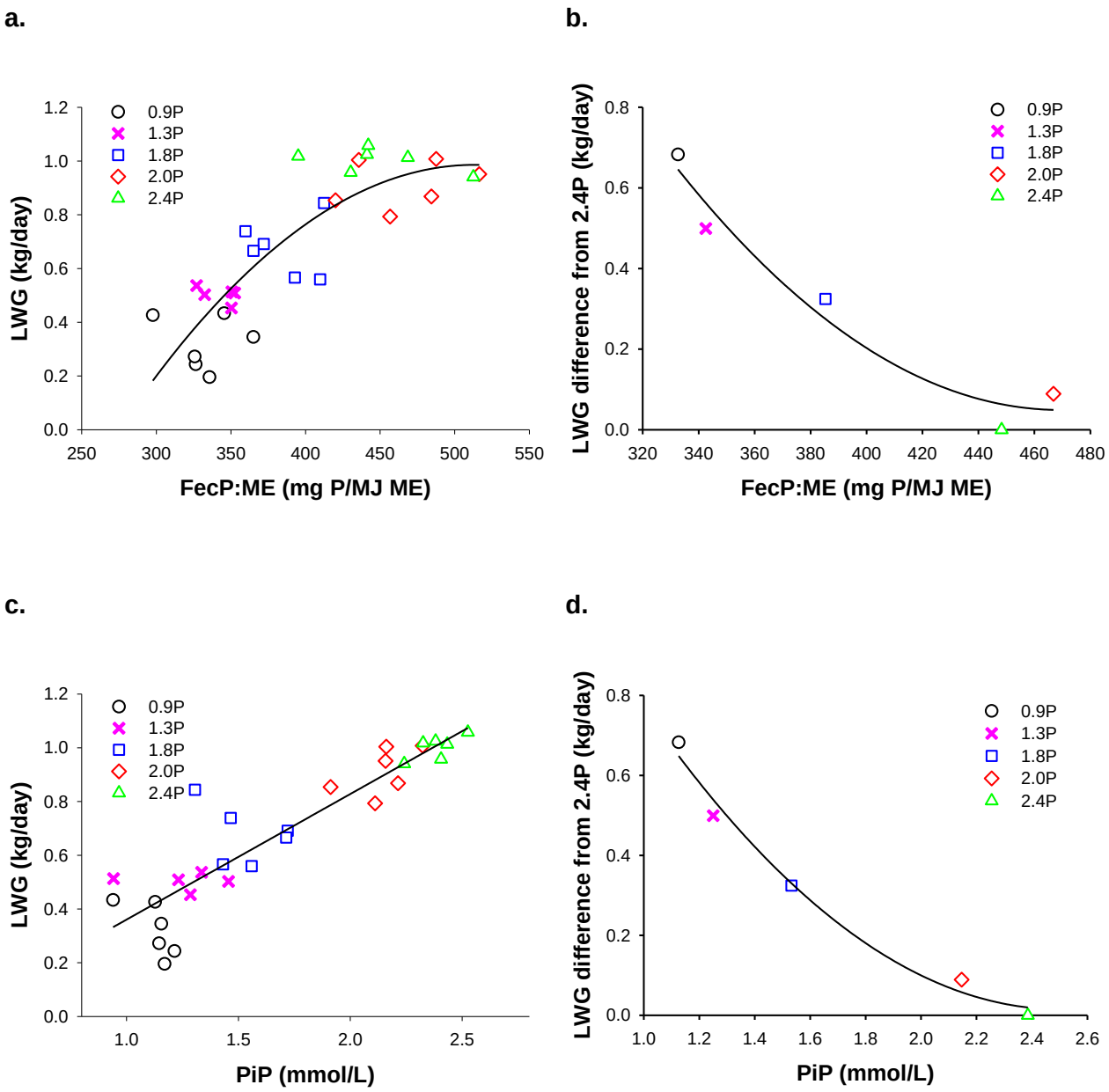


Figure 17. Relationship between faecal P:diet ME (FecP:ME) and liveweight (LW) gain (LWG) (a.) and the difference in mean LWG of each treatment from LWG of 2.4P steers (b.) and between plasma inorganic P (PiP) and LWG (c.) and the difference in mean LWG of each treatment from LWG of 2.4P steers (d.) of steers fed diets containing increasing amounts of phosphorus (P) (0.9, 1.3, 1.8, 2.0 and 2.4 g P/kg DM). *Individual symbols represent individual steers (a. and c.) or treatment means (b. and d.). Relationships are based on repeated measures data over the experimental period; equations are presented in Table 5. Difference in LWG is calculated as mean LWG across the entire experimental period between 2.4P and the other treatment groups.*

The effect of dietary phosphorus content on rumen parameters and microbial protein production, rumen and urine mineral concentration and phosphorus balance

Plasma glucose and urea concentrations were not significantly different between treatment groups (Table 7). Similarly, VFA concentration and the molar percentage of individual VFA in the rumen fluid of steers was not significantly different between treatments. The concentration of NH_3N in the rumen of steers offered the five treatment diets ranged between 50 and 104 mg/L. Steers offered the 2.0P and 2.4P treatment diets had lower concentration of NH_3N in the rumen

compared with steers offered the 1.3P and 1.8P treatments. Microbial crude protein production was highest for steers offered the 2.0P and 2.4P treatments with no significant differences between the other three treatments. The efficiency of MCP production (EMCP) determined in this experiment was lower than the 130 g MCP/kg DOMI suggested by Freer *et al*. (2007) to be the minimum level of EMCP when N supply is adequate. The EMCP was highest in steers offered the 2.0P treatment compared to steers offered the 0.9P and 1.3P treatments. P intake during the collection period was slightly lower than P intake measured when steers were in the outdoor pens over the majority of the experimental period. This was due to lower DM intake when steers were in the metabolism crates compared to when they were in the individual outdoor pens. Nevertheless, P intake was significantly different between treatment groups during the collection period. Steers that were offered the 2.4P treatment retained more P than steers offered the other treatment diets, with no difference in P balance between steers offered the other treatment diets.

The concentration of minerals in the faeces, urine and rumen fluid of steers varied between P treatments (Appendix 8.1). While many of the minerals in the urine and rumen fluid were numerically significantly different between treatments these differences were unlikely to be biologically important given the very low concentrations measured. As reported elsewhere excretion of P in the urine was relatively small compared with faecal P excretion (Appendix 8.1). Steers offered the 2.4P treatment had significantly higher urine P excretion (61 mg P/kg urine) than the 0.9P, 1.3P and 1.8P treatments (all less than 10 mg P/kg urine), with no difference between the other four treatments, suggesting that excess P was available in the 2.4P diet. Meta-analysis of P studies with cattle indicated that urinary P excretion was low when PiP was below 1.55 mmol/L (Ternouth *et al*., 1996). In Experiment 1, urinary P excretion increased significantly at a PiP of 2.0 mmol/L and a dietary P content of 2.4 g/kg DM (Table 7) which demonstrates why the relationship between P intake and PiP or FecP is considered less reliable at higher P intakes as an increasing amount of endogenous P is excreted via the urine rather than the faeces or maintained in the circulation.

Table 7. The concentration of phosphorus (P), glucose and urea in the plasma, the concentration of NH_3N and volatile fatty acids (VFA) in the rumen, the molar percentage of VFA and branched-chain fatty acids (BCFA), microbial protein (MCP) production and the efficiency of MCP production (EMCP), and P balance of steers offered diets with increasing P content over a seven day collection period[1,2,3]

	Treatment				
Parameter	0.9P	1.3P	1.8P	2.0P	2.4P
Plasma P (mmol/L)	1.00 ± 0.1^{a}	1.21 ± 0.1^{ab}	1.53 ± 0.1^{b}	1.88 ± 0.1^{c}	2.04 ± 0.1^{c}
Plasma glucose (mmol/L)	4.83 ± 0.4	4.63 ± 0.4	4.45 ± 0.4	4.62 ± 0.4	5.10 ± 0.4
Plasma urea (mmol/L)	5.09 ± 0.4	5.27 ± 0.5	4.18 ± 0.4	4.58 ± 0.4	4.93 ± 0.4
NH_3N (mg/L)	69.6 ± 12.4^{ab}	99.4 ± 12.4^{ab}	103.5 ± 12.4^{b}	61.1 ± 11.1^{ab}	49.7 ± 11.1^{a}
Total VFA (mmol/L)	55.3 ± 5.6	62.4 ± 5.6	61.9 ± 5.6	55.5 ± 5.0	51.7 ± 5.0
Acetate (%)	71.7 ± 1.3	71.3 ± 1.3	71.6 ± 1.3	70.3 ± 1.1	70.9 ± 1.1
Butyrate (%)	14.0 ± 1.0	14.5 ± 1.0	14.3 ± 1.0	16.4 ± 0.9	14.7 ± 0.9
Propionate (%)	11.7 ± 0.7	11.4 ± 0.7	10.6 ± 0.7	10.5 ± 0.6	11.2 ± 0.6
BCFA (%)	2.5 ± 0.3	2.8 ± 0.3	3.4 ± 0.3	2.8 ± 0.3	3.2 ± 0.3
MCP (g/day)	121 ± 35^{a}	155 ± 31^{a}	250 ± 35^{a}	450 ± 31^{b}	395 ± 31^{b}
MCP (g/kg LW.day)	0.39 ± 0.1^{a}	0.51 ± 0.1^{a}	0.73 ± 0.1^{ab}	1.18 ± 0.1^{c}	0.97 ± 0.1^{bc}
EMCP (g MCP/kg DOMI)	64 ± 9^{a}	62 ± 9^{a}	80 ± 8^{ab}	110 ± 8^{b}	89 ± 8^{ab}
P intake (g/day)	3.3 ± 0.7^{a}	5.4 ± 0.7^{a}	8.8 ± 0.6^{b}	13.6 ± 0.6^{c}	20.5 ± 0.6^{d}
Faecal P output (g/day)	3.5 ± 0.6^{a}	5.0 ± 0.6^{ab}	7.1 ± 0.5^{b}	13.0 ± 0.5^{c}	14.4 ± 0.5^{c}
Urine P output (g/day)	0.01 ± 0.06	0.01 ± 0.06	0.02 ± 0.05	0.12 ± 0.05	0.20 ± 0.05
P balance (g/day)	-0.22 ± 0.6^{a}	0.38 ± 0.6^{a}	1.68 ± 0.5^{a}	0.38 ± 0.5^{a}	5.9 ± 0.5^{b}
P balance (mg/kg LW.day)	-0.9 ± 1.5^{a}	1.0 ± 1.5^{a}	4.7 ± 1.4^{a}	1.1 ± 1.4^{a}	14.9 ± 1.4^{b}

[1]0.9P (0.9 g P/kg DM), 1.3P (1.3 g P/kg DM), 1.8P (1.8 g P/kg DM), 2.0P (2.0 g P/kg DM) and 2.4P (2.4 g P/kg DM)
[2]Data are least-squares means with standard error of the mean (SEM). Different alphabetical superscripts across each row indicate a significant difference between treatments (P<0.05).
[3]Determined on samples collected during and upon completion of the collection period in metabolism crates.

The effect of dietary phosphorus content on cortical bone thickness, specific gravity and mineral content, bone cellularity, cannon bone measurements and plasma osteocalcin and insulin-like growth factor-1 concentration

Cortical bone thickness of the 12th rib increased with increasing dietary P content and P intake, and was significantly thicker at the conclusion of the experiment in steers that were offered the 2.4P diet compared with steers offered the 0.9P and 1.3P treatments (Table 8). In contrast, specific gravity of bone did not vary with dietary P content. Similarly, thickness of the cortical bone of the cannon bone was greater in steers offered 2.0P and 2.4P diets compared with steers offered the 1.3P treatment (Table 9). In contrast to the change in HH in response to dietary P content, cannon bone length, physeal width and epiphyseal height were unaffected by dietary P content, suggesting this bone is relatively less sensitive to changes in P and DM intake than bones in the hind-leg that drive changes in HH.

The concentration of total OCN, and the Glu- and Gla forms of OCN in the plasma were unaffected by dietary P content (Table 10). Osteocalcin is secreted by osteoblasts and is considered a marker of bone formation, with the majority incorporated into hydroxyapatite in bone (Lee *et al*., 2000). It was expected that total, Glu- and Gla OCN would have increased with increasing P intake which presumably resulted in higher bone formation and altered glucose metabolism between the steers. These results are contradictory to responses measured on the steers (HH) or on bone samples (CBT and histology) which indicated that bone formation was increasing with increased P intake. There was very high variation in the results of these assays in Experiment 1. There are other examples in the literature where OCN has not changed in relation to changes in P and Ca content of the diet in dairy cows (Peterson *et al*., 2005; Moreira *et al*., 2009) and in rapidly growing lambs that displayed faster growing bones, which were heavier and stronger at slaughter (Nicodemo *et al*., 1999). It is possible that rates of incorporation of OCN into bone also affected the circulating concentrations measured in Experiment 1. This could be assessed through gene expression or proteomic studies of bone biopsies collected during the experiment, however based on the results of this experiment we see no value in the use of OCN as a marker for bone turnover in growing steers.

Plasma IGF1 concentration was higher in steers at the end of the experiment compared to the start of the experiment, and was significantly higher in steers offered the 2.0P and 2.4P treatments compared to steers offered the 0.9P treatment. Insulin-like growth factor-1 acts in an autocrine, paracrine and endocrine manner on a range of cell types (muscle, bone, fat, mammary tissue and reproductive organs), mainly to enhance cellular proliferation and differentiation, as well as stimulating glucose and amino acid uptake. Infusion of IGF-1 decreased protein degradation and increased protein gain in sheep (Oddy and Owens, 1996) and reduced the concentration of 3-methylhistidine in the plasma of cattle fed low protein diets (Hill *et al*., 1999). The concentration of circulating IGF1 responds to energy intake but not the form of energy in the diet (Houseknecht *et al*., 1988). The concentration of IGF1 in the circulation is also responsive to compensatory LWG after a restriction in energy intake (Hayden *et al*., 1993), feed deprivation (Wu *et al*., 2008), dietary protein supply (Liu *et al*., 1997), the administration of hormonal growth promotants (Pampusch *et al*., 2003) and bovine somatotropin (BST) (Lemal *et al*., 1989).

Table 8. Thickness and specific gravity of cortical bone biopsies collected from the 12th rib of steers offered diets with increasing phosphorus (P) content[1,2]

	Treatment				
Stage of experiment	0.9P	1.3P	1.8P	2.0P	2.4P
	12th rib cortical bone thickness (mm)				
Commencement	2.42 ± 0.15	2.54 ± 0.17	2.53 ± 0.17	2.74 ± 0.15	2.38 ± 0.15
Conclusion	2.54 ± 0.20^{a}	2.65 ± 0.23^{a}	3.24 ± 0.20^{ab}	3.29 ± 0.20^{ab}	3.65 ± 0.20^{b}
Change	0.11 ± 0.23^{a}	0.11 ± 0.25^{a}	0.68 ± 0.25^{ab}	0.56 ± 0.23^{ab}	1.27 ± 0.23^{b}
	12th rib cortical bone specific gravity				
Commencement	1.68 ± 0.02	1.63 ± 0.02	1.64 ± 0.02	1.62 ± 0.02	1.63 ± 0.02
Conclusion	1.64 ± 0.03	1.60 ± 0.03	1.65 ± 0.03	1.62 ± 0.03	1.64 ± 0.03
Change	-0.04 ± 0.03	-0.04 ± 0.04	0.01 ± 0.04	0.00 ± 0.03	0.02 ± 0.03

[1]0.9P (0.9 g P/kg DM), 1.3P (1.3 g P/kg DM), 1.8P (1.8 g P/kg DM), 2.0P (2.0 g P/kg DM) and 2.4P (2.4 g P/kg DM).
[2]Data are least-squares means with standard error of the mean (SEM). Different alphabetical superscripts across each row indicate a significant difference between treatments (P<0.05).

Table 9. Cannon bone length, cortical bone thickness, physeal width and epiphyseal height of steers offered diets with increasing phosphorus (P) content[1,2]

	Treatment				
Stage of experiment	0.9P	1.3P	1.8P	2.0P	2.4P
			Cannon bone length (mm)		
Commencement	212.5 ± 2.5	209.6 ± 2.7	203.3 ± 2.5	203.0 ± 2.5	210.5 ± 2.5
Conclusion	233.0 ± 2.7^{b}	230.2 ± 3.0^{ab}	221.0 ± 2.7^{a}	223.3 ± 2.7^{ab}	231.5 ± 2.7^{ab}
Change	20.5 ± 2.2	20.6 ± 2.5	17.7 ± 2.2	20.3 ± 2.2	21.0 ± 2.2
			Cannon bone cortical bone thickness[3] (mm)		
Commencement	5.2 ± 0.2	5.7 ± 0.2	5.4 ± 0.2	5.3 ± 0.2	5.3 ± 0.2
Conclusion	5.7 ± 0.2^{a}	6.0 ± 0.2^{a}	6.4 ± 0.2^{ab}	7.0 ± 0.2^{b}	6.4 ± 0.2^{ab}
Change	0.43 ± 0.19^{ab}	0.26 ± 0.20^{a}	1.02 ± 0.19^{abc}	1.77 ± 0.20^{c}	1.12 ± 0.19^{bc}
			Physeal width (mm)		
Commencement	40.5 ± 0.8	40.0 ± 0.9	38.5 ± 0.8	37.8 ± 0.8	40.7 ± 0.8
Conclusion	45.2 ± 1.2^{ab}	48.2 ± 1.4^{b}	42.2 ± 1.2^{a}	45.8 ± 1.2^{ab}	44.7 ± 1.2^{ab}
Change	4.7 ± 1.5	8.2 ± 1.6	3.7 ± 1.5	8.0 ± 1.5	4.0 ± 1.5
			Epiphyseal height (mm)		
Commencement	34.5 ± 0.9	33.8 ± 1.0	32.8 ± 0.9	32.5 ± 0.9	34.5 ± 0.9
Conclusion	39.2 ± 1.0	39.2 ± 1.1	36.3 ± 1.0	38.2 ± 1.0	39.5 ± 1.0
Change	4.7 ± 1.1	5.4 ± 1.2	3.5 ± 1.1	5.7 ± 1.1	5.0 ± 1.1

[1]0.9P (0.9 g P/kg DM), 1.3P (1.3 g P/kg DM), 1.8P (1.8 g P/kg DM), 2.0P (2.0 g P/kg DM) and 2.4P (2.4 g P/kg DM).
[2]Data are least-squares means with standard error of the mean (SEM). Different alphabetical superscripts across each row indicate a significant difference between treatments (P<0.05).
[3]Cortical bone thickness of the dorsal cannon bone 50% of the length.

Table 10. Plasma osteocalcin and insulin-like growth factor-1 (IGF1) concentration of steers offered diets with increasing phosphorus (P) content[1,2]

	Treatment				
Stage of experiment	0.9P	1.3P	1.8P	2.0P	2.4P
			Total osteocalcin ng/mL)		
Day 1	380.0 ± 57.7	394.7 ± 64.5	285.4 ± 57.7	293.3 ± 57.7	340.5 ± 57.7
Day 172	313.4 ± 47.2	285.3 ± 52.8	225.4 ± 47.2	342.9 ± 47.2	245.7 ± 47.2
Change	-66.6 ± 65.6	-109.4 ± 73.3	-60.0 ± 65.6	49.6 ± 65.6	-94.7 ± 65.6
			Glu-osteocalcin (ng/mL)		
Day 1	43.3 ± 14.6	42.4 ± 16.3	34.4 ± 14.6	61.9 ± 14.6	46.4 ± 14.6
Day 172	24.0 ± 15.5	36.4 ± 17.4	21.2 ± 15.5	57.3 ± 15.5	51.9 ± 15.5
Change	-19.4 ± 12.4	-6.1 ± 13.9	-13.3 ± 12.4	-4.6 ± 12.4	5.5 ± 12.4
			Gla-osteocalcin (ng/mL)		
Day 1	223.9 ± 71.2	142.9 ± 79.7	102.5 ± 71.2	273.6 ± 71.2	143.7 ± 71.2
Day 172	204.2 ± 25.7	202.4 ± 28.8	223.6 ± 25.7	198.9 ± 25.7	209.7 ± 25.7
Change	-19.7 ± 60.2	59.5 ± 67.3	121.2 ± 60.2	-74.8 ± 60.2	66.0 ± 60.2
			Glu- plus Gla-osteocalcin (ng/mL)		
Day 1	267.2 ± 81.8	185.4 ± 90.9	136.8 ± 81.8	335.5 ± 81.8	190.1 ± 81.8
Day 172	228.1 ± 30.3	238.8 ± 33.8	244.8 ± 30.3	256.1 ± 30.3	261.7 ± 30.3
Change	-39.1 ± 65.2	53.4 ± 72.9	108.0 ± 65.2	-79.4 ± 65.2	71.6 ± 65.2
			IGF1 (ng/mL)		
Day 1	70.8 ± 13.0	101.3 ± 14.5	53.4 ± 13.0	89.2 ± 13.0	100.0 ± 13.0
Day 172	88.9 ± 14.0^{a}	155.3 ± 15.7^{b}	130.7 ± 14.0^{ab}	164.3 ± 14.0^{b}	232.4 ± 14.0^{c}
Change	18.1 ± 15.4^{a}	53.9 ± 17.2^{a}	77.3 ± 15.4^{ab}	75.1 ± 15.4^{ab}	132.4 ± 15.4^{b}

[1]0.9P (0.9 g P/kg DM), 1.3P (1.3 g P/kg DM), 1.8P (1.8 g P/kg DM), 2.0P (2.0 g P/kg DM) and 2.4P (2.4 g P/kg DM).
[2]Data are least-squares means with standard error of the mean (SEM). Different alphabetical superscripts across each row indicate a significant difference between treatments (P<0.05).

4.2 Experiment 2. Field study

Reproductive performance of cows and growth of steers that were offered dry and/or wet season P supplements and the validity of FecP:ME to indicate a P deficient diet under a commercial cattle production system

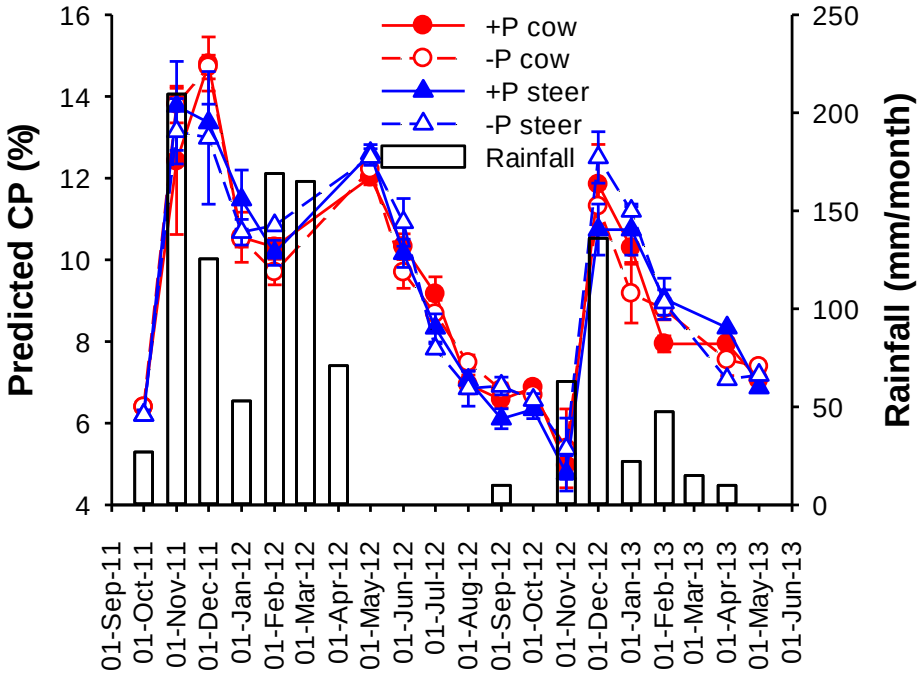
Rainfall, diet quality and supplement intake

The experiment commenced in October 2011 prior to the commencement of the wet season; this followed an above average 2010/2011 wet season (848 mm November 2010 to April 2011, inclusive). There was 793 mm of rain during wet season 1 (November 2011 to April 2012, inclusive) at the Brunchilly outstation homestead (approximately 10 km from Stud paddock). Only 10 mm of rain fell during dry season 1 (May 2012 to October 2012, inclusive) and 293 mm of rain during wet season 2 (November 2012 to April 2013).

The NIRS predictions of dietary CP, DMD and non-grass percentage are presented in Figure 18. Predicted dietary CP content responded to wet season rain as expected, and was elevated to 10 to 14% for a prolonged period of approximately 7 months over wet season 1 and decreased to a minimum of approximately 5% at the end of dry season 1 (November 2012). The peak in predicted dietary CP content in wet season 2 was relatively short compared to wet season 1 and corresponded to the lower rainfall experienced in wet season 2. The predicted DMD of the diet was maintained at between 60 to 65% during wet season 1 and the first 2 months of wet season 2. Diet DMD decreased to a minimum of approximately 55% at the end of dry season 1. The proportion of non-grass species in the diet of cattle was highest at the end of wet season 1 (May/June 2012) and peaked at between 50 to 60% of the diet at this time. By the end of dry season 1 it was predicted that there was less than 5% of non-grass species in the diet consumed by cattle. While non-grass proportion did increase in response to rain in wet season 2, the proportion in the diet peaked at a much lower level and decreased much more rapidly compared with wet season 1 due to the lower rainfall that was experienced in the 2012/2013 wet season at Helen Springs. Clearly the experiment was conducted over two very different wet seasons, following on from a very good wet season prior to the commencement of the experiment, with the majority of the data collected in conjunction with two very good wet seasons (2010/2011 and 2011/2012). Only the 2012/2013 wet season was comparable to the longer-term average rainfall at Helen Springs (440 mm/year). There was no difference in predicted diet quality between cows and steers and between +P (P supplemented) and -P (P unsupplemented) cattle at any stage of the experiment (Figure 18). Therefore P supplementation had no influence on diet selection by cattle in Stud paddock over the course of the experiment.

Supplement intake followed rain and pasture quality, with lower supplement intakes associated with periods of higher rainfall (wet season 1) and higher supplement intakes associated with periods of lower rainfall (dry season 1 and end of wet season 2). Supplement intakes were generally much lower than targeted during the wet season (100 g/head.day targeted) but approached target intakes in the dry season (150 g/head.day targeted) (Figure 19). During the wet season cattle entered the water/lick yards less frequently than during the dry season and this would have contributed to the lower than expected lick intakes in the wet season. Despite selecting a site that had minimal potential to hold surface water it appears that after rainfall events cattle were able to obtain sufficient water from sources other than water troughs in the supplement yards. It was anticipated that if the cattle in Stud paddock were P deficient that they would have sought supplement to meet P demands, particularly during the wet season. This was not the case in Experiment 2, and may suggest that the steers and cows were obtaining adequate P from either their own reserves of from early wet season forbs/legumes that may have responded quicker to rains particularly on the Wonorah land type (red soil area) within Stud paddock. Intake of supplement followed a similar pattern for both +P and -P supplements over the course of Experiment 2. The data presented in Figure 18 is based on number of animals allocated to treatments at that point in time and it is unadjusted for draft accuracy. One possible reason for the higher intake of the -P supplement was that the -P draft was always the default option for any breakdowns or mis-reads of EIDs, therefore it is possible that over any period there were more animals drafted to -P supplement and less animals to +P supplement than what was actually allocated. An alternative reason may be related to the palatability of the +P supplement. The only difference between the +P and -P supplements was the inclusion of an inorganic P supplement (Biofos). A sweetener (EC Feed) was added to both +P and -P wet season supplements, with CSM also added to the wet season 2 supplements in an attempt to stimulate higher supplement intakes.

a.


Predicted CP (%)
Rainfall (mm/month)
+P cow
-P cow
+P steer
-P steer
Rainfall
16
14
12
10
8
6
4
250
200
150
100
50
0
01-Sep-11
01-Oct-11
01-Nov-11
01-Dec-11
01-Jan-12
01-Feb-12
01-Mar-12
01-Apr-12
01-May-12
01-Jun-12
01-Jul-12
01-Aug-12
01-Sep-12
01-Oct-12
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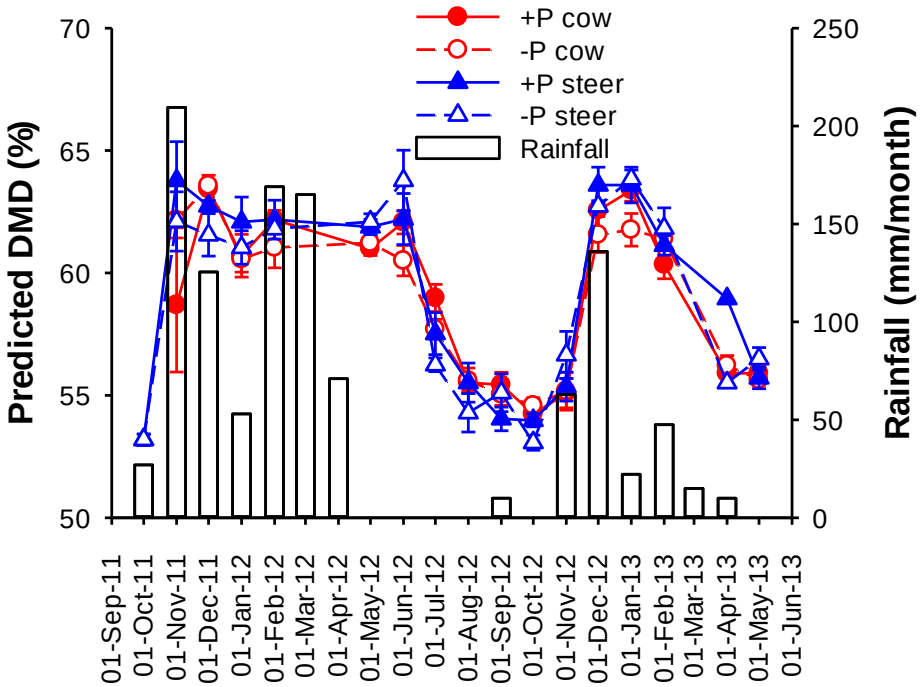
01-Apr-13
01-May-13
01-Jun-13

b.


Predicted DMD (%)
Rainfall (mm/month)
+P cow
-P cow
+P steer
-P steer
Rainfall
70
65
60
55
50
250
200
150
100
50
0
01-Sep-11
01-Oct-11
01-Nov-11
01-Dec-11
01-Jan-12
01-Feb-12
01-Mar-12
01-Apr-12
01-May-12
01-Jun-12
01-Jul-12
01-Aug-12
01-Sep-12
01-Oct-12
01-Nov-12
01-Dec-12
01-Jan-13
01-Feb-13
01-Mar-13
01-Apr-13
01-May-13
01-Jun-13

c.

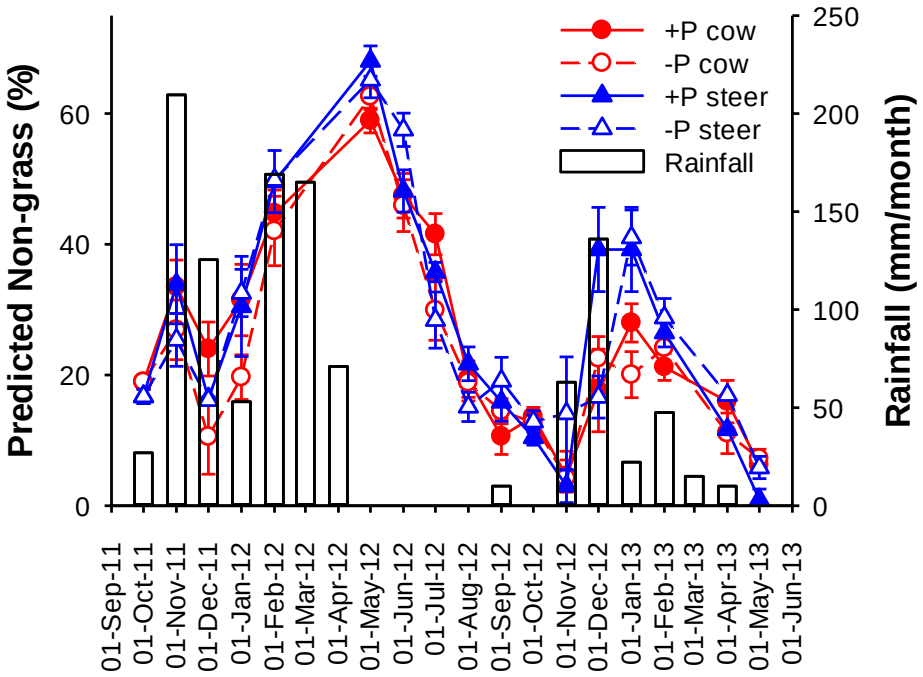


Figure 18. Rainfall and NIRS predictions of crude protein content (a. CP), dry matter digestibility (b. DMD) and percentage of non-grass species (c. Non-grass) in the diet consumed by cows and steers grazing Stud paddock over the duration of the experiment. *Description of number of faecal samples analysed is presented in Appendix 8.2*.

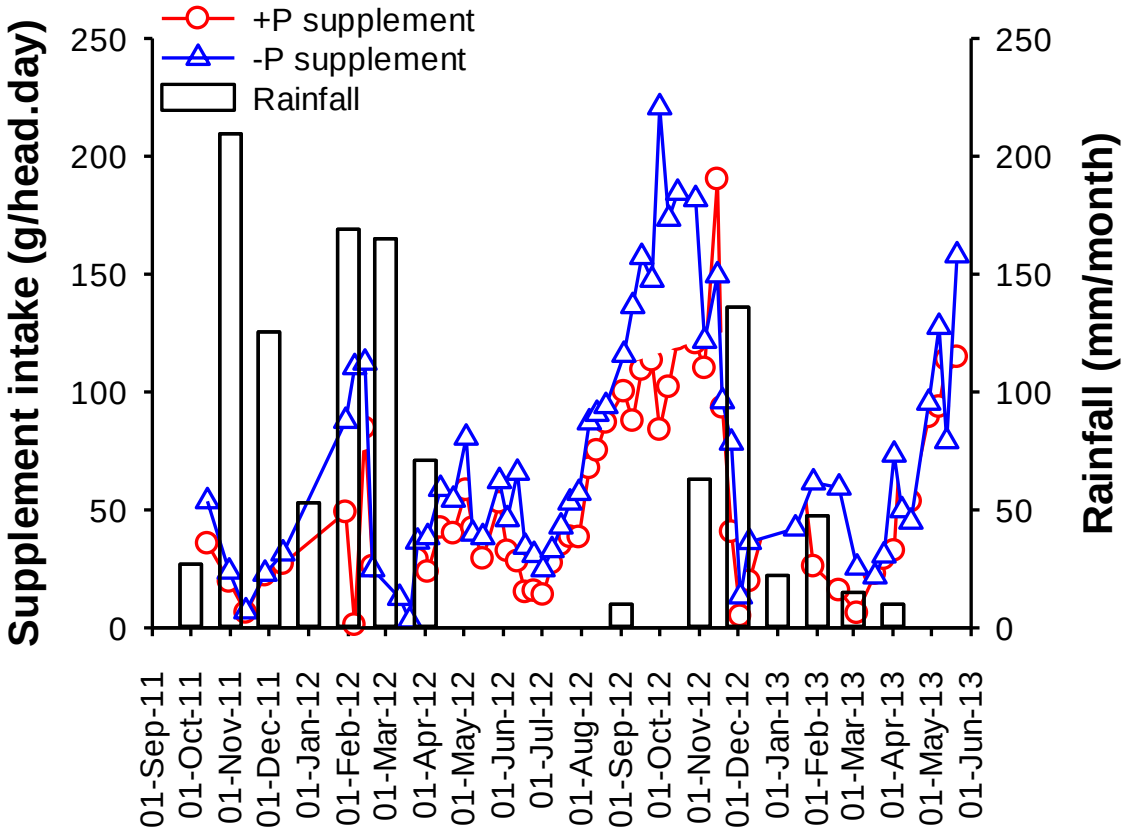


Figure 19. Intake of +P and -P supplements and rainfall in Stud paddock, Brunchilly. *This data is based on number of animals allocated to treatments at any period of time and assumes 100% draft accuracy to treatments.*

Accuracy of auto-drafters and walk-over-weighing: implications for this experiment
The auto-drafters made it possible to implement different supplementation treatments within the same paddock for this experiment. At the start of the project this was quite new technology and it had not been used for this purpose on such a large scale before. As it was new technology, different prototypes of auto-drafters were used at the two water points and modifications were made to the auto-drafters over time. The auto-drafter at the Stud bore was an older prototype and proved to be much less reliable than the one at No. 19 bore which was fitted out with newer equipment and updated designs.

There were many problems with the auto-drafters over the duration of the experiment. There were times when some or all of the auto-drafting, walk-over-weighing and the data recording features stopped working. The telemetry system that was fitted to enable data to be viewed remotely seldom worked. Table 11 shows a summary of when the auto-drafters were not working at each site. The auto-drafters were being used for a total of 604 days (between 2/10/2011 and 28/5/2013) for this work and the Stud drafter was out of action for 226 days (37% of the time) during this period while the No. 19 drafter was out of action for 53 days (9% of the time). Whenever the auto-drafters broke down they were set to draft all cattle to the -P enclosure so that -P treatment animals would not get P supplement during the experiment. The newer equipment at No. 19 was much more reliable than the older prototype at Stud bore and fortunately only a small number of cattle watered at the Stud bore during the trial and so the many breakdowns there had less of an impact on the experiment than if they had occurred at No. 19 bore.

Table 11. Summary of periods of time when auto-drafters were not working in Stud paddock at Brunchilly.

		Number of days that the auto-drafters were not working at each site	
Date from	Date to	Stud	No. 19
28/11/2011	7/01/2012	40	0
8/02/2012	15/02/2012	0	7
13/02/2012	14/02/2012	1	0
12/03/2012	16/03/2012	0	4
24/03/2012	29/03/2012	0	5
1/04/2012	5/04/2012	0	4
12/04/2012	16/04/2012	0	4
2/05/2012	4/05/2012	0	2
3/05/2012	15/06/2012	43	0
17/06/2012	13/07/2012	26	0
23/07/2012	10/08/2012	18	0
20/08/2012	15/10/2012	56	0
24/09/2012	25/09/2012	0	1
19/11/2012	27/11/2012	8	8
17/01/2013	18/01/2013	1	1
29/01/2013	30/01/2013	1	0
11/03/2013	17/03/2013	0	6
11/03/2013	4/04/2013	24	0
28/03/2013	4/04/2013	0	7
7/04/2013	8/04/2013	1	0
15/04/2013	16/04/2013	0	1
6/05/2013	7/05/2013	1	0
12/05/2013	13/05/2013	0	1
21/05/2013	27/05/2013	6	0
20/05/2013	21/05/2013	0	1
26/05/2013	27/05/2013	0	1
Total		226	53

In addition to the times when the auto-drafters were not working, there were times when they appeared to be drafting correctly but data (recording of EID number and/or WOW weight) was not being recorded properly. The main time when this happened at No. 19 bore was during dry season 1. For much of the period between 11-May-2012 and 29-Jan-2013 few EIDs were recorded at this auto-drafter. As few EIDs were recorded it is not possible to determine the draft accuracy during this time. However the draft accuracy appears to have been reasonable (>90%) during the periods either side of this time (Table 12), and the auto-drafter appeared to be working correctly during the weekly checks made by DPIF staff during this period (they would swipe test tags past the EID tag reader to confirm if the draft gate moved correctly). Also when the limited data recorded by the auto-drafter showing how many times each animal was drafted correctly

was evaluated and compared to the serum inorganic P results for sample animals, there did not seem to be any evidence that drafting had not been occurring correctly (e.g. -P treatment animals with high serum inorganic P results were not recorded as having being drafted incorrectly many times, and +P treatment animals with low PiP results appeared to have been mostly drafted correctly). Therefore there does not appear to be any evidence to suggest that animals were not being drafted correctly during the 2012 dry season and so it has been assumed that they were drafted correctly, even though there is no conclusive proof of this. While animals may have been drafted incorrectly at times throughout the experiment, and data is missing in some periods, it was decided on the basis of the previously mentioned evidence and the fact that draft accuracy was 92% (Table 12) over the duration of the experiment (where data was available) to assume that drafting was correct in enough cases that the treatment allocation of animals did not need to be retrospectively adjusted.

Overall the draft accuracy for the auto-drafter at the No. 19 bore, where it could be determined, was 92% correct. This is quite good considering the harsh conditions (heat, wind and dust) that it operated under and the large number of animals that used it daily (most of 536 cows, 120 steers, plus bulls and calves when they were present). The makers of the auto-drafter made a number of modifications to the auto-drafters throughout the project and by the end they considered that the No. 19 drafter was much improved and quite reliable. The Stud auto-drafter was considered to be unreliable and will be updated with new equipment to improve its reliability. When the auto-drafter technology was functioning correctly it was a powerful research tool as it enabled a number of nutritional treatments to be implemented within the one paddock hence removing paddock effects. The experience and knowledge gained during this project should result in the auto-drafters being improved and more reliable for future research work.

The walk-over-weighing ultimately failed to provide any useful data during the experiment. Data generated from this technology is highly variable due to animal movement across the scales, meaning that only a percentage (10 to 20%) of data collected from passes over the platform is useable. This coupled with the irregular passing of animals into supplement enclosures in the wet seasons meant that the collection of regular, reliable data using this technique was inconsistent, particularly in the wet season. Of greater concern was the gradual drift in scale accuracy over the experiment which meant that basically no reliable data was collected using this equipment over the final nine months of the experiment. This is discussed in Appendix 8.3.

Table 12. Draft accuracy of the auto-drafter at No. 19 bore

Period	Month	Sum of Correct drafts	Sum of Incorrect drafts	Accuracy
Wet Season 2011	Oct 2011	3049	178	94%
	Nov 2011	6300	253	96%
	Dec2011	5811	158	97%
	Jan 2012	7651	70	99%
	Feb 2012	7153	580	92%
	Apr 2012	5164	1626	76%
	May 2012	8122	706	92%
	WS 2011 Total	43250	3571	92%
Dry season 2012	Data was not recorded from May to Dec 2012			
Wet season 2012	Jan 2013	10323	403	96%
	Feb 2013	12071	239	98%
	Mar 2013	5485	2313	70%
	Apr 2013	7372	895	89%
	May 2013	4228	26	99%
	WS 2012 Total	39479	3876	91%
Overall	Grand total	83263	7639	92%

Note: Draft accuracy was able to be determined by comparing the list of animals that were recorded as passing through the auto-drafter on a day to the list of animals recorded passing through an exit spear on one of the enclosures on the same day.

Serum inorganic P

In Experiment 1, it was demonstrated that PiP reflected P intake at, or near to, the point in time at which the sample was collected. A similar result was found in Experiment 2, where serum P was influenced by most recent P supplement treatment (i.e. within the current season at that sampling time) rather than P supplement across the entire year (i.e. there were no carry-over effects of P treatment on serum inorganic P from one season to the next, as expected). In Experiment 2, steers had higher serum inorganic P concentration than cows at all stages of the experiment ($P<0.05$). Serum inorganic P concentration of growing steers measured throughout the course of Experiment 2 would suggest that a response of steers to P supplementation was unlikely in Stud paddock [if a threshold value of PiP from the jugular vein of 1.6 mmol/L (50 mg/L) is used (Coates, 1994)]. There was little effect of +P or -P supplementation on serum inorganic P concentration of steers or cows at the end of wet season 1 (Figure 20). At the end of dry season 1, steers and cows that received the +P supplement had higher serum inorganic P than steers and cows that received the -P supplement over the dry season ($P<0.05$; Figure 20). At the end of the shorter wet season 2, +P supplemented cows had higher serum inorganic P concentration than -P supplemented cows but supplementation had no effect on serum inorganic P in growing steers. These differences in serum inorganic P concentration reflect differences in supplementary P intake during the dry season, when cows and steers from both +P and -P supplement groups had high supplement intakes. The high P intake by +P cattle during the dry season was a

consequence of increased consumption of the high N dry season supplement at that time. Similarly, supplement intakes were higher in the shorter wet season 2 compared to wet season 1 and this is likely to be responsible for the greater difference in serum inorganic P between +P and -P supplemented cows at the end of wet season 2 compared to wet season 1. There was considerable variation in the serum inorganic P concentration measured for the same class of animal within a treatment group within Experiment 2. For example, the serum inorganic P concentration ranged from 0.59 to 3.26 mmol/L in cows (n=48) that were unsupplemented over the first wet season of the experiment. The variability in serum inorganic P concentration of unsupplemented steers (n=21) at the same time was less but the values still ranged from 1.76 to 2.93 mmol/L, which were well above the PiP concentration at which Coates (1994) proposed no response to supplementation was likely. This variability in serum inorganic P concentration within a mob of cattle will have implications for the number of samples required to get a reasonable estimate of circulating P concentration, and hence the likelihood of a response to P supplementation.

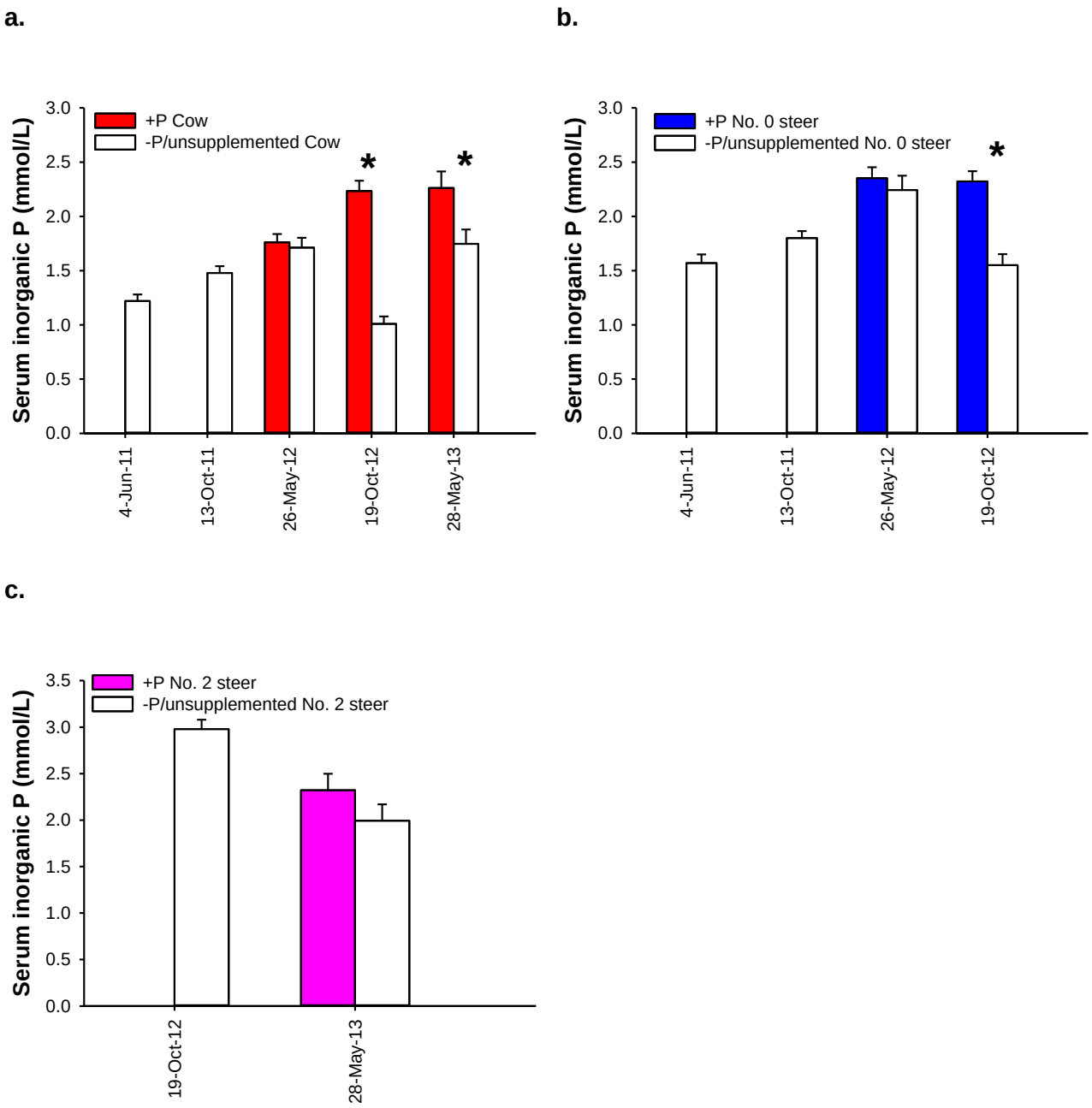


Figure 20. The concentration of inorganic phosphorus (P) in the serum of cows (a.) and No. 0 (b.) and No. 2 (c.) steers that received a supplement either with (+P) or without (-P) inorganic P during the wet or dry season prior to sample collection in Stud paddock, Brunchilly. *An asterisk above columns indicates significant difference in serum inorganic P between cattle offered a supplement with or without inorganic P (P<0.05).*

Faecal P

At sample collections conducted when cattle were mustered to the yards FecP, FecP:predicted ME and FecP:predicted CP were different between +P and -P cows and steers at the end of the 2012 dry season (Oct 2012; Figures 21, 22 and 23). In addition a significant difference in faecal indices was observed between +P and -P supplemented cows at the end of the 2013 wet season (May 2013). The difference at October 2012 likely reflects increased supplement intake during the dry season, which increased both N and P intake in +P cattle but only N intake in -P cattle. Regardless of the differences in FecP and its associated ratio's between cows offered +P or -P supplements the actual values remained low throughout the experiment. In contrast to serum inorganic P, there was little difference in FecP, FecP:ME and FecP:CP between cows and steers except for significantly higher faecal indices of steers in October 2012. There was large variation in the concentration of P measured in the faeces from these grab samples collected from the rectum, with a maximum and minimum concentration of 7080 and 894 mg P/kg respectively measured in two breeders in October 2011; this time of measurement was prior to the commencement of any supplementation.

The low number of samples collected in the supplement yards resulted in significant variability in the results per treatment group at any one time (Figure 24). For example, the very high +P cow FecP concentration in December 2011 is the result of one of the four samples analysed having an extremely high FecP (6080 mg P/kg) concentration relative to the other three samples collected at that time (2976, 3013 and 4166 mg P/kg) which emphasises the importance of collecting a large number of samples for bulking prior to analysis under industry conditions, or individual samples for analysis within a research project. The number of faecal samples analysed at each time point is provided in Appendix 8.2 and while this number per treatment was below the 15 recommended by White *et al*. (2010) for herd management decisions, the greater source of variation here was in the concentration of P in the faeces not the NIR predictions of CP, DMD and Non-grass. The total number of samples analysed most months was approximately 25 (from steers and cows) and this should be more than enough to give an accurate estimate of CP, DMD and Non-grass in a paddock this size (White *et al*., 2010) as there were no differences between sexes and supplementation treatment groups. Unfortunately there was little that could be done to increase the number of samples collected in the supplement enclosures, particularly in the wet season when animals infrequently entered them. There was a requirement to observe defecation, so that steer and cow faeces could be identified, this prevented the collection of faeces from the ground in the supplement enclosures when cattle were not present at the time of visit and it was difficult to follow cattle around Stud paddock in the wet season to observe defecation outside of the enclosures and identify what treatment to which they were allocated.

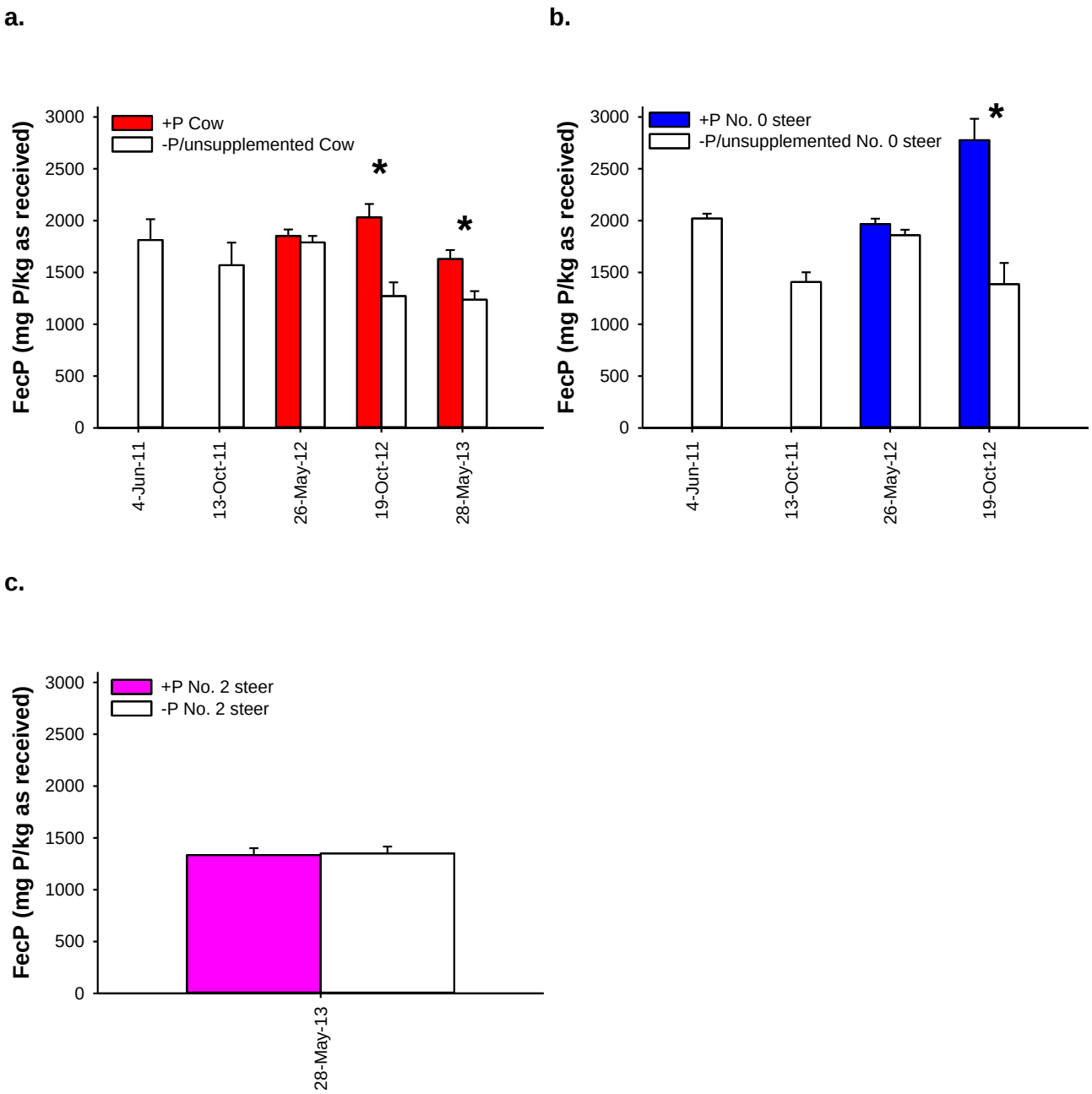


Figure 21. The concentration of inorganic phosphorus (P) in the faeces of cows (a.) and No. 0 (b.) and No. 2 (c.) steers that received a supplement either with (+P) or without (-P) inorganic P during the wet or dry season prior to sample collection in Stud paddock, Brunchilly. *Faecal grab samples collected at musters to yards only presented. An asterisk above columns indicates significant difference in FecP between cattle offered a supplement with or without inorganic P (P<0.05).*

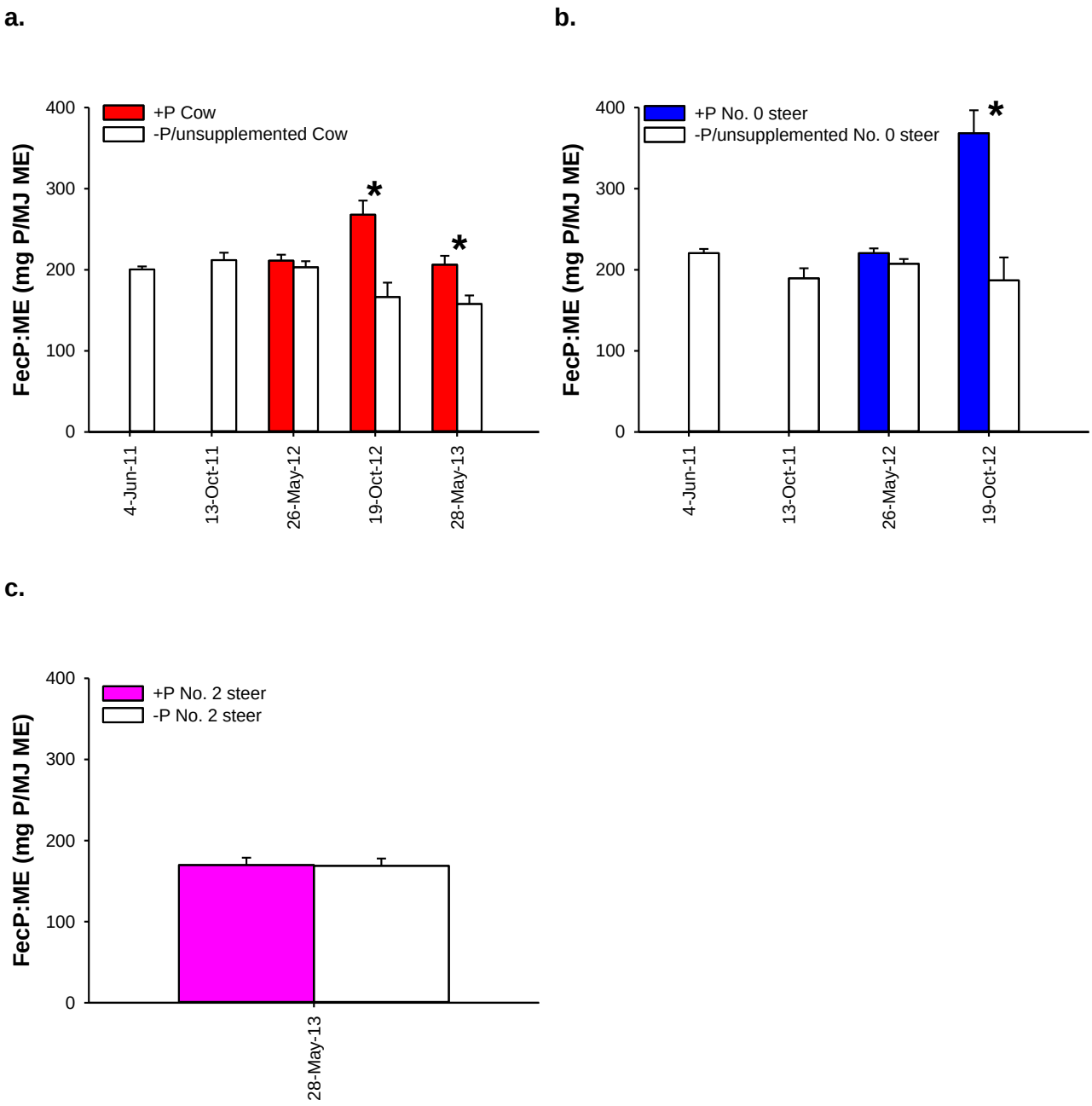


Figure 22. The concentration of inorganic phosphorus (P) in the faeces relative to predicted dietary metabolisable energy (ME) content of cows (a.) and No. 0 (b.) and No. 2 (c.) steers that received a supplement either with (+P) or without (-P) inorganic P during the wet or dry season prior to sample collection in Stud paddock, Brunchilly. *Faecal grab samples collected at musters to yards only presented. An asterisk above columns indicates significant difference in FecP:ME between cattle offered a supplement with or without inorganic P (P<0.05). Results of faecal analysis were reported on an as received basis.*

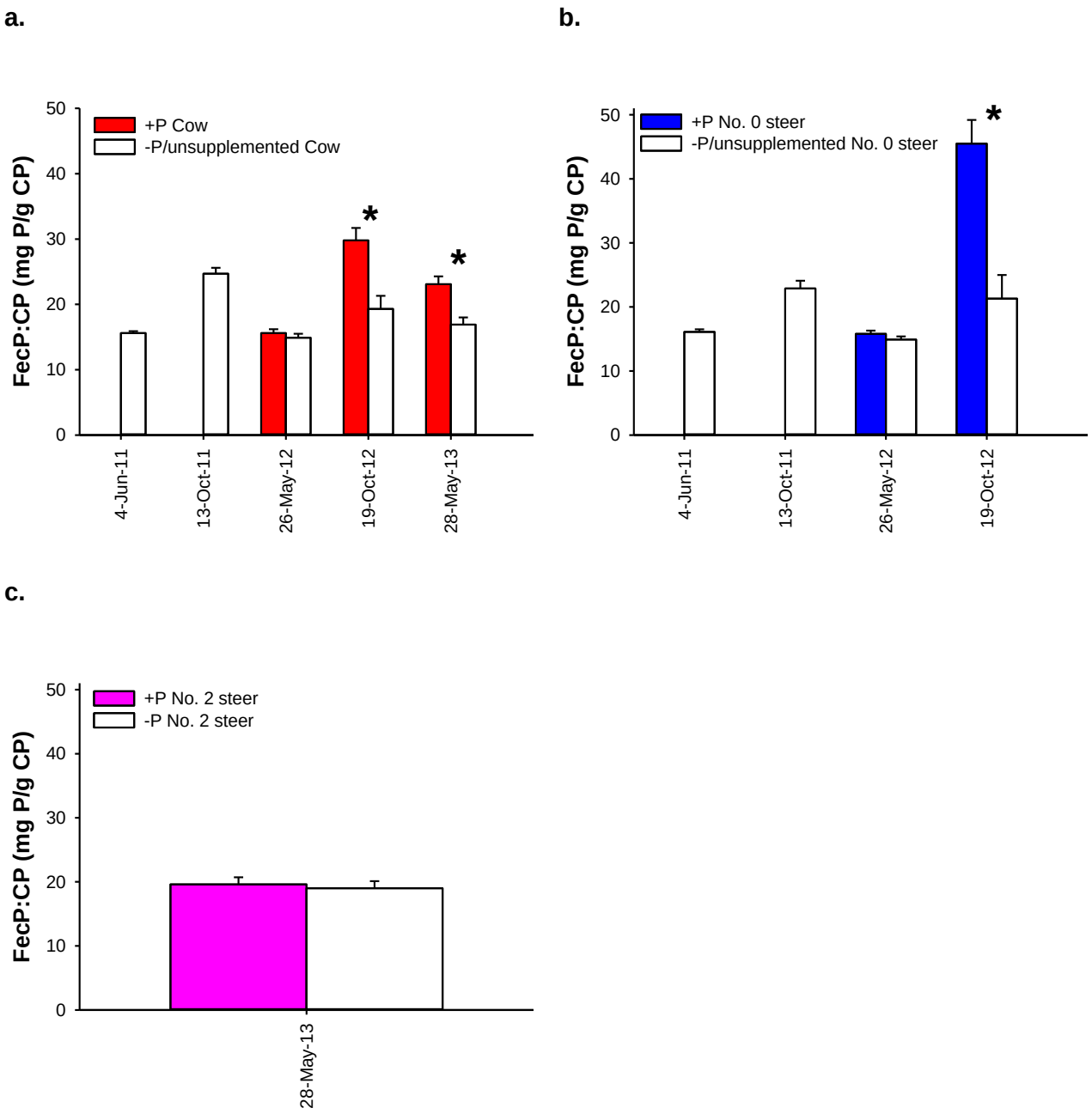


Figure 23. The concentration of inorganic phosphorus (P) in the faeces relative to predicted dietary crude protein (CP) content of cows (a.) and No. 0 (b.) and No. 2 (c.) steers that received a supplement either with (+P) or without (-P) inorganic P during the wet or dry season prior to sample collection in Stud paddock, Brunchilly. *Faecal grab samples collected at musters to yards only presented. An asterisk above columns indicates significant difference in FecP:CP between cattle offered a supplement with or without inorganic P (P<0.05). Results of faecal analysis were reported on an as received basis.*

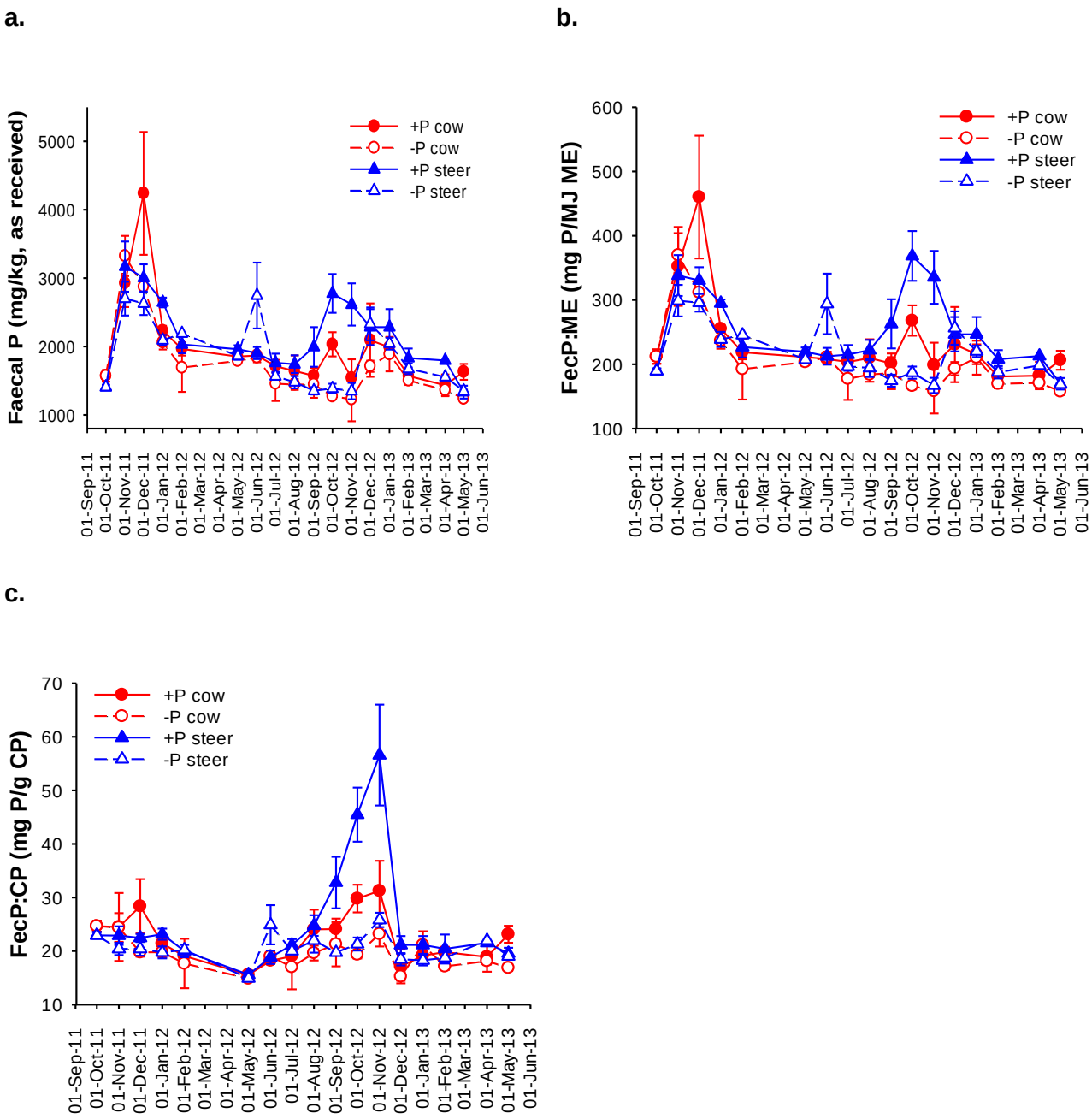


Figure 24. Faecal phosphorus (P) (a. FecP), FecP:predicted dietary metabolisable energy (ME) content (b. FecP:ME) and FecP:predicted dietary crude protein (CP) content (c. FecP: CP) of cows and steers offered supplements with (+P) or without (-P) P in the wet/dry seasons in Stud paddock, Brunchilly. *Faecal grab samples collected at musters to yards in October 2011, May 2012, October 2012 and May 2013 and samples collected from supplement yards in other months presented. Results of faecal analysis were reported on an as received basis.*

Based on the threshold values provided in Dixon and Coates (2012) the FecP:ME measured in Experiment 2 (typically 200 mg P/MJ ME) would indicate that supplemented and unsupplemented cows were consuming diets that were deficient in P for most of the year. A more exhaustive data set on the ability of FecP:ME to predict the likelihood of poor reproductive performance in northern breeder herds suggested a threshold FecP:ME value of ~450 mg P/MJ ME below which there is an increased risk of a P deficiency having a negative effect on reproductive performance (McGowan, *pers. comm.*). It is important to note that the FecP data from the Brunchilly site was reported on an as received basis while the majority of FecP data would be reported on a DM basis. The Brunchilly samples were dried to a constant weight at

65^{o}C before submission for analysis, so would likely be at approximately 90% DM at the time of analysis, which would not explain the large differences in FecP measured in Stud paddock and those reported elsewhere. Data from Experiment 2 is extremely different to the Cashcow findings where FecP:ME was consistently below 250 in Stud paddock breeders, whilst weaning rates were in excess of 85%. The Phosphorus Manual (Jackson *et al*., 2012) states that a FecP:ME of 370 would be required to maintain LW of a non-lactating 400 kg breeder in the final trimester when DMD was 52%. The results from Experiment 2 suggested that non-supplemented cows, typically in the final trimester at the end of the dry season (~54% DMD) were able to essentially maintain LW with a FecP:ME of less than 250. The reason for this discrepancy is unknown and clearly warrants further examination. Herein lies a discrepancy between the FecP:ME measurement and all other available information from cattle in Stud paddock. Approximately 34% of the area of Stud paddock contains soils that would be considered adequate (approximately 9 ppm P) (the other 66% of Stud paddock would be considered acutely deficient), with a high proportion of shrubs, forbs and non-grass species all of which would probably respond quickly to early wet season rain. Growing steers had serum inorganic P concentrations which would indicate a low risk of P deficiency and showed no LWG response to P supplementation over the wet seasons in which the experiment was conducted. Cows had high serum inorganic P concentrations and showed no response in LWG, BCS or reproductive parameters to P supplementation at any stage of the experiment, or cumulative across the experiment. Overall, the only suggestion that a P deficiency was present was the FecP (and associated ratio's) and all other evidence would suggest that cattle in Stud paddock were not P deficient during the years in which the experiment was conducted.

Based on the lack of an animal production response and serum inorganic P concentration it appears that the cattle in Stud paddock were not P deficient during the period of the experiment yet FecP:ME values would be indicative of a P deficiency. The work that underpins FecP:ME as an indicator of dietary P content, i.e. FecP is related to diet P content and P requirements to achieve production targets are linked to ME intake. This relationship between FecP and diet P is based on data from the extensive research conducted on unsupplemented cattle grazing fertilized or unfertilized tropical grass-stylosanthes pastures in northern Australia (Dixon and Coates, 2012) and it is under these pasture systems that the relationship will be most reliable. The relationship between diet P and faecal P was also evaluated by Dixon and Coates (2012) across a range of published data sets with a comparable result to that found across the northern Australian data sets for most of the data sets The diet consumed by cattle in Stud paddock would have been different to those diets, with a high CP, ME and proportion of non-grass species (near to 70% at certain times) predicted to be in the diet of cattle in Stud paddock throughout the year; the non-grass component of the diet was most likely shrubs and forbs that grew predominately on the higher P red soil country and would have responded quickly to early wet season rains and may have been preferentially grazed by the cattle during the experiment. It is unknown what the exact species were on the red soil areas and how these non-grass species may have influenced the resultant FecP:ME. If the actual species/ingredient mixture of the diet is influencing the relationship between FecP and diet P and the subsequent FecP:ME then it will need to be used with caution in areas with different diet characteristics to those under which it was originally developed.

Preferential grazing of higher P red soil areas in Stud paddock, that likely responded first to early wet season rain, is likely to have provided sufficient P to build up P reserves of non-lactating cows before parturition. The above average rainfall at Brunchilly within two of the three wet seasons the experiment was conducted may have resulted in greater feed availability, which was of higher CP, ME and P which persisted for a longer period of time than years with average, or below average, rainfall. The breeders used in this experiment were selected on fetal age to calve between November and April (predominately December to February) over the initial wet season. Barnes and Jephcott (1955) showed greatest effects of P deficiency in cows that lactated during the dry season, when P demands would be highest and P content of pasture lowest, this coupled with depressed intake (due to N deficiency) would have further exasperated already low P

intakes. In contrast, by weaning at the start of the dry season (as occurred on Brunchilly), P demands are reduced during a period of low P intake, if cows start lactating after the wet season has started there may be sufficient time for them to replete P from grazing higher P areas before parturition. If the high soil P area of Stud paddock did support higher P content pasture at the start of the wet season, the effect of P deficiency on intake would be negated as well allowing for an increase in ME intake and further recovery of BCS prior to calving. Preliminary data from the bone biopsies suggest that the animals were able to maintain CBT over a 12 month period, suggesting that repletion of mineral reserves was possible in non-lactating cows. It is possible that the experiment did not proceed for a long enough period of time to induce a response. Work in South Africa reported decreased reproductive performance of cows on acutely P deficient country in the second year of calving (Read *et al*., 1986), so if Stud paddock was only marginally P deficient (or at least had some areas of higher P soils) then it is not surprising that no effects on reproductive performance were observed after three calving's.

Bone

Mean CBT for sample cows prior to the commencement of the experiment was 4 mm. This is significantly higher than the 3 mm suggested by Little (1984) to indicate an adequate P supply. This suggests that these cows had sufficient P reserves to mobilise in the face of a period of P deficiency and that P intake earlier in life must have been high to build up these mineral reserves. There was no significant difference in CBT between treatments over the full 12 month period when seasonal carry-over effects of P supplementation were tested (May-2012 to May-2013). Over this period CBT change was 0.74 $\pm$ 0.27, 0.33 $\pm$ 0.28, 0.40 $\pm$ 0.26 and 0.31 $\pm$ 0.28 mm for +P+P, +P-P, -P+P and -P-P cows respectively (11^{th} near/off-side rib comparison). Interestingly, CBT increased more during the dry season (11^{th} near-side/12^{th} off-side rib comparison) compared to the wet season (12^{th} off-side/11^{th} off-side rib comparison); this result should be viewed with some caution as they are confounded by rib number. This suggested that cows were able to replete bone mineral reserves during the dry season when they were not lactating, and this occurred regardless of P supplement treatment and indicates the importance of herd management as one tool available to producers to manage P mobilisation and deposition of breeders. The current best practice management on Brunchilly with respect to calving and weaning time and season provides a good example that bone P reserves can be maintained and/or manipulated in the absence of P supplements by simple management of the nutrient demands on the cow. Specific gravity of bone biopsies did not change over the 12 month treatment cross-over period and was not affected by P supplement treatment. The lack of response of SG to P intake is consistent with Experiment 1 results.

Reproduction

Table 14 summarises the reproductive performance of the breeders over the experiment. Overall there were no significant effects of treatment on the reproductive parameters studied. There were no significant differences in weaning rate in either 2012 or 2013, in the percentage of cows pregnant at the last data recording in August 2013, or in the average stage of pregnancy of pregnant cows at the end of the experiment.

Table 14. Summary of the reproductive performance of cows supplemented with (+P) or without (-P) phosphorus in the wet or dry season (wet/dry).

	Treatment			
Parameter	+P/+P	+P/-P	-P/+P	-P/-P
Number of cows WR1 2012	137	137	131	131
Calf loss rate 2011/12 (%)	14.6	14.6	13.0	14.5
Weaning rate 2012 (%)	85.4	85.4	87.0	85.5
Pregnant by end of 2012 (%)	97.7	94.7	96.9	96.9
Number of cows WR1 2013	132	132	130	130
Calf loss rate 2012/13 (%)	14.7	16.0	10.3	12.7
Weaning rate 2013 (%)	83.3	79.5	86.9	84.6
Pregnant by end of 2013 (%)	72	75	75	67
Average number of months pregnant at end of trial	4.5	4.7	4.5	4.4

As all cows were pregnant at the start of the experiment (in August 2011) the weaning rate in 2012 is the same as the calf loss rate subtracted from 100%. There was no significant difference in calf loss rate over the 2011/12 calving season and so there were also no significant differences in 2012 weaning rate between treatments. Only a small percentage of cows (3.4% overall) failed to conceive by the second round muster in 2012. This is extremely good reproductive performance for the NT and reinforces how good the seasonal conditions were over the 2011/12 season. There were no significant differences in calf loss between treatments. Overall calf loss averaged 13.4% which is at the higher end of the normal range for calf loss for breeders in this region (the Cash Cow project found that the 25th to 75th percentile value range was from 5% to 14% for calf loss in the northern downs region; McGowan *et al*., 2013).

The average LW of weaners at the first round muster in May 2012 was 208 kg and in May 2013 it was 209 kg. Calves were not mothered up so it was not possible to determine which treatment their dams were in. Tail hair samples were collected from both cows and calves for DNA parentage analysis but since there were no differences in pregnancy rates between treatments it was decided that the considerable expense of DNA parentage testing for all cows and calves would not be worthwhile.

It may be possible that the extended period without P supplement was starting to have an effect on the -P-P group by the end of the experiment as their pregnancy rate was 7% lower than the overall pregnancy rate of the other 3 groups that had received P supplement during the experiment. However this difference was not significantly lower than the other treatment groups. In general, despite the FecP:ME indicating that the diet was P deficient, all cows (including the -P-P group) had adequate nutrition to give good reproductive performance over the course of the experiment, which followed and included above average wet seasons. The reproductive performance of cows is strongly influenced by their LW (or BCS) and the LW of all cows, adjusted for stage of pregnancy (O'Rourke *et al*., 1991) remained high throughout the experiment with no observed differences between treatment groups (Table 15).

Table 15. Average liveweight (kg) of cows, adjusted for stage of pregnancy, supplemented with (+P) or without (-P) phosphorus in the wet or dry season (wet/dry).

	Treatment			
Muster date	+P/+P	+P/-P	-P/+P	-P/-P
21-Aug-2011	465.6	469.2	471.0	469.2
26-May-2012	489.1	491.6	484.5	481.9
19-Oct-2012	485.4	483.3	493.0	473.9
28-May-2013	450.5	462.8	451.8	448.2

Steer liveweight gain

The No. 0 (weaned in 2010) steers were only intended to be in the experiment until its original scheduled completion in October 2012, however they were left in the paddock for the extension of the experiment over the 2012/2013 wet season so growth could be calculated over this period as well. However blood and faecal samples were not collected from these animals at the final muster on 26-May-2013. There were 20 No. 0 steers allocated to each of the 4 treatments but one of the steers in the -P+P treatment was missing at the musters on 26-May-2012 and 28-May-2013, so it was not possible to calculate growth data for this animal. The average LWG of steers for each treatment between musters is shown in Table 16. None of the differences between treatments were statistically significant.

In Table 17 the two treatments that contained P during each season were combined ("+P during season") and the two treatments that did not contain P ("-P during season") during each period were combined. The LWG over the 2011/2012 wet season and the 2012 dry season tended to be higher in the treatments that contained P although these differences were not statistically significant. Liveweight gain was higher in the "-P during season" treatments over the 2012/13 wet season but again this difference was not statistically significant. An additional 40 No. 2 (weaned in 2012) steers (n=20 in each wet season P treatment group) were added to the experiment in October 2012 for the extension of the experiment over the 2012/2013 wet season. The LWG over the wet season for steers that received the +P (98.1 kg) or -P (93.7 kg) supplement was not significantly different.

Table 16. The average liveweight gain (LWG) of No. 0 steers supplemented with (+P) or without (-P) phosphorus in the wet or dry season (wet/dry).

	Treatment			
Parameter	+P/+P	+P/-P	-P/+P	-P/-P
LWG (kg) - 21-Aug-2011 to 26-May-2012	153.5	161.0	155.4	146.6
LWG (kg) - 30-May-2012 to 19-Oct-2012	27.0	24.3	27.1	25.2
LWG (kg) - 19-Oct-2012 to 28-May-2013	51.1	50.3	46.8	60.7
LWG (kg) - 21-Aug-2011 to 28-May-2013	231.6	236.7	230.1	232.5

Table 17. The average liveweight gain (LWG) of steers during the periods between musters when the two +P and -P treatments are combined in each season.

Parameter	+P during season	-P during season
LWG (kg) – wet season 1	157.2	151.0
LWG (kg) – dry season 1	27.0	24.8
LWG (kg) – wet season 2	50.4	53.9

Note, "+P during season" is the treatments +P+P and +P-P during the wet season, and +P+P and -P+P during the dry season.

4.3 Overall discussion

In Experiment 1 there was an increase in FecP in response to increasing dietary P content and P intake. The relationship was improved the longer the steers consumed their allocated treatment diets. At the final sample collection, after 172 days on treatment diets, the relationship between diet and faecal P content for individual steers was comparable to that in the data sets of Coates from unsupplemented growing and breeder cattle (reviewed in Dixon and Coates, 2011) and for growing unsupplemented steers fed a range of forages (Holechek *et al*., 1985) (Figure 25). However the relationship between diet and faecal P content was different in Experiment 1 when mean FecP across the entire experiment was used. The diet used in Experiment 1 contained an inorganic source of P and this does not appear to have altered the relationship between diet and faecal P, at least at the end of the experiment. The current recommendations are for removal of any inorganic P supplements for 1 to 2 weeks prior to faecal sample collection. At a practical level this is unlikely to be an issue as most producers that are already providing a P supplement to their animals are doing so because they are aware of the beneficial responses to supplementation of their animals and are unlikely to be testing cattle that they are supplementing. The results from the end of experiment 1 would suggest that inorganic P in the diet has not had an abhorrent effect on the relationship between diet and faecal P content. It is unknown if the inclusion of the inorganic P source in the diet contributed to the lack of strong relationship between diet and faecal P at earlier stages of the experiment. Interestingly FecP increased immediately in response to P supplementation which followed immediately at the end of the pen experiment (see Appendix 8.7) for steers that were considered to be P deficient at the end of the pen experiment described above. This may suggest that FecP responds to diet P (or P intake) independently of depletion and repletion of P in bone.

A similar response of increasing concentration of P in faeces was observed in Experiment 2, when intake of supplements, and hence P, increased markedly in the late dry season. In Experiment 1, ME content was similar between treatments and remained relatively constant throughout the duration of the experiment and a relationship between P intake and FecP:ME was observed. In Experiment 2, the FecP:ME indicated that the animals were deficient at most stages of the experiment. All other evidence from Experiment 2 would suggest that the animals were not P deficient as there was no difference in responses between +P and -P supplemented animals. This discrepancy between FecP:ME and measured animal response may be related to the composition of the diet and mixture of land types within Stud paddock (Barkly Tableland, NT) compared to where much of the data was generated for the FecP:ME test (North Queensland). Wadsworth *et al*. (1990) also concluded that threshold values for PiP, FecP and CBT indicating the likely response to supplementation were best made within sites rather than using a common threshold value applied widely across northern Australia.

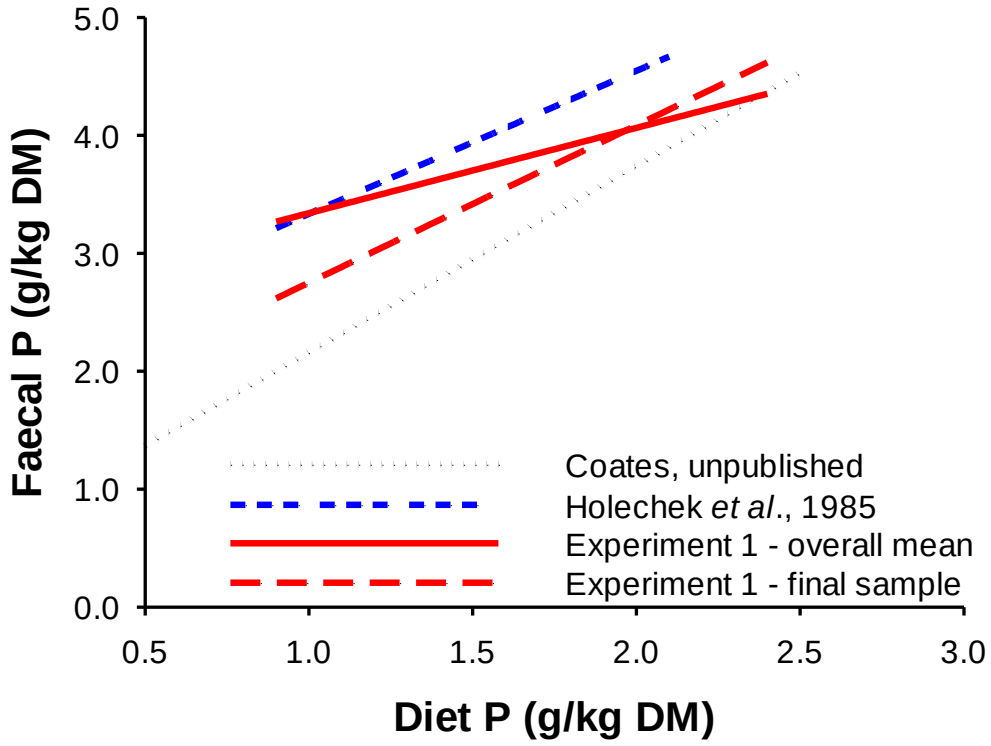


Figure 25. The relationship between diet and faecal phosphorus (P) content of cattle from various experiments. *Relationships are for unsupplemented growing and breeder cattle grazing fertilised/unfertilised tropical pastures (Y=1.58x+0.58, R^2=0.81; Coates, unpublished data; presented in Dixon and Coates, 2012), growing steers in pens fed a range of forages (Y=1.21x+2.12, R^2=0.65; Holechek et al., 1985, data is the treatment mean for five forages, with the high P content (3.4 g P/kg DM) Sainfoin removed from the dataset presented here) and data from Experiment 1 in this report for either the samples collected across the entire experiment (Y=0.72x+2.62, R^2=0.68) or final sample collection only (Y=1.34x+1.42, R^2=0.73).*

A key issue regarding the use of FecP:ME is related to the timing of when a sample is taken to give the best indication of dietary P content. Current thoughts are that FecP:ME test is valid only in the wet season when P is limiting and animals are most likely to be subject to a P deficiency. Experiment 1 demonstrated that the relationship between P intake and FecP varied based on the length of time that the steers had consumed their treatment diet, even when ME and CP were maintained at high levels (typical of wet season pasture) throughout the experiment. In Experiment 2, the only time that a difference in FecP:ME was evident was at the end of the dry season, reflecting increased P intake at this time when CP was likely to be the first limiting nutrient, not P. These variable responses of FecP:ME suggest that it may not be as simple as recommending a sample be collected at a single point in time and that repeated sampling within a paddock across different years/seasons may be required to indicate if a response to P supplementation is likely.

It appears that FecP:ME provides a good indicator of P intake in some scenarios, however the conditions under which it is used appear more restrictive than what the producer manual '*Phosphorus management of beef cattle in northern Australia*' (Jackson *et al*., 2012) would indicate. Since this project commenced a number of conditions have been relayed to the project regarding the use of FecP:ME including,

- The relationship between diet P content and FecP is based on data collected from specific land types/pasture mixtures
- Only useful in the wet season

- Questionable use when there is a high non-grass component of the diet
- Questionable use when inorganic P is included in the diet
- Questionable use when synthetic/concentrate diets/supplements are used as part of the overall diet

The above conditions are all justifiable but due to the specificity of these conditions around the use of the FecP:ME it is questionable that an industry wide recommendation for its use or the use of common threshold values is appropriate without further validation under a much wider range of land types/pasture mixes and across different seasonal conditions than the single site studied in Experiment 2 of this project.

If the concentration of inorganic P in the circulation is to be used as an indicator of the likely response of animals to P supplementation then uniform protocols need to be adopted – class of animal sampled, number of animals to be sampled, time of sampling (in relation to diet quality), site of sampling (jugular or tail) and form of sample (plasma, serum, whole blood). While PiP may be a more reliable indicator of P intake by the grazing ruminant there are a number of issues around sample collection and interpretation of results that have contributed to the lack of commercial adoption. Firstly, traditionally, collection, processing, storage and transport of blood samples on-farm are considered to be difficult. Secondly, interpretation of results may also be complicated by the timing of sample collection and the class of animal sampled, particularly with lactating cows that may be mobilising large quantities of P which would be incorporated into the inorganic pool. It is likely that if PiP was considered the most reliable indicator of P intake then solutions to the above issues could be developed. Recent advances in technology allow for the extraction of DNA from blood spot cards and test strips have been developed to test the concentration of any number of metabolites in blood or urine, so there is no reason to think that similar technologies could not be developed for a rapid PiP test. If larger sample volumes are required, logistics in the storage and transport of samples have developed considerably over recent years that would allow immediate cooling and processing of samples and express freight from the farm to the testing laboratory. The running of a small cohort of growing animals in paddocks with breeders would overcome the issue around complications that arise from sampling mobs of breeders. Probably the one issue that needs to be resolved is around the timing of the sample collection. Current recommendations are for sampling to occur in the wet or growing season. Such a definition is ambiguous and often draws the response that cattle are not mustered, and hence available for sampling, during the wet season; a more specific recommendation for timing of sample collection related to some indices of diet quality (e.g. samples must be taken from cattle growing at a certain rate of LWG or when NIRS predictions of diet DMD or CP content are above a certain level). The number of animals to be sampled also requires some clarification. Under the controlled conditions of Experiment 1 there was minimal variation in PiP but under the grazing conditions of Experiment 2 the variability in serum inorganic P between individuals allocated to the same treatment was large.

The Phosphorus Manual (Jackson *et al*., 2012) currently reports results as mg P/L whole blood collected from the tail whereas most commercial laboratories would report P concentration in mmol/L. The Phosphorus Manual states that animals are unlikely to respond to P supplementation when the concentration of P in whole blood collected from the tail is above 55 mg/L, based on conversions given in the manual this would equate to approximately 1.9 mmol PiP/L in jugular samples. Coates (1994) and Ternouth (1990) suggest that growing cattle are unlikely to respond to supplementation at a PiP concentration above 50 and 60 mg P/L in jugular samples respectively(1.6 and 1.9 mmol/L). In Experiment 1, ME intake and LWG increased in response to diet P content (2.0P and 2.4P) up to a PiP concentration of approximately 2 mmol/L, which aligns closely with the values in the P Manual. Clearly there is more work to be done in standardisation of protocols in the use of PiP to indicate the likely response of cattle to supplements under field conditions. Wadsworth *et al*. (1990) demonstrated that threshold values for detecting a P deficiency varied between sites and suggested that threshold values for likely response to supplementation be developed within each site.

No clear conclusions can be made regarding the carry-over effects of dry season P supplementation of cows due to the overall lack of response of the cattle in Stud paddock to supplementation at any stage of the experiment due to the likely adequacy of P to meet the requirements of the cattle that were within the site. Clearly the area of high P soil in Stud paddock has provided sufficient P for the cattle. This does suggest that a mixture of country types within a single paddock may reduce, or eliminate, the need for P supplementation, providing one of these land types has adequate P content soils, and appropriate stocking rates and herd management strategies are used.

In light of the findings from Experiment 2, and the clear response of growing steers to dietary P content in Experiment 1, the most useful indication of a response to P supplementation is probably the conduct of a an on-farm trial to observe if a response to P supplementation occurs. Circulating P also appears to give a more reliable indication of P intake than FecP but protocols for sample collection for PiP require standardisation and threshold values need to be refined for different sites. Soil P analysis conducted in Stud paddock appeared a viable option for indicating the P status of the paddock, and areas within a paddock of variable soil P concentration, with detection of soil P at 2 ppm possible. The use of satellite imagery could help identify areas within paddocks where soil samples could be collected and what areas of a paddock are P adequate and deficient, and recommendations for supplementation be based on the proportion of different soil P content within a paddock, coupled with knowledge of grazing behaviour of animals within the paddock.

4.4 Recommendations for future work

Potential areas of work/further questions that have arisen from this project include

1. *Repeat of the cow phosphorus work conducted at Brunchilly on areas where a response to P has been previously confirmed (PiP concentration near to 1 mmol/L in growing animals at the start of the dry season) to determine if carry-over effects of dry season P supplementation of breeders is a possible strategy to build up P reserves in breeders for mobilisation in the wet season.*
2. *Further validation of the FecP:ME test under different land types/diet composition and different breeder management systems (segregated vs. year round calving mobs) and development of site specific thresholds*.
3. *The relationship between dietary N and dietary P and LWG on PiP requires further evaluation (i.e. at what dietary N or DMD is P no longer the first limiting nutrient?) and may provide more specific guidelines for the timing of blood sample collection and the interpretation of results*. *The issue of site specific threshold values for PiP could also be evaluated, with this possibly done on existing samples.*
4. *A simplified and standardised method for collection, storage and transport of blood samples for PiP testing could be developed to overcome some of the perceived logistical issues around the technique*. The use of blood spot cards, dip sticks or simple crush side tests could be explored.
5. *The collection of faecal samples for FecP analysis also needs to be examined in more detail around the timing of sample collection and the number of samples collected and analysed*. Current recommendations are for early wet season sampling, whereas the pen experiment found that the relationship between P intake and FecP changed with time spent on a diet representative of early wet season pastures.
6. *A detailed understanding of the gene regulation of bone response to P intake could be undertaken.* Next Generation Sequencing of bone biopsies collected during Experiment 1 could be conducted to determine the key differentially expressed transcripts and gene pathways involved in a P mediated response in bone growth. Appropriate samples are currently stored at -80^{o}C.
7. *The importance of soft tissue P to repletion/depletion of cattle to changes in P intake could be investigated.*

8. *Further work may be required on the effect of DM content of samples submitted to commercial laboratories responsible for faecal P and NIRS analysis*.
9. *The use of soil P analysis to indicate P status of paddocks needs to be revisited and examined in conjunction with simple currently available satellite imagery. The development of remote technologies from Precision Agriculture that could be employed to assess P status on a paddock scale could be investigated*.

5 Conclusions

Dietary P content regulates DM intake, and hence LWG, in growing cattle when it is the first limiting nutrient as is the case in the wet season across large areas of northern Australia. Growing cattle that consume low P diets with adequate ME and CP will take longer to achieve target weights if they do not receive and consume P supplements during the growing season. Decreased production due to a P deficiency may take as little as five weeks. The measurement of the concentration of inorganic P in the plasma will provide an immediate indication of P intake of growing cattle consuming diets with above 10% CP and 9 MJ ME/kg DM but also indicated P intake of grazing P supplemented steers and cows under field conditions in the late dry season (5% CP, 7.8 MJ ME/kg DM). The measurement of the concentration of P in the faeces and its associated ratio's with diet quality will provide an indication of dietary P content but the relationship improves the longer the animals have consumed a diet. This project failed to answer the question regarding carry-over effects of dry season P supplementation of breeders and that will need to be revisited in the future. The project has successfully demonstrated that impressive LWG can be achieved in growing animals if a P supplement is provided during the wet/growing season. Within this project PiP provided a better indication of the P intake by cattle and the likely response to P supplementation of growing cattle than FecP and it is concluded that this is a more reliable indicator of likely response to P supplementation than FecP and its associated ratio's. The use of FecP and its associated ratio's with predicted diet quality as an indicator of the likely response of cattle to P supplementation may need refinement across different land types and cattle classes. A practical outcome from the pen work was that it takes approximately five weeks for a low P diet to start having a negative impact on LWG of P adequate animals but for P deficient animals the response to a P supplement was much quicker (approximately one week). This means that it is important to have P supplements available before the start of the wet season if one is to maximise use of the P supplement.

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8 Appendices

8.1 Appendix 1. Additional data from Experiment 1

Additional data generated from Experiment 1 which is not presented in the main body of this report is presented in this Appendix. The data includes

1. mineral content of faeces, urine and rumen fluid collected from steers during the digestibility collection period,
2. excretion of P in urine and faeces of steers in metabolism crates and
3. response equations to P intake on a g P/day basis, as this relates better to values presented in Jackson *et al*. (2012)

Table 8.1.1. Concentration of minerals in the urine and faeces of steers offered diets with increasing phosphorus (P) content [1,2,3]

	Treatment				
Parameter	0.9P	1.3P	1.8P	2.0P	2.4P
Urine P (mg/kg)	9.2 ± 12.5^{a}	8.3 ± 12.5^{a}	7.2 ± 12.5^{a}	24.7 ± 11.2^{ab}	60.8 ± 11.2^{b}
Urine Ca (mg/kg)	270 ±143	225 ± 143	489 ± 143	710 ± 128	789 ± 128
Urine Mg (mg/kg)	336 ± 84^{a}	399 ± 84^{a}	460 ± 84^{ab}	597 ± 75a^{b}	751 ± 75^{b}
Urine K (mg/kg)	4694 ± 875	4639 ± 875	6310 ± 875	5709 ± 782	7089 ± 782
Urine Na (mg/kg)	90 ± 39^{ab}	105 ± 39^{ab}	103 ± 39^{ab}	58 ± 35^{a}	207 ± 35^{b}
Urine S (mg/kg)	440 ±112^{a}	384 ± 112^{a}	463 ± 112^{a}	571 ± 101^{ab}	952 ± 101^{b}
Urine Fe (mg/kg)	0.24 ± 0.09	0.33 ± 0.09	0.27 ± 0.09	0.50 ± 0.08	0.39 ± 0.07
Urine Zn (mg/kg)	2.51 ± 2.82^{a}	2.94 ± 2.82^{a}	6.15 ± 2.82^{ab}	9.49 ± 1.81^{b}	10.60 ± 1.81^{b}
Faecal P (g/kg DM)	3.42 ± 0.15^{a}	3.82 ± 0.16^{ab}	3.98 ± 0.15^{b}	4.95 ± 0.14^{c}	5.54 ± 0.14^{c}
Faecal Ca (g/kg DM)	26.9 ± 0.9^{b}	9.3 ± 1.0^{a}	45.5 ± 0.9^{c}	6.7 ± 0.9^{a}	7.5 ± 0.9^{a}
Faecal Mg (g/kg DM)	2.74 ± 0.11^{ab}	3.23 ± 0.12^{b}	3.52 ± 0.11^{b}	2.75 ± 0.10^{a}	2.47 ± 0.10^{a}
Faecal K (g/kg DM)	4.77 ± 0.48^{a}	5.44 ± 0.51^{ab}	5.18 ± 0.48^{a}	7.67 ± 0.46^{b}	7.43 ± 0.46^{b}
Faecal Na (g/kg DM)	1.52 ± 0.19^{b}	1.43 ± 0.20^{ab}	1.21 ± 0.19^{a}	1.42 ± 0.18^{ab}	1.45 ± 0.18^{b}
Faecal S (g/kg DM)	3.17 ± 0.08^{c}	2.74 ± 0.07^{b}	3.48 ± 0.08^{d}	2.24 ± 0.07^{a}	2.37 ± 0.07^{a}
Faecal Fe (g/kg DM)	2.00 ± 0.35^{b}	1.07 ± 0.31^{ab}	1.51 ± 0.35^{ab}	1.05 ± 0.31^{ab}	1.00 ± 0.31^{a}
Faecal Mn (g/kg DM)	0.10 ± 0.004^{b}	0.13 ± 0.003^{c}	0.14 ± 0.004^{d}	0.09 ± 0.003^{a}	0.13 ± 0.003^{c}
Faecal Zn (g/kg DM)	0.15 ± 0.1^{b}	0.22 ± 0.01^{d}	0.19 ± 0.01^{cd}	0.09 ± 0.01^{a}	0.17 ± 0.01^{bc}
Rumen P (mg/kg)	536 ± 58	529 ± 58	666 ± 58	505 ± 52	598 ± 52
Rumen Ca (mg/kg)	615 ± 50^{b}	224 ± 50^{a}	775 ± 50^{c}	165 ± 45^{a}	187 ± 45^{a}
Rumen Mg (mg/kg)	88 ± 8^{b}	70 ± 8^{ab}	81 ± 8^{b}	41 ± 7^{a}	47 ± 7^{a}
Rumen K (mg/kg)	905 ± 45^{b}	783 ± 45^{b}	812 ± 45^{b}	568 ± 40^{a}	557 ± 40^{a}
Rumen Na (mg/kg)	930 ± 48	1054 ± 48	980 ± 48	1097 ± 43	1110 ± 43
Rumen S (mg/kg)	78 ± 33	91 ± 33	50 ± 33	78 ± 29	111 ± 29
Rumen Fe (mg/kg)	23 ± 10	20 ± 10	47 ± 10	8 ± 9	13 ± 9
Rumen Mn (mg/kg)	15 ± 3^{b}	9 ± 3^{ab}	12 ± 3^{ab}	3 ± 3^{a}	1 ± 3^{a}
Rumen Zn (mg/kg)	1.4 ± 0.7	2.4 ± 0.7	2.5 ± 0.7	0.9 ± 0.6	2.7 ± 0.6

[1]0.9P (0.9 g P/kg DM), 1.3P (1.3 g P/kg DM), 1.8P (1.8 g P/kg DM), 2.0P (2.0 g P/kg DM) and 2.4P (2.4 g P/kg DM)
[2]Data are least-squares means with standard error of the mean. Different alphabetical superscripts across each row indicate a significant difference between previous P diets (P<0.05).
[3]Determined on samples collected during the collection period in metabolism crates.

Table 8.1.2. Phosphorus (P) intake and faecal (Fec) and urine excretion by steers offered diets with increasing P content[1,2]

	Treatment				
Parameter	0.9P	1.3P	1.8P	2.0P	2.4P
P intake (mg/kg LW.day)	10.9	16.1	24.9	34.7	50.7
Faecal P output (mg/kg LW.day)	11.6	14.7	20.1	33.5	35.8
Urine P output (mg/kg LW.day)	0.11	0.08	0.10	0.33	0.49
P intake (g/day)	3.3	5.4	8.8	13.6	20.5
Faecal P output (g/day)	3.5	4.9	7.1	13.0	14.4
Urine P output (g/day)	0.03	0.03	0.04	0.14	0.20

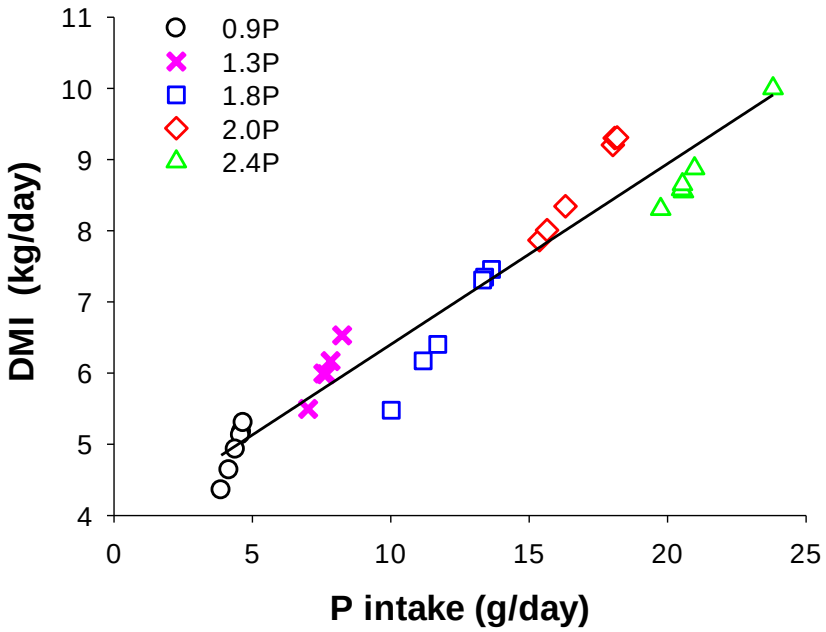
[1]0.9P (0.9 g P/kg DM), 1.3P (1.3 g P/kg DM), 1.8P (1.8 g P/kg DM), 2.0P (2.0 g P/kg DM) and 2.4P (2.4 g P/kg DM)
[2]Determined on samples collected during the collection period in metabolism crates.

Table 8.1.3. Response of dry matter intake (DMI), liveweight (LW), hip height (HH), plasma inorganic phosphorus (PiP), faecal P (FecP) and associated ratio's with diet metabolisable energy (ME) and crude protein (CP) content to increasing P intake over the entire experimental period[1,2]

Parameter (*Y*)	Equation	R^2	Root MSE[3]	*P-value*
	P intake (g/day) (*X*)			
DMI (kg/day)	$Y = 0.25x + 3.86$	0.93	0.46	<0.001
DMILW (g/kg LW.day)	$Y = 0.07x^2 + 0.99x + 12.98$	0.90	1.25	0.037
PiP (mmol/L)	$Y = 0.08x + 0.71$	0.89	0.18	<0.001
FecP (mg/kg DM)	$Y = 62.2x + 3057$	0.68	275	<0.001
FecP:ME (mg P/MJ ME)	$Y = -0.1x^3 + 3.8x^2 – 31.9x + 425.7$	0.75	36.2	0.021
FecP:CP (mg P/g CP)	$Y = -0.0357x^2 + 1.56x + 23$	0.74	2.76	0.041
LWG (kg/day)	$Y = -0.001x^2 + 0.066x + 0.04$	0.95	0.06	0.020
LW change (kg)	$Y = -0.209x^2 + 13.33x + 2.18$	0.96	10.6	0.003

[1]0.9P (0.9 g P/kg DM), 1.3P (1.3 g P/kg DM), 1.8P (1.8 g P/kg DM), 2.0P (2.0 g P/kg DM) and 2.4P (2.4 g P/kg DM)
[2]Quadratic and cubic terms were removed from the model if not significant (P>0.05)
[3]Root mean squared error

a.



b.

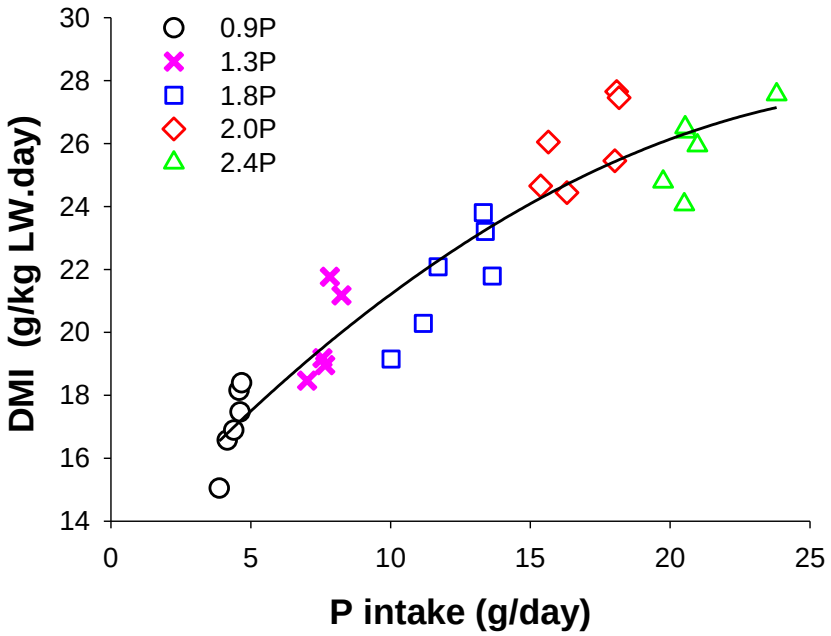
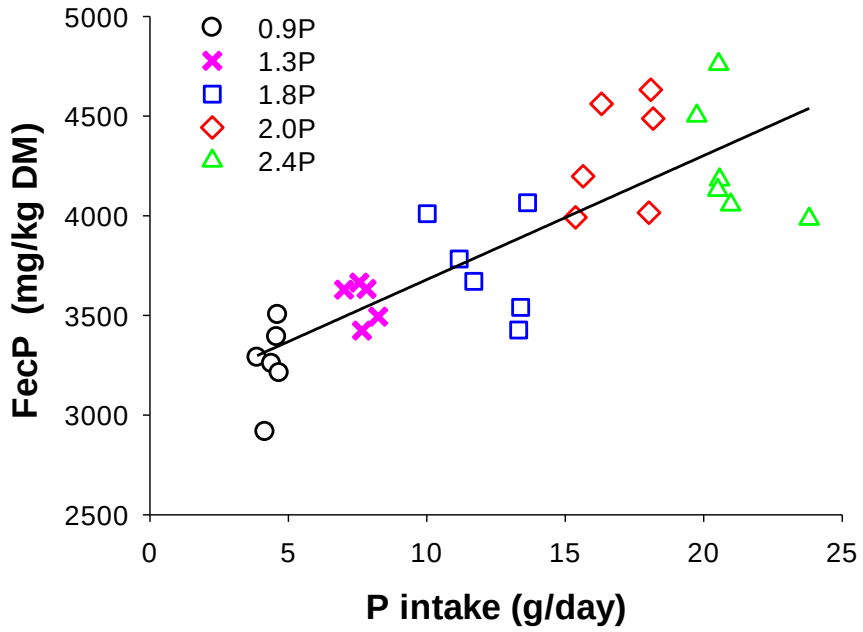


Figure 8.1.1. Relationship between phosphorus (P) intake and dry matter intake (DMI) (a. kg/day; b. g/kg LW.day) of steers fed diets containing increasing amounts of P (0.9, 1.3, 1.8, 2.0 and 2.4 g P/kg DM). *Individual symbols represent individual steers. Relationships are based on repeated measures data over the experimental period; equations are presented in Table 8.1.3.*

a.



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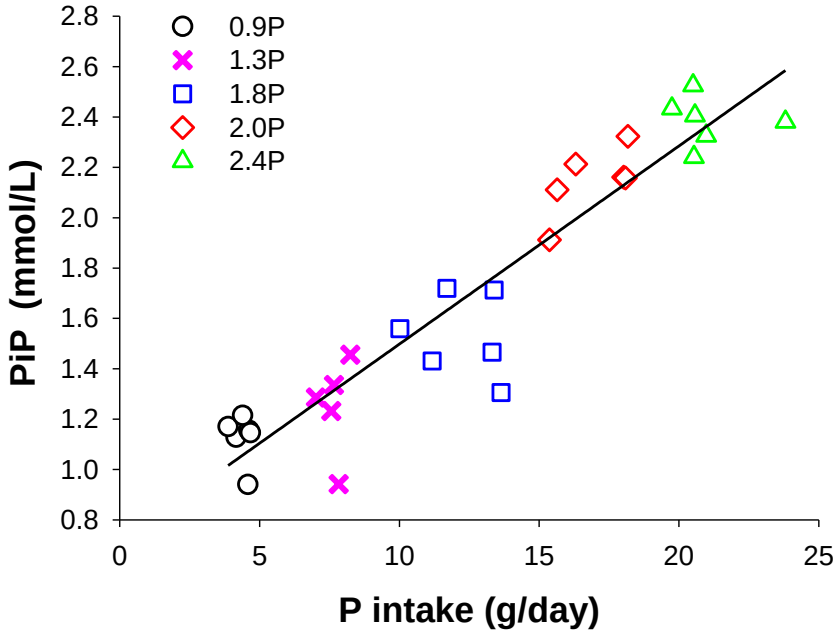
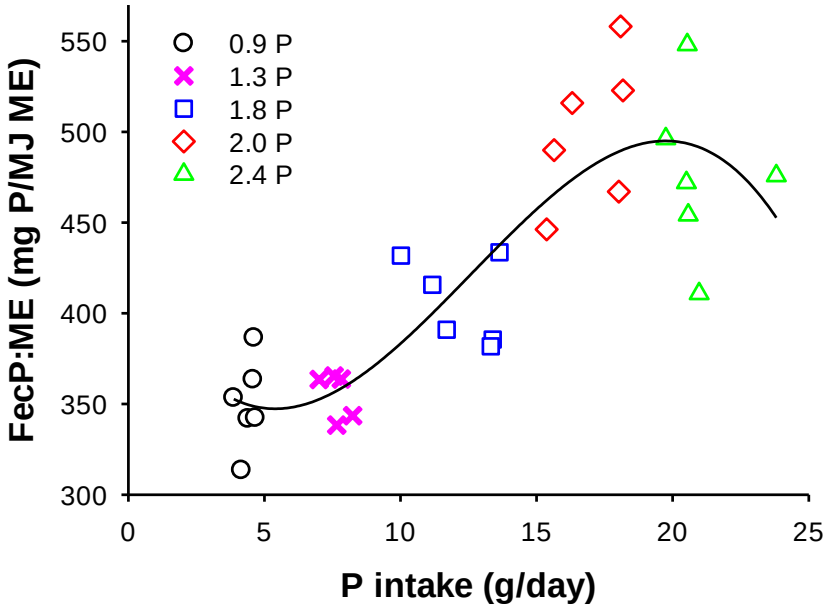


Figure 8.1.2. Relationship between phosphorus (P) intake and the concentration of P in the faeces (a., FecP) and plasma (b., PiP) of steers fed diets containing increasing amounts of P (0.9, 1.3, 1.8, 2.0 and 2.4 g P/kg DM). *Individual symbols represent individual steers. Relationships are based on repeated measures data over the experimental period; equations are presented in Table 8.1.3.*

a.



b.

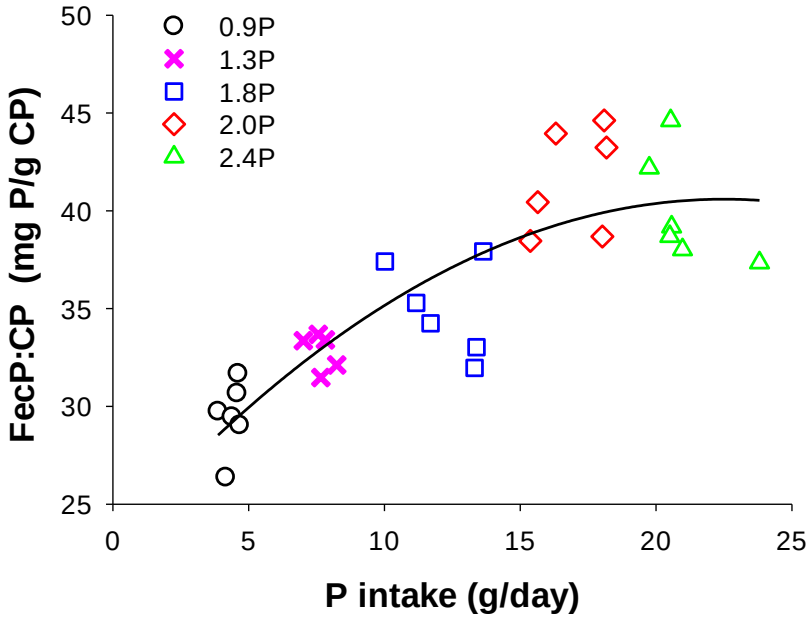
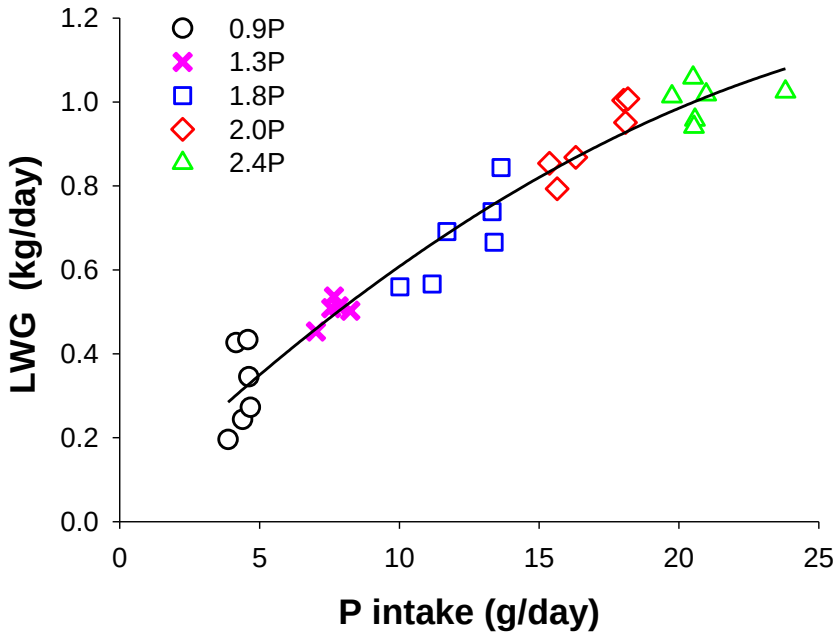


Figure 8.1.3. Relationship between phosphorus (P) intake and the ratio of the concentration of P in the faeces (FecP) to the metabolisable energy (ME) content of the diet (a., FecP:ME) and to the crude protein (CP) content of the diet (b., FecP:CP) of steers fed diets containing increasing amounts of P (0.9, 1.3, 1.8, 2.0 and 2.4 g P/kg DM). *Individual symbols represent individual steers. Relationships are based on repeated measures data over the experimental period; equations are presented in Table 8.1.3.*

a.



b.

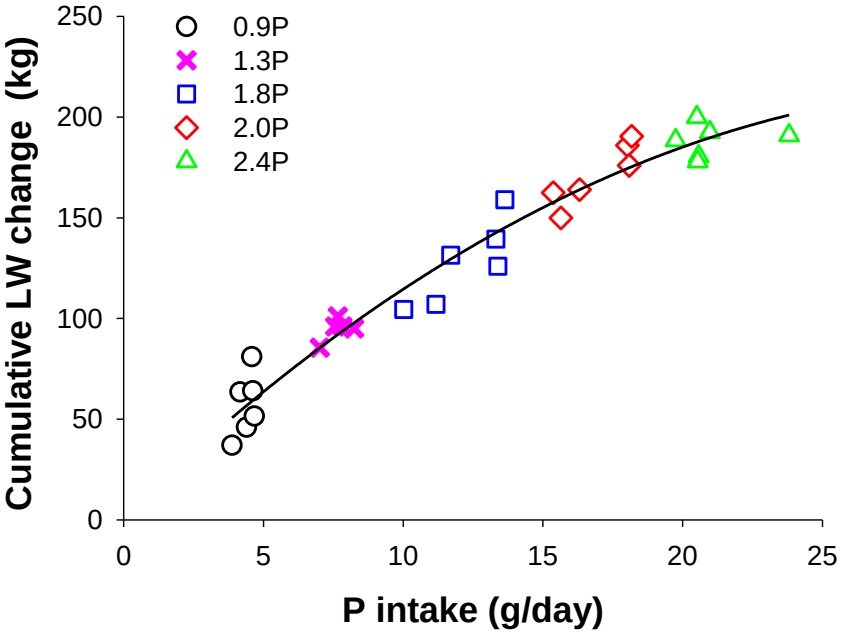


Figure 8.1.4. Relationship between phosphorus (P) intake and the liveweight (LW) gain (LWG) (a., kg/day) and cumulative LW change (b., kg) of steers fed diets containing increasing amounts of P (0.9, 1.3, 1.8, 2.0 and 2.4 g P/kg DM). *Individual symbols represent individual animals. Relationships are based on repeated measures data over the experimental period; equations are presented in Table 8.1.3.*

8.2 Appendix 2. Additional information from Experiment 2

Requirements for site selection for Experiment 2
The requirements that were considered in selecting a site for Experiment 2 are described in the main part of the report. The decision to select Stud paddock on Brunchilly was partly based on historical FecP:ME data collected in the Cashcow project and are presented in Figure 8.2.1.

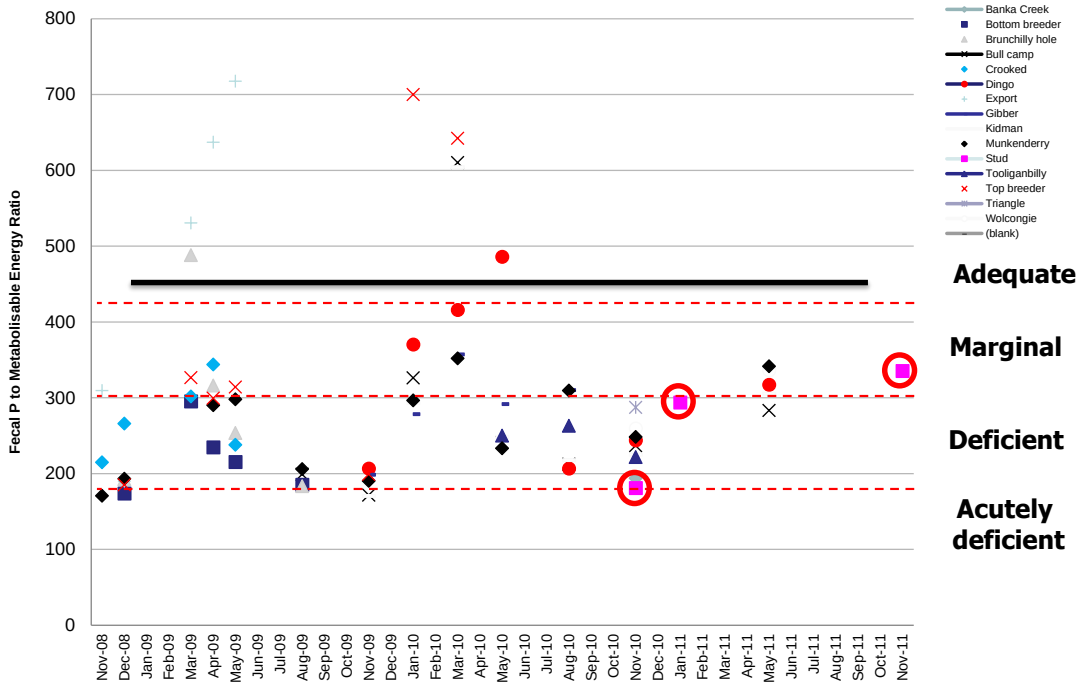


Figure 8.2.1. Faecal phosphorus to metabolisable energy (ME) ratio of paddocks on Brunchilly, November 2008 to November 2011. *Stud paddock values are circled in red; Threshold values (acutely deficient, deficient, marginal and adequate) are indicated by the dashed lines and are sourced from Table 2.1 in Jackson et al. (2012).*

Properties of soils and water of Stud paddock, Brunchilly

Table 8.2.1. Red and black soil profile analysis in Stud paddock, Brunchilly

Parameter	Units	Black soil bulk[1]	Red soil bulk[1]
Moisture	% w/w	3	2
pH (1:5)	pH Units	7.5	7
Electrical Conductivity (1:5)	µS/cm	67	73
TDS	mg/kg	220	240
pH - $CaCl_2$ (1:5)	pH units	6.7	6.3
Chloride, Cl (1:5)	mg/kg	<10	16
Sulphur, S	mg/kg	3	8
Total Oxidised Nitrogen (as N)	mg/kg	<0.1	4.8
Total Kjeldahl Nitrogen (as N)	mg/kg	290	400
Total Nitrogen	mg/kg	290	400
Total Kjeldahl Phosphorus	mg/kg	57	140
Colwell Phosphorus	**mg/kg**	**2**	**9**
Total Organic Carbon	% w/w	0.32	0.49
Organic Matter	% w/w	0.55	0.84
Carbon-Nitrogen Ratio		11	12
Sodium, Na	mg/kg	40	31
Sodium (meq%)	meq%	0.17	0.13
Exchangeable Sodium	%	<1	<1
Potassium, K	mg/kg	790	670
Potassium (meq%)	meq%	2	1.7
Exchangeable Potassium	%	8	11
Calcium, Ca	mg/kg	2600	1800
Calcium (meq%)	meq%	13	9
Exchangeable Calcium	%	49	60
Magnesium, Mg	mg/kg	1400	520
Magnesium (meq%)	meq%	11	4.3
Exchangeable Magnesium	%	43	28
CEC	meq%	27	15
CEC (Sol Salts Removed)	meq%	26	15
Iron, Fe (DTPA)	mg/kg	36	66
Manganese, Mn (DTPA)	mg/kg	41	49
Copper, Cu (DTPA)	mg/kg	0.7	1.1
Zinc, Zn (DTPA)	mg/kg	0.5	0.6

[1]Each soil type is the bulk of 10 core samples collected randomly within Stud paddock from the surface 10 cm.

Table 8.2.2. Red and black soil Colwell P of individual core samples collected in Stud paddock, Brunchilly

	P content (mg/kg)	
Core Number	Black soil	Red soil
1	3	7
2	3	6
3	3	6
4	3	5
5	2	8
6	2	20
7	2	9
8	3	10
9	4	6
10	3	20
Mean	**2.8**	**9.7**
Maximum	4	20
Minimum	2	5
Median	3	7.5

Table 8.2.3. Properties of water collected at Stud and No. 19 bores in Stud paddock, Brunchilly, November 2011

Parameter	Units	Limit	Stud	No. 19
pH	pH units	0.04	8.2	7.9
Electrical Conductivity	uS/cm	10	2500	2300
Total Alkalinity	mg/L $CaCO_3$	14	66	160
Chloride	mg/L	7	540	460
Hardness	mg/L $CaCO_3$	1	490	600
Calcium Carbonate Saturation Index			0.7	0.81
Sodium Adsorption Ratio			5.9	4
Turbidity	NTU	0.07	0.51	0.64
Ammonia-N	mg/L	0.02	0.083	0.33
Free Reactive Phosphorus	mg/L	0.008	0.011	<0.008
Nitrate-N	mg/L	0.02	<0.02	0.32
Nitrite-N	mg/L	0.01	<0.01	0.038
Total Nitrogen	mg/L	0.08	0.44	1.1
Total Phosphorus	mg/L	0.05	<0.05	<0.05
Total Elements				
Aluminium	mg/L	0.05	<0.05	<0.05
Arsenic	mg/L	0.05	<0.05	<0.05
Boron	mg/L	0.05	0.56	0.45
Calcium	mg/L	0.03	54	92
Cadmium	mg/L	0.01	<0.01	<0.01
Cobalt	mg/L	0.01	<0.01	<0.01
Chromium	mg/L	0.01	<0.01	<0.01
Copper	mg/L	0.01	<0.01	<0.01
Iron	mg/L	0.01	<0.01	<0.01
Potassium	mg/L	0.08	30	27
Magnesium	mg/L	0.01	88	90
Manganese	mg/L	0.01	<0.01	<0.01
Molybdenum	mg/L	0.02	<0.02	<0.02
Sodium	mg/L	0.07	300	220
Nickel	mg/L	0.01	<0.01	<0.01
Phosphorus	mg/L	0.04	<0.04	<0.04
Lead	mg/L	0.03	<0.03	<0.03
Sulfur	mg/L	0.1	140	100
Selenium	mg/L	0.05	<0.05	<0.05
Zinc	mg/L	0.01	<0.01	0.01

Table 8.2.4. Number of samples analysed for faecal NIRS and phosphorus.

Month	+P cows (*n*)	-P cows (*n*)	+P steers (*n*)	-P steers (*n*)
October 2011[1]	74		40	
November 2011	2	5	7	7
December 2011	4	6	2	2
January 2012	7	10	5	7
February 2012	10	10	7	1
March 2012	0	0	0	0
April 2012	0	0	0	0
May 2012[1]	39	38	16	18
June 2012	10	10	4	4
July 2012	10	9	3	5
August 2012	10	10	5	4
September 2012	10	10	6	5
October 2012[1]	39	37	18	18
November 2012	7	10	5	6
December 2012	6	9	5	5
January 2013	10	9	5	3
February 2013	10	10	2	4
March 2013	0	0	0	0
April 2013	10	9	1	1
May 2013[1]	32	35	14	14

[1]Samples collected in October 2011, May 2012, October 2012 and May 2013 were grab samples collected from cattle in the yards. All other samples were collected after observation of defecation by cattle in respective supplement yards.

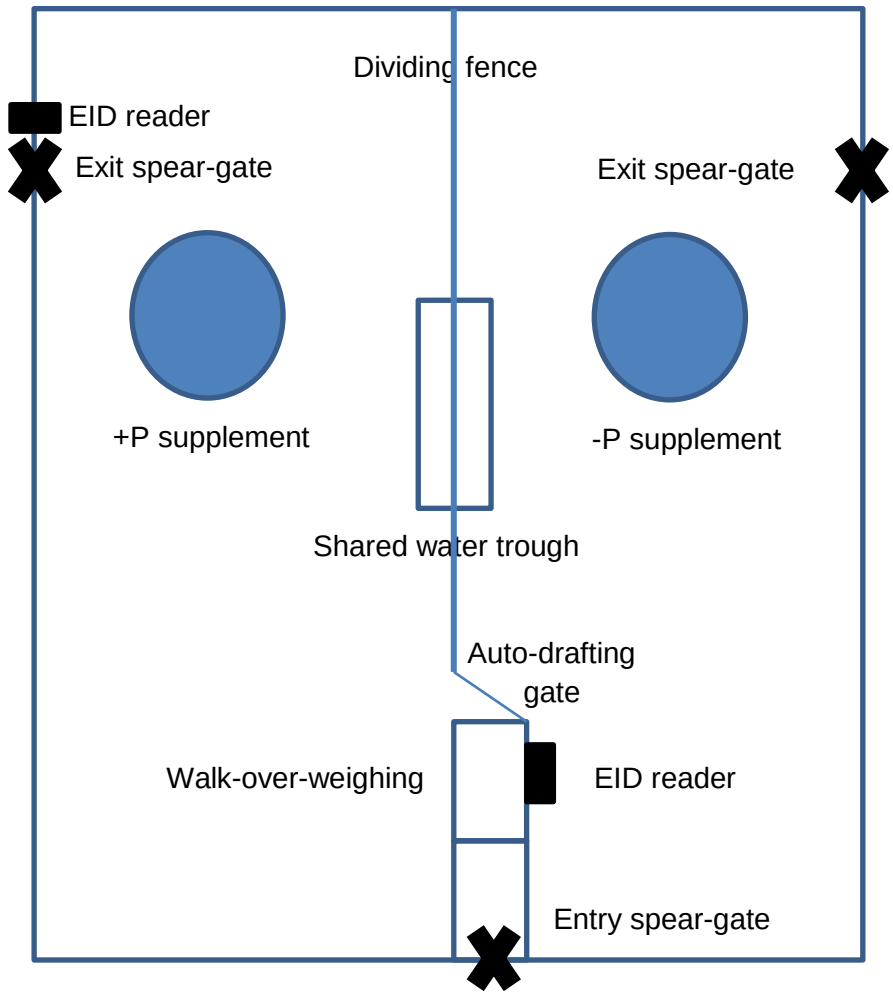


Figure 8.2.3. Schematic of supplementation yards with associated infrastructure at No. 19 bore in Stud paddock, Brunchilly.

a.

b.

Figure 8.2.4. Auto-drafter with walk-over-weighing, solar panels, batteries, telemetry, back-up gas bottle and EID readers (a.) and cattle drafted into supplement enclosures (b.) at Stud paddock, Brunchilly (Source: T Schatz and S Quigley).

Figure 8.2.5. Steer drafted into allocated supplement enclosure (a.), steer exiting enclosure via spear-gate with exit reader (b.), cattle consuming supplements (c.) and checking the accuracy of the walk-over-weighing (d.) at Stud paddock, Brunchilly (Source: S Quigley).

8.3 Appendix 3. Remote Livestock Management System

The following information was prepared by Tim Diver (Precision Pastoral, Pty Ltd).

Background of technology. Where it has come from and what it can do?
Precision Pastoral Pty Ltd has developed an innovative remote cattle management technology called RLMS. The RLMS emerged from research within the Desert Knowledge Cooperative Research Centre's 21st Century Pastoralism Project. The Northern Territory government was a core partner of the 21st Century Pastoralism Project. The RLMS consists of a combination of hardware and software (Figure 8.3.1.) capable of remotely identifying, weighing and drafting individual animals within a cattle herd without labour inputs. The RLMS is solar powered and can transmit data from a remote location to support timely and profitable management decision making within a beef enterprise. The RLMS has the capacity to increase beef enterprise production whilst reducing operating costs.

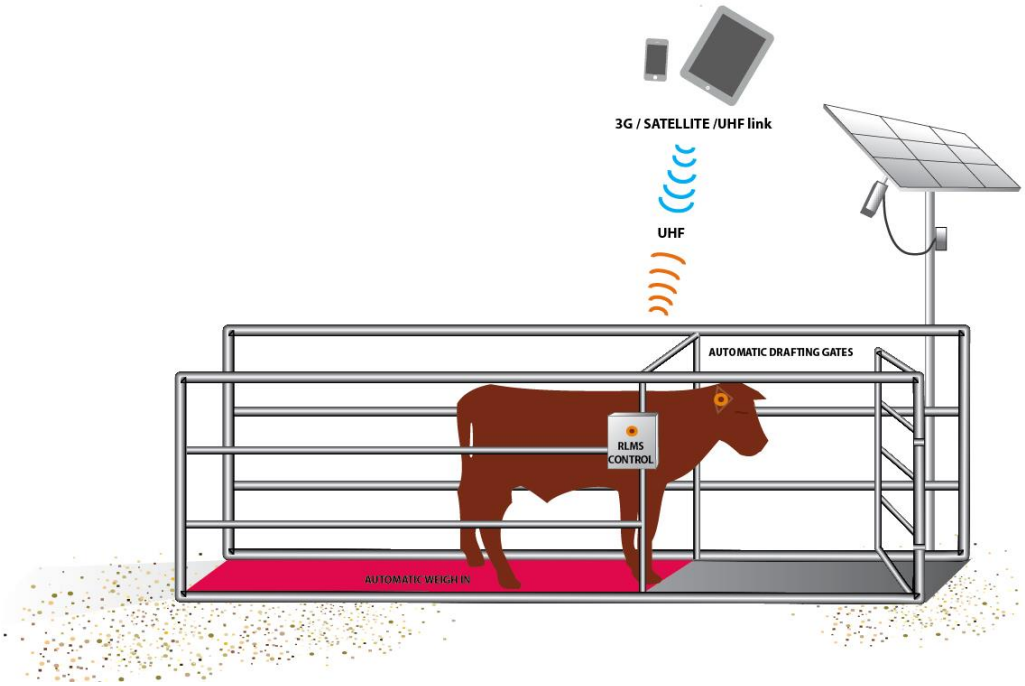


Figure 8.3.1. Diagram representing the components of the Remote Livestock Management System.

The technology is typically placed between cattle and watering points (Figure 8.3.2). As cattle leave watering points they walk over a platform which collects the animal's liveweight which is subsequently cross referenced with its ear tag (EID), simultaneously identifying the animal. This provides management with more accurate and frequent information about individual animals. Examples of field set-up of the RLMS are presented in Figure 8.3.3.

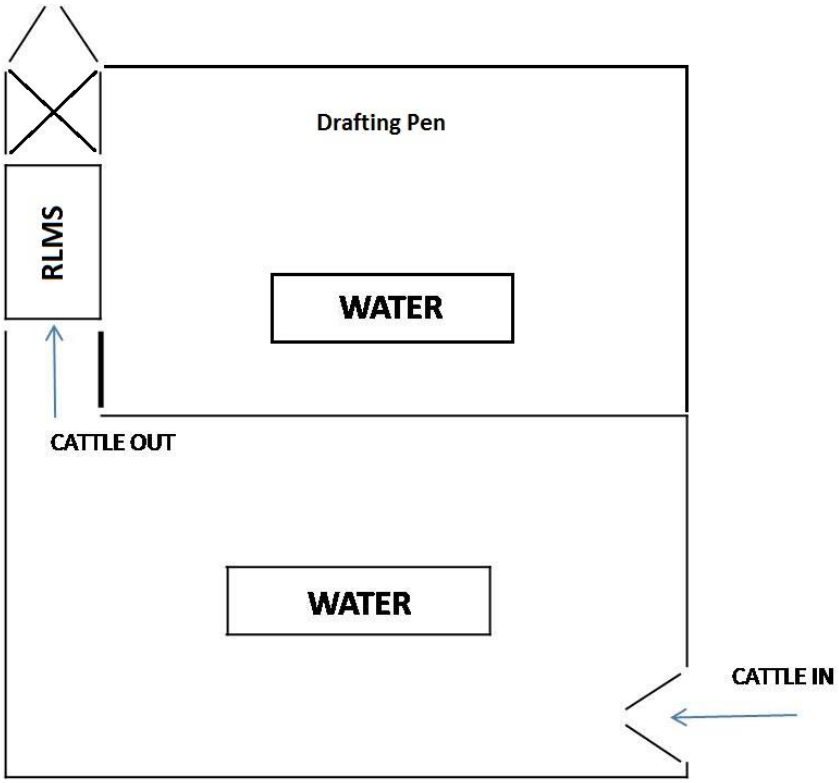


Figure 8.3.2. Typical placement of Remote Livestock Management System. *Note, the placement at Brunchilly was different to this with the Remote Livestock Management System situated at entry to water points to allow animals to be drafted to supplement enclosures.*

a.

b.

Figure 8.3.3. Remote Livestock Management System on site at No. 19 bore, Brunchilly during the training phase of the trial (a.) and at Newcastle Waters station (b.) Northern Territory.

Once the baseline information has been collected, indicators such as their weight, sex, health, breed and calving, all of which typically relies on visual inspections, can be more regularly and accurately assessed. This information is collected remotely and can be used to better optimise the timing of mustering and the selection of cattle for sale. Cattle meeting a preferred profile can be selected for mustering and can be automatically and remotely drafted into holding yards for collection, without the weight loss associated with the stress of mustering. A significant benefit of the RLMS technology is likely to be its use as a tool for producers to easily implement new management practises. If technology is used to implement new management practises the benefit will likely be greater, as there will also be the benefit of the new management practices.

Table 8.3.1. Potential benefits of the Remote Livestock Management System

Priority	Area of Impact	Specific information	Practice	Benefits
High	Animal Management: Sale Heifers/ Steers/ Cows	Performance recording: Tracking animals growth to improve sale weights through weighing frequently	Target specific markets most suited to animals being sold Mustering when 'the most' animals are making the weight	Higher prices per kg More efficient handling/transport costs per kg
High	Animal Management: Breeders	Timing of supplementation: Drafting individuals into supplement pen based on weight trajectory	Effective segregated supplementation based on time of calving or intended time of joining Spike feeding to bring on cycling Getting heifers up to joining weight	Reduced supplementation cost Increased calving % Increased calving %
High	Animal Management: Weaners/ Breeders	Automated weaning through drafting based on weight or ear tag identification	Wean group (truck load etc.) of calves based on weight/ age/ cow condition	Weaner specific paddock (high quality feed etc.) Weaner only supplement feeding Improved cow condition Increased cows cycling Increased calving %
High	Animal Management: Breeders	Performance recording: Time of calving	Using weight identify when Pregnancy Tested In Calf cows have calved	Increased herd efficiency through higher calving percentage
Medium	Animal Management: Breeders	Controlling mating (i.e. non-continuous mating)	Tighter calving pattern away avoiding summer births	Increased weaning % Targeted genetic improvement
Low	Land Condition Management: Improving pasture condition	Accurate grazing pressure (kg feed:kg cattle)	Using animal performance for indication of quality/ quantity of pasture	Maintaining/ improving land condition
Low	Land Condition Management: Improving pasture condition	Feral animal and non-production animal control	Exclude or trap non production animals from water	Increasing available feed for production animals

Low	Land Condition Management: Improving pasture and animal production	Alternate grazing strategies (rotational/ spell/ cell)	Drafting animals through alternate grazing system as they pass through RLMS	Reduced labour input for non-continuous grazing strategies

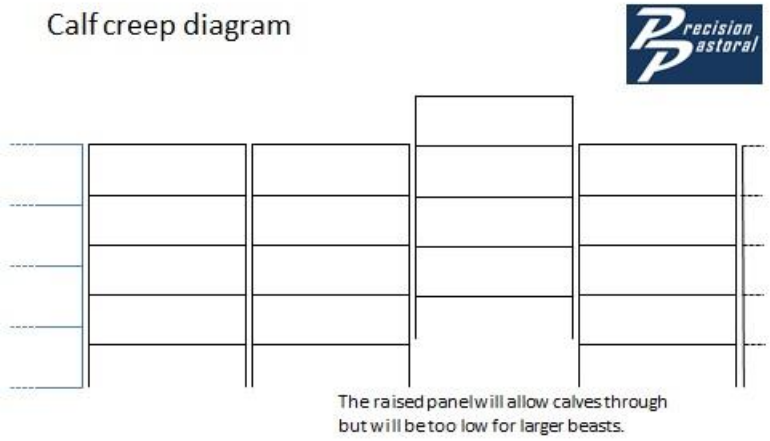
How involvement in the P trial was initiated and input into the design
The Phosphorus experiment at Brunchilly was dependent on the use of the RLMS to remove any paddock effects on response to imposed experimental treatments. Significantly, the work would not have been able to proceed without the technology being made available and supported by Precision Pastoral. Initial project design meetings occurred in September and October 2010 with the installation of the prototype RLMS undertaken in March 2011. Operating the RLMS at Brunchilly, in a commercial setting, over the extended period of time has allowed for refinement of the design to occur thus resulting in increased reliability.

Prior to the P Trial the RLMS utilised a pneumatic gate which was achieving an accuracy of 96% on all classes of cattle. Initially it was thought that smaller animals may have lower levels of draft accuracy due to their shorter length giving the gate less time to draft. When animals were not placed under any pressure this did not appear to pose any problems. Due to the high numbers of drafts that were anticipated at Brunchilly the gate powering system had to be significantly modified. The pneumatic system initially operated on commercially supplied oxygen cylinders which required changing every 3000 drafts. Over the entire trial at Brunchilly 228,000 drafts were performed. The modified gate system ran on solar power for the entire trial period (May-2011 to June-2013). The draft accuracy of the system during the trial, when not operating under a failure mode, was 95%. Given the prototypic nature of the RLMS at the time, particularly having regard to the modified gate powering system, Precision Pastoral undertook a large amount of the servicing at its own expense.

Operation of the RLMS during the trial
The RLMS became operational in May 2011 and collected 142,000 weights and performed 228,000 drafts over a period of 23 months.

To ensure that cattle were familiar with the use of the RLMS, and in order that animal welfare issues would not compromise the P Trial, a training period of 6 weeks was initiated during March/April 2011. The training procedure began with training cattle to use spear traps as the trial animals had never previously been exposed to spear traps. The animals quickly adapted to the spear trap use, with all of the animals trained to the spears within a two week period. The next step was to train the cattle to cross the weigh bridge without draft gate movement. This enabled the cattle to familiarise with the race structure they would be entering daily during the trial and took approximately one week for animals to adapt to the system. Once the cattle were comfortable with the walking through the weigh bridge the draft gate movement was introduced. During the training phase the gate moved every time an animal entered the RLMS with all animals ending up in the same location. This was performed over a two week period. A number of animals did not respond to the training and were transferred out of the trial. To ensure that no calves were excluded from water or isolated away from their mothers a calf creep was installed and displayed at Figure 8.3.4.

a.



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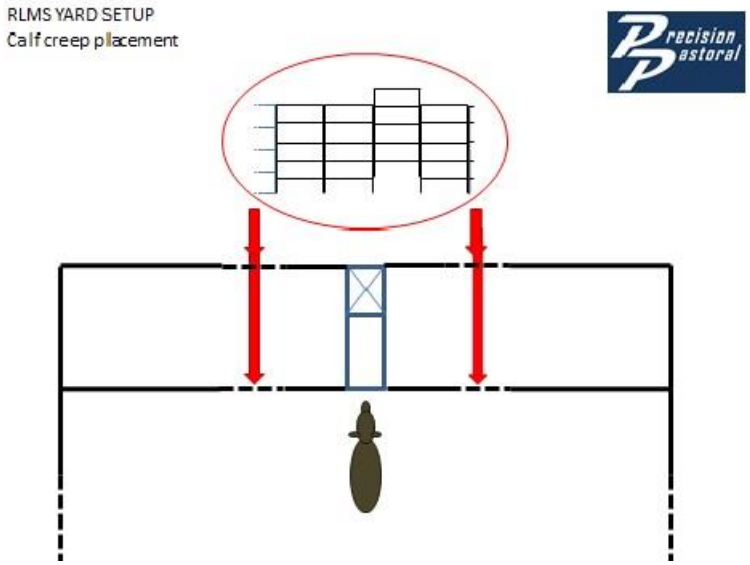


Figure 8.3.4. Calf creep design (a.) and placement (b.) to allow calf movement between both supplement enclosures and the paddock.

Operational issues experienced with the RLMS over the period of prototype deployment varied and were often a result of third party equipment not being suited to the harsh environment of the cattle yards. Problems with the electrical system were cables being chewed by cattle and cockatoos. The solution to this problem was to design cable protection and fence off sensitive areas from cattle with portable panels.

The use of third party equipment for the weighing functions of the RLMS resulted in some operational issues. Primarily these were restricted to the data logger failing to record a complete array of data, for example recording weights, time and dates but not recording every RFID, even when RFID reader was operating correctly. A load cell failure mode was identified and resulted in the under weighing of cattle, which got progressively worse as the project proceeded. This fault was not identified by either Precision Pastoral or research staff. Overall, it was evident that the third party equipment was not designed for continuous operation rather it has been designed for “crush side” operations. Failure mode windows varied from 3 weeks to 4 months. A change to the brand of RFID reader was implemented on the RLMS and resulted in a large improvement in reliability. Failure modes still occurred but were able to be repaired easily on site and were much less frequent.

Initially, draft inaccuracy was experienced whilst the required custom built solar system was being tested. The previous pneumatic gate RLMS was not suitable for use in the P trial due to the need for 600 - 700 head of cattle to be drafted on a daily basis. The majority of the draft inaccuracies occurred when the RFID reader failed to read the RFID tag resulting in the RLMS inability to draft cattle into the correct yard. Very few inaccuracies were caused by a failure in the custom built solar system.

Through the use of the RLMS in the Phosphorus experiment it has been shown that naïve herds unfamiliar with trapping can be trained to use the system in a relatively short timeframe. The Phosphorus experiment allowed the collection of critical failure mode information around the third party equipment, which prompted Precision Pastoral to seek further investment from industry and the Commonwealth Government (via the Commercialisation Australia Program) for the reengineering of the RLMS. The Phosphorus experiment provided evidence that the RLMS concept of remote weighing and drafting within large production systems was viable and highlighted potential benefits of the RLMS to producers. The results from the Phosphorus experiment when the RLMS was fully operational have provided Precision Pastoral with the confidence to continue investing in further development of the RLMS. The Phosphorus experiment also highlighted the need for dedicated RLMS management software for producers and researchers consisting of:

- a “day to day” management dash board (to be used at a 5 min glance) monitoring vital information such as RLMS operational indicators e.g. Battery voltage, draft gate working, weight and EID recording correctly; and
- basic “day to day” cattle production information such as weight trajectory and missing or present cattle.

It has been evident through the trial process that producers and researchers want more, in the sense that once they receive one report, they want something else added or another report entirely. This has highlighted the need for “goal posts” to be kept in place so the machine that was built to do the job remains the right machine. The Phosphorus experiment demonstrated how simple the accompanying infrastructure for weighing and drafting can be as within the P Trial a combination of portable panels and 4 barbwire fencing was utilised effectively.

Future opportunities for the use of the RLMS by industry and researchers.
Since the first deployment of the RLMS in 2011 continual development, testing and improvement has been taking place. The full commercially available system will be deployed in 2014. Improvements in available technologies have resulted in changes to the electrical control circuits by re-engineering failing components and improvements to the draft gate control module and mechanisms. Future opportunities exist for researchers to utilise the RLMS include determining water (or other attracting) visitation and gestation analyses. With the drafting module attached, drafting based on any combination of any metrics is possible, assuming a thorough and reliable database exists. Opportunities also exist for further research on multiple treatments vs. control group, social and behavioural analyses and production analyses. Major opportunities producers for the commercial beef industry that will result from this include the ability to:

- target specific markets most suited to animals being sold
- muster when ‘the most’ animals are at the optimum weight or weight demanded by the target market
- wean groups (truck load etc.) of calves based on liveweight, age or cow body condition.

8.4 Appendix 4. Concentration of P in blood

Background
Despite a perception that logistical issues exist around the collection of blood under field conditions (including the timing of collection in relation to season and feeding/curfews, technical skills required to collect a viable sample, the number of samples required to be representative of a mob of cattle, processing of samples on site and transport of samples to a laboratory for testing) the concentration of inorganic P in the circulation does appear to be the most reliable indicator of P intake, and hence the requirement for P supplementation, in growing cattle. While previous workers have examined sample site and blood fraction differences (Teleni *et al*., 1976), time after feeding (Monteil *et al*., 2007) and level of excitation on the concentration of inorganic P in plasma these experiments were conducted on cattle fed the same diet. It is unknown if these relationships exist when growing cattle are fed diets that differ markedly in P content. If circulating inorganic P is to be recommended as a method to assess the likely response of cattle to P supplements in a paddock then a uniform sampling and testing methodology needs to be developed.

Materials and methods
Bos indicus crossbred steers were fed increasing amounts of P in a diet that was comparable in CP and DMD, as described in the detailed materials and methods section of the main report. On day 172 of the experiment blood samples were collected from the jugular and coccygeal veins of each steer into separate lithium heparin vacutainers; an additional blood sample was collected from the jugular vein of each steer into a SST^{TM} (clot activator and serum separator) vacutainer. Samples collected into lithium heparin tubes were inverted 6 to 8 times and placed on ice, samples collected into the SST tubes were inverted 5 to 6 times and sat at room temperature for 30 to 60 min. Samples were centrifuged at 2250 *g* for 10 min with plasma or serum stored at -80^{o}C for subsequent analysis. Samples were given subjective visual haemolysis scores of 0 (none), 1 (minor), 2 (marked) or 3 (severe). The inorganic P concentration in plasma and serum was determined on an Olympus AU400 auto-analyzer (Beckman Coulter) using Beckman Coulter inorganic phosphorus reagents.

A similar activity was undertaken at the end of dry season 1 in Experiment 2, where blood samples were collected from the jugular vein into lithium heparin and SST^{TM} tubes and from the coccygeal vein into SST^{TM} tubes of the 32 No. 2 steers that were included in the experiment at that time.

Results
Minor and marked haemolysis was observed in 4 and 8 of the 30 samples collected from the coccygeal vein respectively in Experiment 1; no haemolysis was observed in either the plasma or serum samples collected from the jugular vein. In Experiment 1, haemolysis in tail plasma samples had little effect on measured plasma inorganic P concentration (Figure 8.4.1); there appears to be one sample with minor haemolysis that has affected the overall relationship.

There were linear relationships between serum and plasma inorganic P collected from the same site and between plasma inorganic P collected from the jugular and coccygeal vein of steers fed diets with a large difference in P content in Experiment 1 (Figure 8.4.2). The concentration of inorganic P in the serum was approximately 7% lower than the concentration of inorganic P in the plasma across a wide range of P intakes in Experiment 1. The concentration of inorganic P in plasma collected from the coccygeal vein was approximately 6% higher than the concentration of plasma collected from the jugular vein in Experiment 1. In Experiment 2, the concentration of P in the serum was 14% lower than that in the plasma in samples collected from the jugular vein. The concentration of P in serum collected from the coccygeal vein was 5% higher than in the serum collected from the jugular vein of the same steers. It should be noted that there was more variability in the data from Experiment 2, with R^2 of 0.68 (serum v plasma) and 0.62 (jugular v coccygeal).

Conclusions

Good relationships between blood components and sample collection sites were evident across a wide range of P intakes. The most important aspects in the use of circulating inorganic P as an indicator of P intake are likely to be consistency with sample collection procedure and good operator technique. The procedure used to collect samples will vary depending on the conditions. Under field conditions, collection of samples from the coccygeal vein will be easier than from the jugular vein. The use of SST clot activator tubes does influence the measurement of inorganic P but does allow for easier processing in the field. Despite the lack of effect of haemolysis on inorganic P concentration reported in these studies, it is likely that severe haemolysis will elevate inorganic P concentration and that good operator technique will minimise the risk of false high concentration.

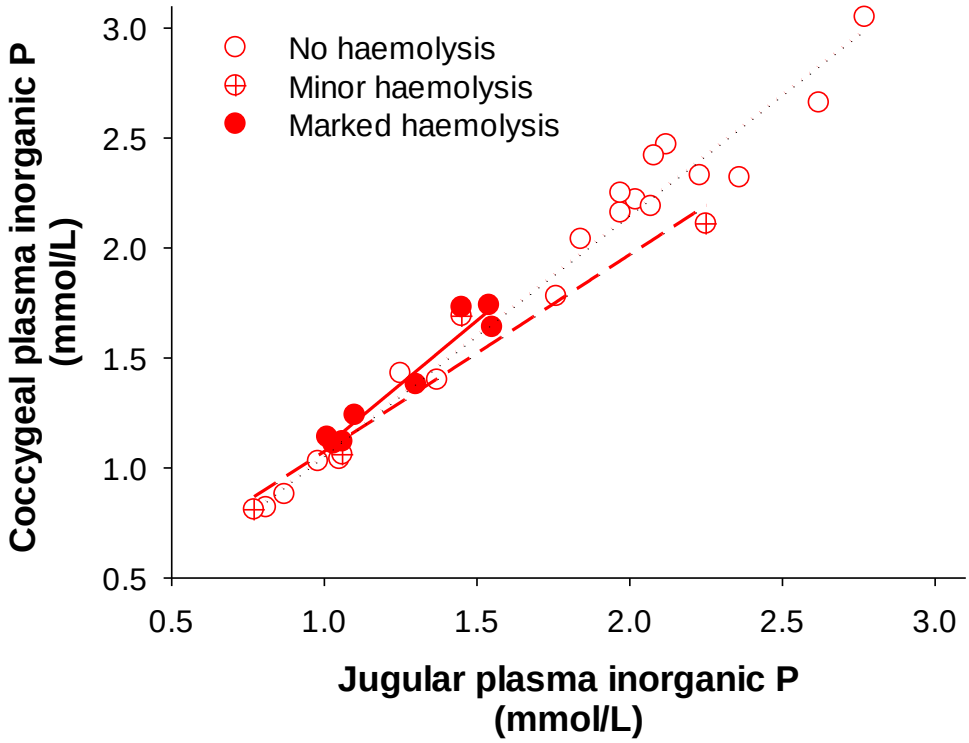
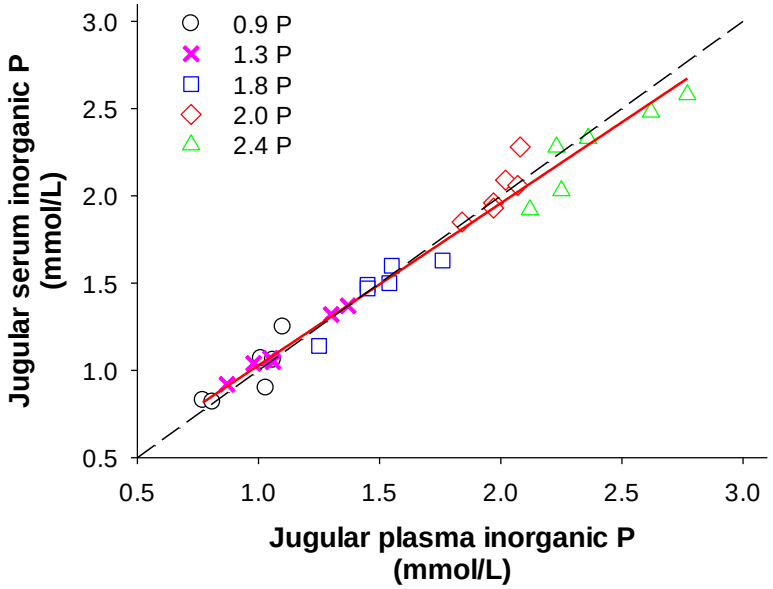


Figure 8.4.1. The effect of haemolysis (**….**, none; **---** minor; —— marked) on the relationship between inorganic phosphorus (P) concentration in plasma collected from the jugular and coccygeal veins of steers consuming increasing amounts of P.

a.



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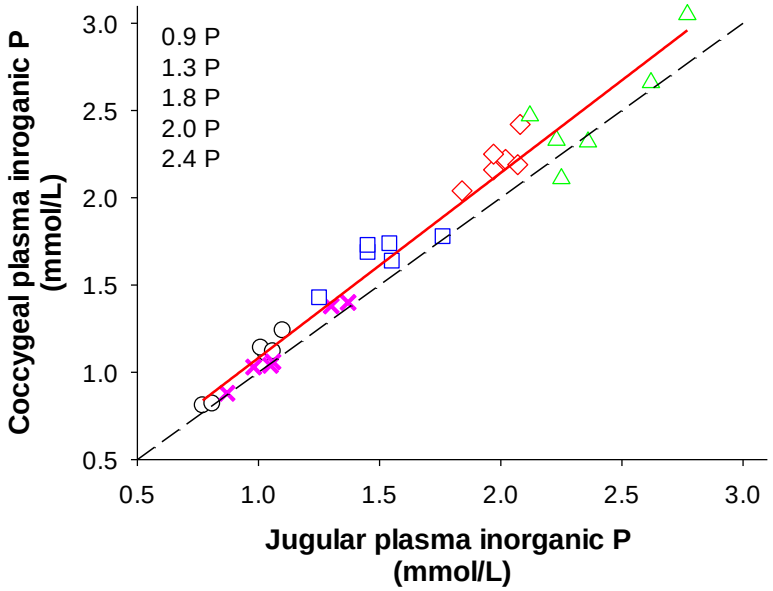


Figure 8.4.2. The relationship between plasma and serum inorganic phosphorus (P) concentration collected from the jugular vein (a.; y=0.93x+0.10, R^2=0.97) and the relationship between plasma inorganic P concentration collected from the jugular and coccygeal veins (b.; y=1.06x+0.02, R^2=0.97) of steers feed diets with increasing P content. *Solid line is observed X-Y relationship; dashed line is parity relationship.*

8.5 Appendix 5. In vitro solubility of various P supplements

Background

The importance of determining the P requirements of cattle and dietary P content are well known and are demonstrated in the pen experiment conducted within this project. Producers in P deficient country are recommended to offer P supplements to cattle over the wet season, when P is the first limiting nutrient. Global P supplies are decreasing and costs are increasing, hence there is a requirement to accurately determine the P requirements of animals and the P availability of supplements. The solubility of P determined *in vitro* is often used as an indicator of P availability and while this is not always reflected in vivo, in vitro P solubility tests do provide a rapid means of comparing the potential availability of P in various supplements. The existing methods used to determine P solubility were developed for fertilisers, where the pH does not reflect that of the abomasum of ruminants (pH 2 to 3) after which P absorption occurs, therefore more acidic conditions may be required to reflect *in vivo* conditions in ruminants. The average retention time of digesta in the abomasum is 20-30 minutes in cattle and this should also be considered when trying to determine the availability of P for ruminants under *in vitro* conditions. The objectives of this study were to evaluate 1) various *in vitro* conditions to determine the solubility of P supplements specific for ruminants and 2) the P solubility of various supplements available for use by the beef cattle industry.

Materials and methods

Phosphorus content and solubility of 25 P sources were determined. The sources were classified as monosodium phosphate (MSP, 25.7% P; n=1), monodicalcium phosphate (MDCP, 21.2 ± 0.2% P; n=9), monocalcium phosphate (MCP, 23.1% P; n=1), dicalcium phosphate (DCP, 19.9 ± 0.4% P; n=6), tricalcium phosphate (TCP, 19.0 ± 1.5% P; n=2) and rock phosphate (RP, 10.8 ± 1.4% P; n=6). Phosphorus solubility of the 25 sources was determined after incubation in water, buffered 0.1M HCl (pH 2.5), 2% citric acid or ammonia citrate at 39^{o}C for 30 or 120 minutes. Phosphorus content was determined after incubation using the Molybdovanadophosphate method (AOAC 958.01) with absorbance measured at 400 nm (Shimadzu UV-1201, Japan). P solubility was expressed relative to total P (%). The effects of P source, solubility method and incubation time were assessed by one-way analysis of variance procedures using the MINITAB software.

Results

Across both times and all P sources, solubility of P was significantly higher ($P<0.05$) for the CA (74.9 ± 4.8%) and HCl (75.2 ± 4.3%) methods than the AC (43.5 ± 5.0%) and H2O (37.6 ± 5.6%) methods, with no significant difference between the CA and HCl methods (Table 8.5.1). Solubility of P was greater in CA and HCl for all P sources evaluated, with the exception of MSP which was completely soluble in all incubation media. There was no difference in solubility between the CA and HCl methods used in this study. Maximum solubility of P in CA and HCl media was achieved after 30 min incubation for MSP, MDCP, MCP and DCP but increased between 30 and 120 min for TCP and RP. Solubility of P in AC and H2O media increased with time for MDCP, MCP and DCP but this was comparable to the solubility achieved after 30 min incubation in CA and HCl. Rock phosphates and TCP were insoluble in AC and H2O, regardless of incubation time. After incubation in CA for 30 minutes there was no difference in solubility between MSP, MDCP, MCP and DCP sources. There was a large difference in P solubility of the two TCP sources (22 and 54%). The RP supplements were lowest in P solubility (1.0 to 27.3%). There was considerable variation in solubility within the DCP and TCP sources studied. The range in solubility of DCP sources in CA was 67.5 to 100% after 30 min incubation, and 94 to 100% after 120 min incubation.

Table 8.5.1. Solubility of P in mono-sodium phosphate (MSP), mono-di-calcium phosphates (MDCP), mono-calcium phosphate (MCP), di-calcium phosphates (DCP), tri-calcium phosphates (TCP) and rock phosphates (RP) incubated in ammonia-citrate (AC), citric acid (CA), water (H2O) or hydrochloric acid (HCl) for 30 or 120 minutes

Source	30 minutes incubation				120 minutes incubation			
	AC	CA	H2O	HCl	AC	CA	H2O	HCl
MSP	100.0	99.1	99.6	98.2	97.7	99.9	100	99.9
MDCP	54.6±2.5	95.7±1.6	76.9±2.6	91.6±2.1	86.7±4.3	97.2±1.0	78.8±2.7	94.2±1.5
MCP	42.9	96.4	80.4	88.8	77.1	98.9	83.8	97.2
DCP	37.7±6.1	92.0±5.2	9.4±5.7	91.9±2.4	60.2±4.2	97.3±0.8	9.8±0.1	97.1±1.4
TCP	0.0±0.0	37.9±15.9	0.0±0.0	34.2±6.3	0.0±0.0	65.4±15.5	0.2±0.1	57.1±10.6
RP	0.0±0.0	17.1±5.1	0.2±0.1	28.1±8.5	0.0±0.0	28.7±7.8	0.1±0.0	36.7±10.4

Conclusions

At a practical level MSP, MDCP, MCP, DCP are very similar in P availability for ruminants. Phosphorus content and price should be considered when determining which is the most suitable P supplement to be used for cattle. Untreated rock phosphates were very low in P solubility and are unlikely to be useful P supplements. The acidic *in vitro* based tests (HCl and CA) were most appropriate to determine P availability for ruminants but there was no advantage in replacing the established CA method with the new HCl method.

Acknowledgements

This work was submitted as a one-page paper at the Northern Beef Research Updates Conference, Cairns (August, 2013). This work was conducted as a small research project in a course-work Master's degree and was funded by The University of Queensland.

8.6 Appendix 6. Validation of laboratory results

During the course of this project, and other P related work, issues were raised over the reliability of results from various laboratories. These issues were not raised by members of the team of researchers involved in this project but by others who had experience with these laboratories in the past or who had reviewed results from these laboratories as this project was in progress. As such several tests were undertaken to validate the reliability of the laboratories used in this project.

We would consider the below results confidential at this stage as they have not been shared with the collaborating laboratories and request that they are not circulated any wider than MLA and the project team.

Validation 1. Inter-assay variation in faecal NIR and faecal P analysis at commercial laboratories

Background
Issues had been raised regarding the reliability of results from faecal NIR analysis conducted at commercial laboratories. It is not clear to the team involved in this project what those issues were or whether the issues were with the analytical process or the interpretation of data.

Materials and methods
Twenty-four faecal samples collected from individual cattle in the experiment conducted at Brunchilly were submitted to one commercial laboratory for faecal NIR and faecal P analysis. The samples were initially submitted unground in April 2013 and then retrieved after analysis. The same 24 ground samples were re-submitted to the same laboratory in August 2013 for faecal NIR and faecal P analysis. Samples were scanned and results reported for CP (diet crude protein), Delta-13C, ESTVIVO (dry matter digestibility), FECASH10 (faecal ash), FECN2 (faecal nitrogen), MEIMEAN (ME intake), MEINTAKE (ME intake/100 kg of liveweight) and mg P/kg, with MJ of ME estimated from ESTVIVO and mg P/MJ of ME calculated.

Results
Repeatability of assays between the two sample submissions was generally good (Figure 8.6.1). Estimated CP content had the highest inter-assay co-efficient of variation (Table 8.6.1) and was lower when the second measurements were conducted (in August compared to the measurements conducted in April). All other parameters had an inter-assay CV of less than 10% with most less than 6%.

Conclusions
Variability between the two assays was low, with the exception of the prediction for CP. These results indicate that the faecal NIR predictions of diet quality conducted by the commercial laboratory are repeatable between assays. It should be noted that assay repeatability is in no way an indicator of assay validity, it simply reflects the consistency with which the analysis is conducted (in this case analysis of the same samples on different dates).

Table 8.6.1. Inter-assay co-efficient of variation (CV) for a range of samples analysed by faecal NIR and for faecal phosphorus for predictions if diet quality (results were reported on an as received basis)

Parameter	Inter-assay CV%
CP	12.1
Delta-13C	2.7
ESTVIVO	1.5
FECASH10	4.6
FECN2	5.5
MEIMEAN	6.9
MEINTAKE	3.9
mg P/kg	6.5
MJ ME	1.8
mg P/MJ ME	6.0

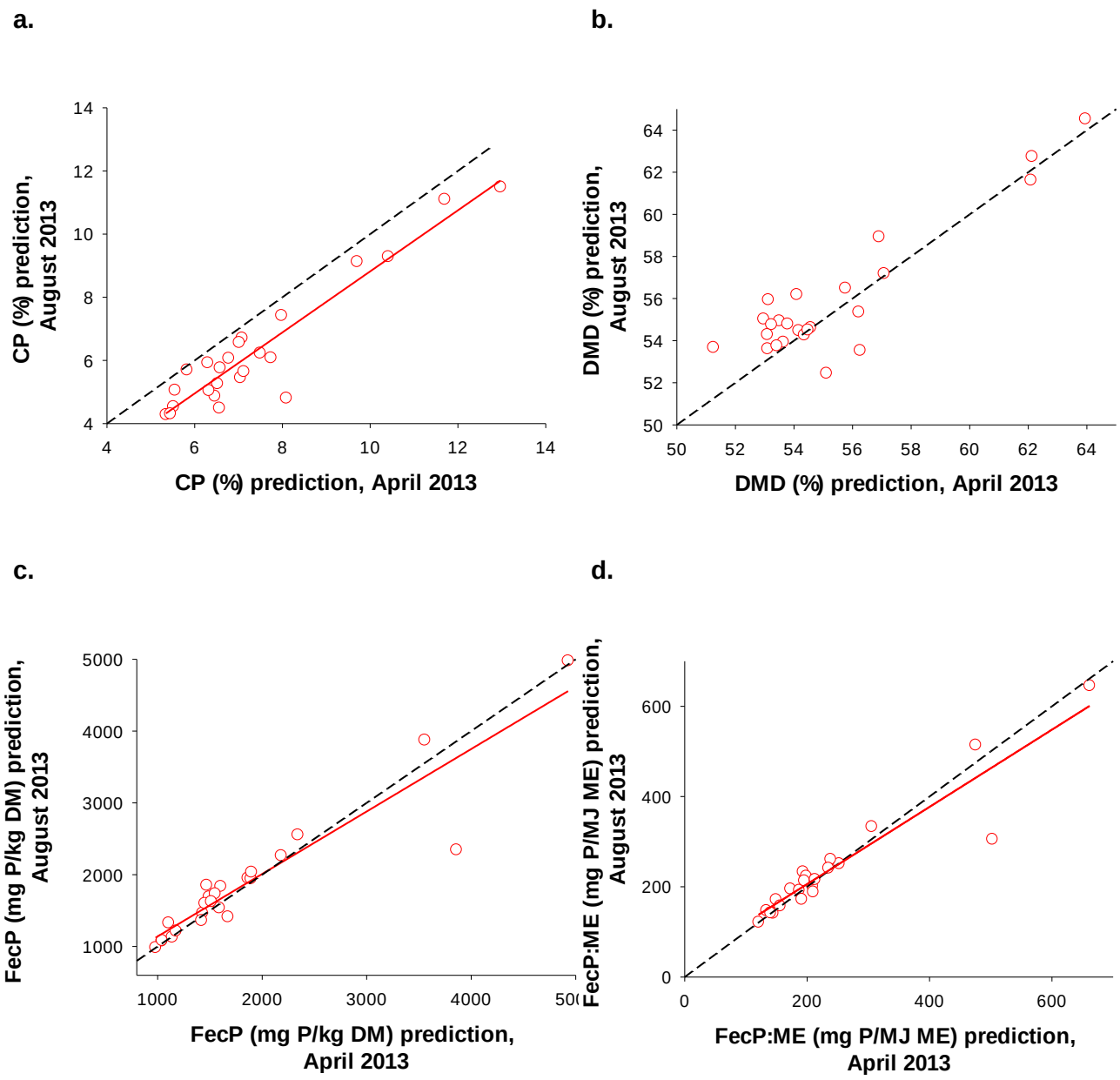


Figure 8.6.1. Comparison of faecal NIRS predictions for dietary crude protein (CP; a.), dry matter digestibility (DMD; b.), faecal phosphorus (FecP; c.) and FecP:metabolisable energy (FecP:ME; d.) of the same 20 samples conducted at the same commercial laboratory on two separate occasions (April and August 2013). *Solid line is observed X-Y relationship; dashed line is parity relationship.*

Validation 2. Faecal P ring-test

Background
During the review of the MLA funded 'Cashcow' project the reviewers raised concerns over the faecal P data reported by the Central Analytical Laboratory (CAL) at the University of Queensland. Other researchers had also previously raised issues regarding an apparent over-estimation of P content of various samples. The CAL had addressed this issue in the past but nonetheless a ring-test was conducted with CAL using its revised method for determining P concentration.

Materials and methods
Twenty separate dried, ground faecal samples of variable P content were thoroughly mixed and four sub-samples taken from each faecal sample. The sub-samples were dispatched to one of three commercial laboratories (Symbio, Eight Miles Plains, QLD; APAL, Magill, SA; Waite Analytical Service, Waite, SA) and CAL (UQ, Gatton, QLD) for determination of P concentration. All laboratories used an ICPS to determine P concentration after an acid digest.

Results
Two of the laboratories (A and B) reported results on a DM basis, while two laboratories (C and D) reported results on an as received basis. The reporting of results on an as received basis by laboratories assumes that all materials are dried to a common DM content. This may be a fair assumption for grains and hays and for research samples that are dried in dehydrators to a constant weight (e.g. faecal samples collected at Brunchilly) but for faeces and fresh grasses the DM content may be highly variable, especially when sun drying is used. For the comparison of laboratories described here all results are corrected for DM content, as determined at CAL by drying samples at 105^{o}C for 16 h. After correcting for DM content of the faecal samples there was good agreement (R^2=0.99) between laboratories in the reporting of faecal P concentration (Table 8.6.2).

Conclusion
CAL at UQ produced faecal P results which were comparable to the other three laboratories. We cannot see any issues with the faecal P results generated by any of the laboratories once they are corrected for DM content of the sample analysed.

Table 8.6.2. Dry matter (DM) content and phosphorus (P) concentration of twenty faecal samples submitted to four different laboratories (A, B, C, D)

Sample	DM	A	B	C	C (DM corrected)	D	D (DM corrected)
	%	mg P/kg DM	mg P/kg DM	mg P/kg	mg P/kg DM	mg P/kg	mg P/kg DM
1	94.2	3.18	3.20	2.90	3.08	2.96	3.14
2	94.4	3.99	3.80	3.20	3.39	3.49	3.70
3	93.7	6.59	6.50	5.70	6.09	5.65	6.03
4	93.0	3.95	3.80	3.30	3.55	3.40	3.66
5	95.2	7.24	7.20	6.90	7.25	6.45	6.77
6	93.4	7.34	7.20	6.50	6.96	6.30	6.75
7	93.7	7.39	7.30	6.70	7.15	6.28	6.70
8	94.4	4.38	4.40	3.90	4.13	3.82	4.05
9	95.2	5.81	5.80	5.40	5.67	5.19	5.45
10	95.8	2.06	2.10	1.80	1.88	1.90	1.98
11	95.9	1.56	1.69	1.40	1.46	1.47	1.54
12	97.0	1.19	1.30	1.20	1.24	1.15	1.18
13	95.6	5.00	5.30	4.80	5.02	4.78	5.00
14	95.4	6.97	7.20	6.20	6.50	6.38	6.69
15	95.3	9.35	9.10	8.80	9.23	8.49	8.91
16	97.0	4.40	4.70	4.20	4.33	4.23	4.36
17	94.2	5.04	5.40	4.80	5.09	4.76	5.05
18	94.7	6.05	6.00	5.60	5.91	5.19	5.47
19	94.9	1.34	1.35	1.20	1.26	1.15	1.21
20	93.5	1.81	1.87	1.70	1.82	1.66	1.78

Validation 3. Dry matter content of samples submitted to a commercial laboratory for faecal NIR and faecal P analysis

Background
In light of the issue of some laboratories reporting faecal P results on an as received basis a test was conducted to validate the influence of DM content on faecal P and faecal NIR results. The following instructions are provided by a commercial laboratory to producers for the drying of faecal samples to be submitted for faecal NIR testing in their laboratory,

DRYING

Oven drying:
Samples can be oven dried at 60-65^{o}C. The samples should be broken up during drying to hasten the process.

Sun drying:
1. The faecal sample to be dried should be placed on a piece of clean, flat galvanised iron sheet or other non-absorbent sheet in a sunny position.
2. The sample should be spread out like pancake to a thickness of about 10 mm or less.
3. After about 4 hours in the sun, the sample should be turned over using an old egg slice. Try to keep the sample in one piece.
4. After another 4 hours the sample should be dry enough to break up and place in the sample jar provided.
5. If conditions are windy, cover samples with chicken wire to prevent the loss of material

Materials and methods
Faecal grab samples were collected from twelve steers, bulked and mixed thoroughly. Four sub-samples were dried by one of the following methods
- In a drying oven at 65^{o}C until a constant weight was achieved (**65C**)
- Sundried for 12 h (note, this sample was originally dried for 8 h but the DM content was below 50%, so it was dried for an additional 8 h) (**SD12**)
- Sundried for 48 h (**SD48**)
- Sundried for 96 h (**SD96**)

Samples were then crumbled and submitted to a commercial laboratory in zip-lock bags. Residual DM content of the samples was determined by drying at 105^{o}C for 24 h.

Results
The different drying approaches employed resulted in four samples with variable DM content (Table 8.6.3). Despite the commercial laboratory stating on their instructions for sample submission that 'Wet samples are not suitable for testing', the SD12 sample was analysed with results reported on an as received basis with no reference to the sample made in the results reported or no indication if the sample was further dried by the commercial laboratory upon receipt of the sample by them. The DM content of the samples analysed has influenced the wet chemistry faecal P assay. The faecal NIR predictions of diet quality (DCP and ESTVIVO) appear to be less influenced by the DM content of the sample submitted. Although it is assumed that the calibration equations used for the predictions are based on faecal samples dried to a constant DM content to minimise the interference of water content of the sample on the NIRS signal. The use of the faecal P results on either an as received or on a DM corrected basis gave very different FecP:ME results, resulting in a very different interpretation which may influence the decision of producers to provide supplementary P to their cattle or not. It should be noted that the results are somewhat different to our expectation and that they were only done with four samples and with no replication. This issue warrants further investigation.

Table 8.6.3. Phosphorus (P) concentration in faeces (FecP) and the ratio of FecP to estimated dietary metabolisable energy (ME) ratio (FecP:ME) in faecal samples of variable dry matter (DM) content (65C, dried to constant weight in a 65°C oven; SD12, sundried for 12 h; SD48, sundried for 48 h; SD96, sundried for 96 h)

Sample	Residual DM %	FecP (as received) mg P/kg	FecP (DM corrected) mg P/kg DM	CP %	ESTVIVO %	FecP:ME[1] (as received) mg P/MJ	FecP:ME[2] (DM corrected) mg P/MJ
65C	96.0	3048	3175	5.57	51.5	426	444
SD12	60.6	3527	5820	6.24	50.3	508	839
SD48	89.3	4301	4816	6.45	52.8	583	653
SD96	94.9	3663	3860	6.33	50.7	522	550

[1]FecP:ME calculated using mg P/kg (reported on as received basis).
[2]FecP:ME calculated using mg P/kg DM (reported after corrected for residual DM).

Conclusions

Results generated from wet chemistry procedures, such as the concentration of P in the faeces, should be reported on a DM basis rather than an as received basis. Further evaluation of the effect of DM content of samples submitted for faecal NIR and faecal P on results and interpretations is warranted to make recommendations to commercial laboratories regarding the reporting of results on an as received or DM basis. It is recommended that further validation of this issue be conducted with a much larger number of samples over a range of DM content. This may improve the reliability of analytical procedures and the accuracy of the interpretation of the results. This issue is unlikely to be of importance for research, where relative differences between treatments are of importance and most likely the samples analysed will be processed under similar conditions (most likely dried to a constant weight in a dehydrator). Of more concern are the implications for industry where only a few samples might be processed under less controlled conditions and it is the absolute value of the results that is of importance. This is where over- or under-estimates of P content may influence interpretation of results and recommendations made to producers.

Validation 4. Comparison of faecal NIR conducted at three separate laboratories

Background
Issues had been raised regarding the reliability of results from faecal NIR analysis conducted at commercial laboratories. It is not clear to the team involved in this project what those issues were or whether the issues were with the analytical process or the interpretation of data.

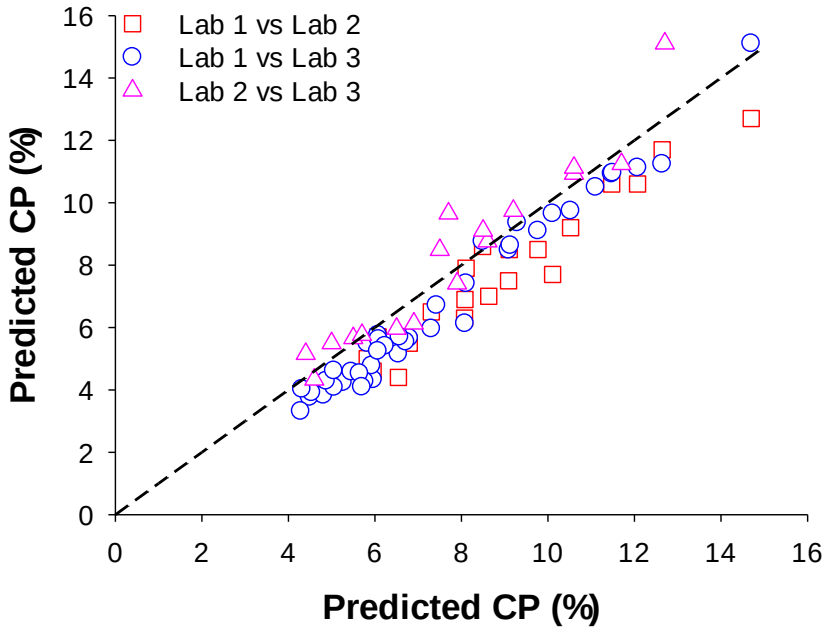
Materials and methods
Twenty faecal samples collected as part of the 'Cashcow' project were analysed for faecal NIRS by Symbio, QDPI and UQ (formerly QDPI machine]. An additional 24 samples from Experiment 2 at Brunchilly were analysed for faecal NIRS using the Symbio and UQ machines. Data comparisons were made between laboratories for CP, DMD, Non-grass, FecN, FecP:ME and MEINTAKE.

Results
There were good relationships between all three laboratories for predictions of CP and DMD (Figure 8.6.2). Relationships between laboratories for non-grass [(deltaC-13.5)*7.14] and FecN were not as good as those for CP and DMD, with laboratory 1 consistently predicting higher and lower values than the other two laboratories for FecN and non-grass respectively; there was good agreement between laboratories 2 and 3 (Figure 8.6.3). There was also good agreement between all laboratories for ME intake and FecP:ME (Figure 8.6.4).

Conclusions
We conclude that for the key indicators of diet quality on which producers would make management decisions (CP, DMD, MEINTAKE, FecP:ME) there is good agreement between laboratories/machines. There does appear to be an issue with the FecN and non-grass predictions in one of the laboratories. Based on the results here we see no issues with the predictions generated by Symbio for the important indicators of diet quality listed above. We cannot comment on the validity of the technique itself but simply on the repeatability of the technique between laboratories. Similarly, we cannot comment on the interpretations and recommendations that are subsequently made and provided to producers based on this data.

Acknowledgements
We acknowledge Prof. Michael McGowan, Dave Smith and Kieren McCosker for access to faecal samples and data that were generated in the MLA funded 'Cashcow' project.

a.



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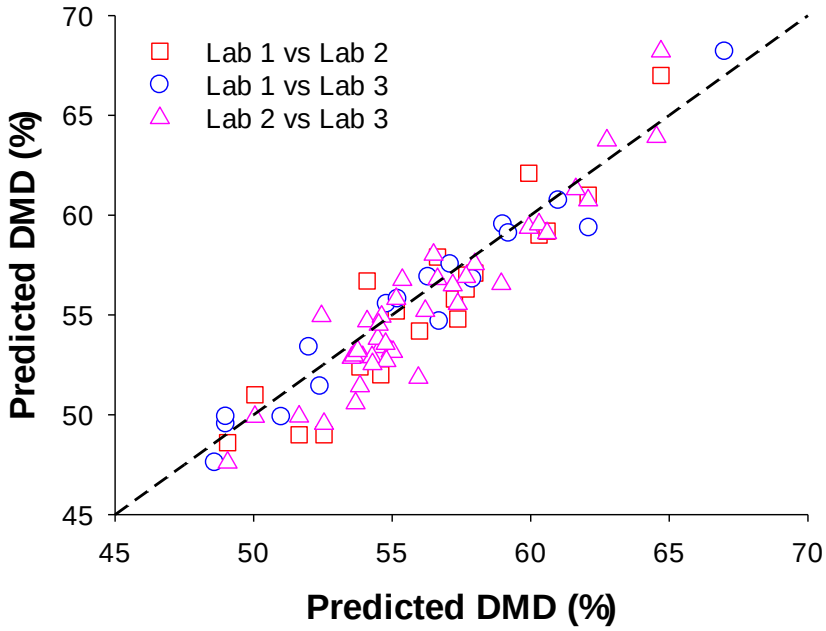
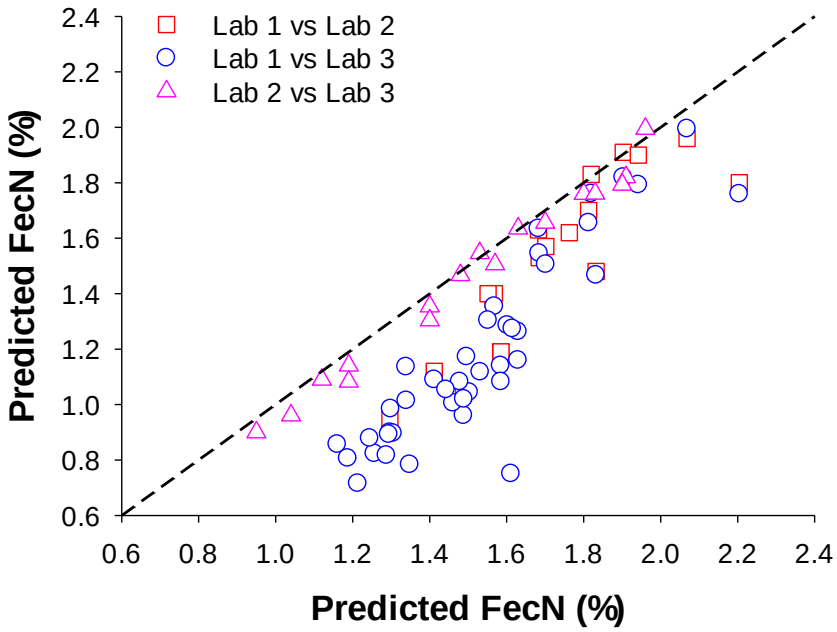


Figure 8.6.2. Comparison between three laboratories in the prediction of dietary crude protein (CP; a.) and dry matter digestibility (DMD; b.) by faecal NIRS. *Dashed line is parity relationship.*

a.



b.

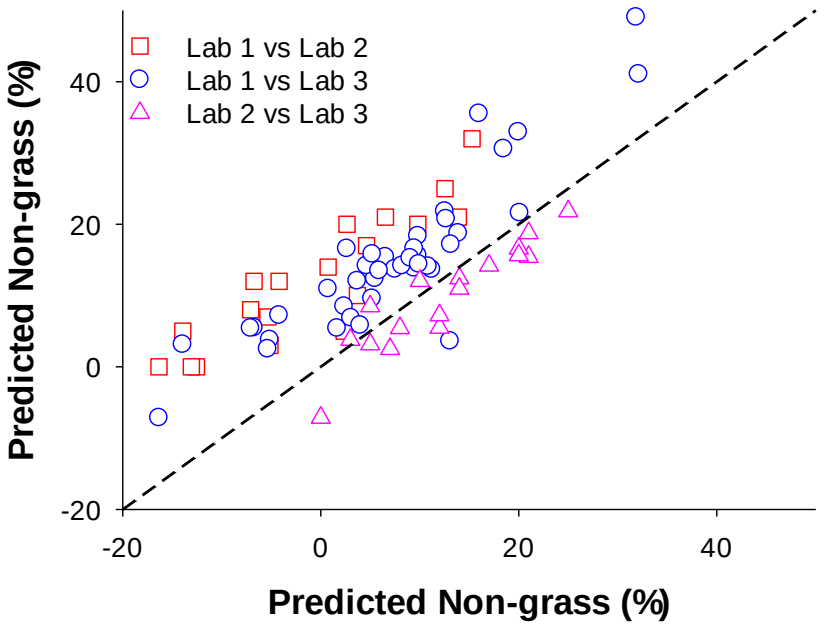
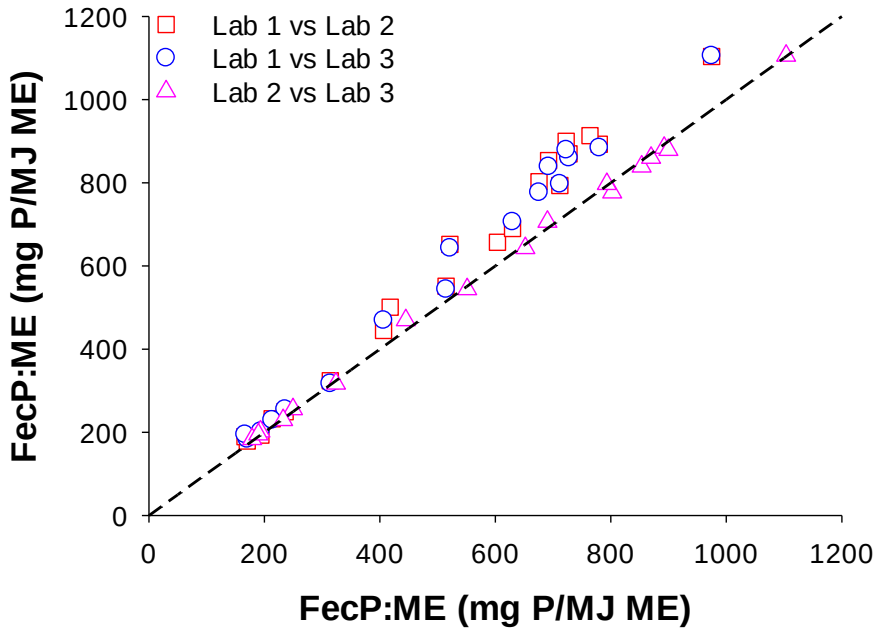


Figure 8.6.3. Comparison between three laboratories in the prediction of faecal N (FecN; a.) and non-grass (b.) by faecal NIRS. *Dashed line is parity relationship.*

a.



b.

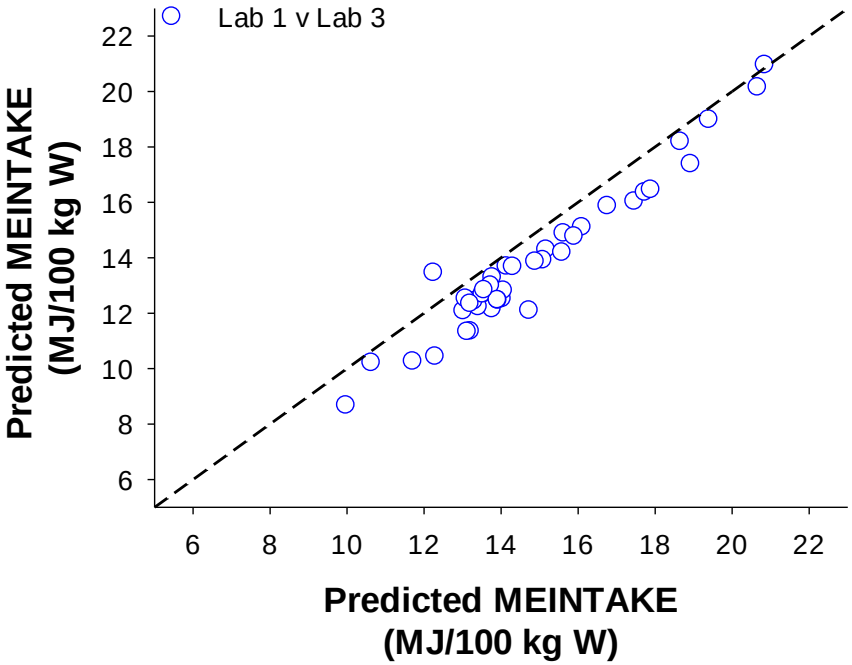


Figure 8.6.4. Comparison between laboratories in the prediction of faecal P:ME (FecP:ME; a.) and ME intake (b.) by faecal NIRS. *Faecal P concentration used in FecP:ME for Lab 2 was measured by Lab 3. No predictions for MEINTAKE were available from Lab 2. Dashed line is parity relationship.*

8.7 Appendix 7. Re-alimentation of P deficient steers

Background
At the conclusion of Experiment 1 of this project an extension was granted by MLA (Project BNBP.0565) to examine re-alimentation of the steers when fed a high P diet (*Phase 2*). The results from that study are published as a final report submitted to MLA. A summary of data presented here shows the response of animals undergoing depletion (Experiment 1; *Phase 1*) and repletion (*Phase 2*) of P.

Materials and methods
Upon completion of Experiment 1, described within this report, all steers were introduced to the P-1 pellet (106 g CP, 914 g OM, 400 g NDF, 270 g ADF and 1 g P/kg DM) and a supplementary P source [Biofos, 21% P; and monosodium phosphate (MSP), 24% P, were both used in the experiment] over two weeks, to allow recovery from the biopsies and adaptation to the new feeding regimen. *In vivo* DMD of the P-1 pellet used in *Phase 2* was determined in *Phase 1* of the experiment and was assumed to be the same (63% DMD) as the same batch of pellets were used in both phases of the experiment. Steers were then fed the P-1 pellet *ad libitum* (previous days intake + 1 kg), with MSP/Biofos offered at 6 or 7 g/kg DM, respectively, to supply a dietary P content of approximately 0.25% P/kg DM intake (*Phase 2*). The supplementary P source was mixed in equal proportion with sugar (w/w) to improve palatability and encourage consumption of the total allocation of supplementary P and was fed before the pellets each morning. Steers remained in individual pens for five weeks, with intake measured daily, and were then moved to feedlot pens (n=5/pen) with each pen containing one of the original animal blocks, such that steers of each of the original five P dietary treatments (0.9P, 1.3P, 1.8P, 2.0P and 2.4P) were represented in each feedlot pen. In the feedlot pens, the P supplement was offered first each morning (at the same rate as in the individual pens) and was spread out along the feed trough, allowing access by all steers, with pellets offered *ad libitum* (previous days intake + approximately 5 kg) after the daily allowance of P supplement was consumed. After a total of 12 weeks, steers were slaughtered (Marcelford Meats, Churchill Abattoir; Churchill, QLD) after an overnight curfew, with carcass measurements and Meat Standards Australia grading conducted.

Results
There were immediate (within one week) responses of DM intake and LWG to a change from a low P diet (0.9P) to a high P diet (2.5 g P/kg DM) (Figures 8.7.1 and 8.7.2). Steers that were fed the 0.9P treatment in *Phase 1* showed some compensatory LWG in *Phase 2*. Although they never fully compensated in LW this was due to the shorter duration of *Phase 2* compared to *Phase 1*. Similar to LW and DM intake, PiP and FecP:ME responded immediately to a change in diet P content (Figures 8.7.3 and 8.7.4). The immediate response of FecP:ME to change in P intake in *Phase 2* (steers with depleted P reserves consuming a high P diet) is in contrast to the observations in *Phase 1*, where a more gradual response in FecP:ME to P intake occurred (steers with adequate P reserves consuming a low P diet).

Conclusions
Steers that are P depleted will respond immediately to a P replete diet. The less time steers have consumed a P deficient diet prior to P supplementation, the less likely they will be to display production losses and more likely they will fully compensate to recover any losses. Steers that undergo re-alimentation after consuming a P deficient have lighter carcasses but similar carcass characteristics to those that were not P deficient.

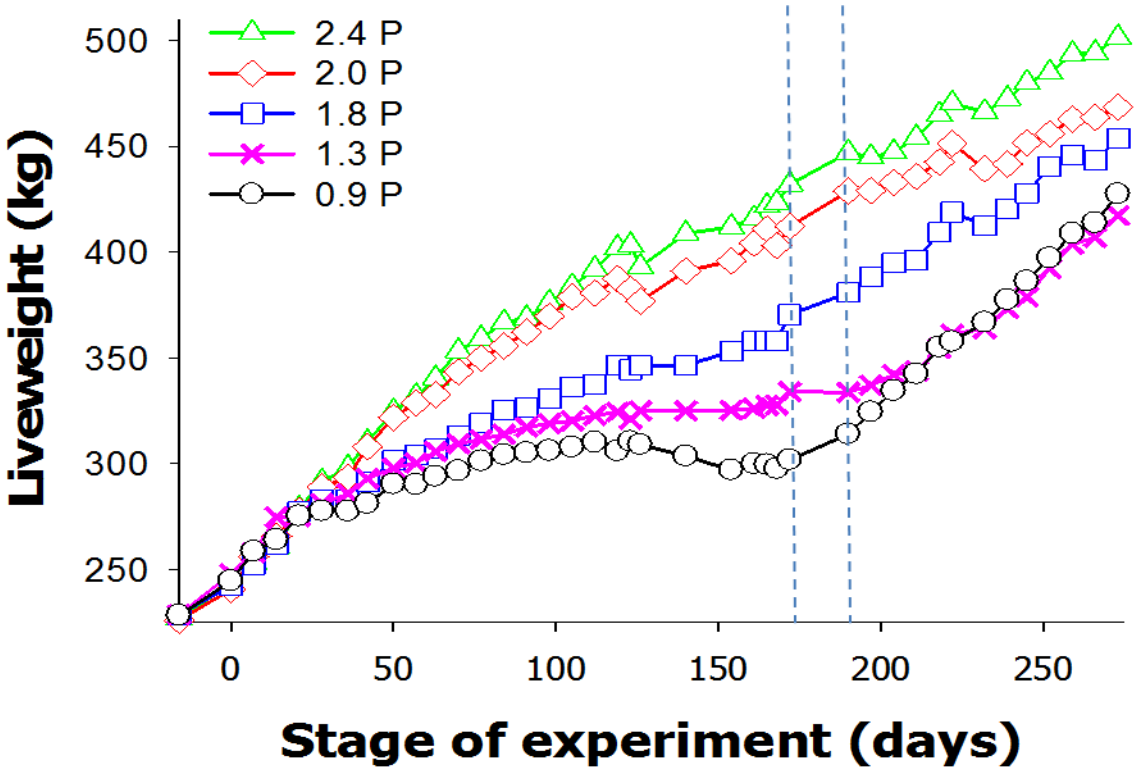


Figure 8.7.1. Change in liveweight of steers offered a diet with similar metabolisable energy (ME) and crude protein (CP) content with increasing phosphorus (P) content (0.9, 1.3, 1.8, 2.0 and 2.4 g P/kg DM) (*Phase 1*) followed by a high P diet (2.4 g P/kg DM) with similar ME and CP content (*Phase 2*). *Dashed line indicate end of treatment feeding in Phase 1 and commencement of full treatment feeding in Phase 2.*

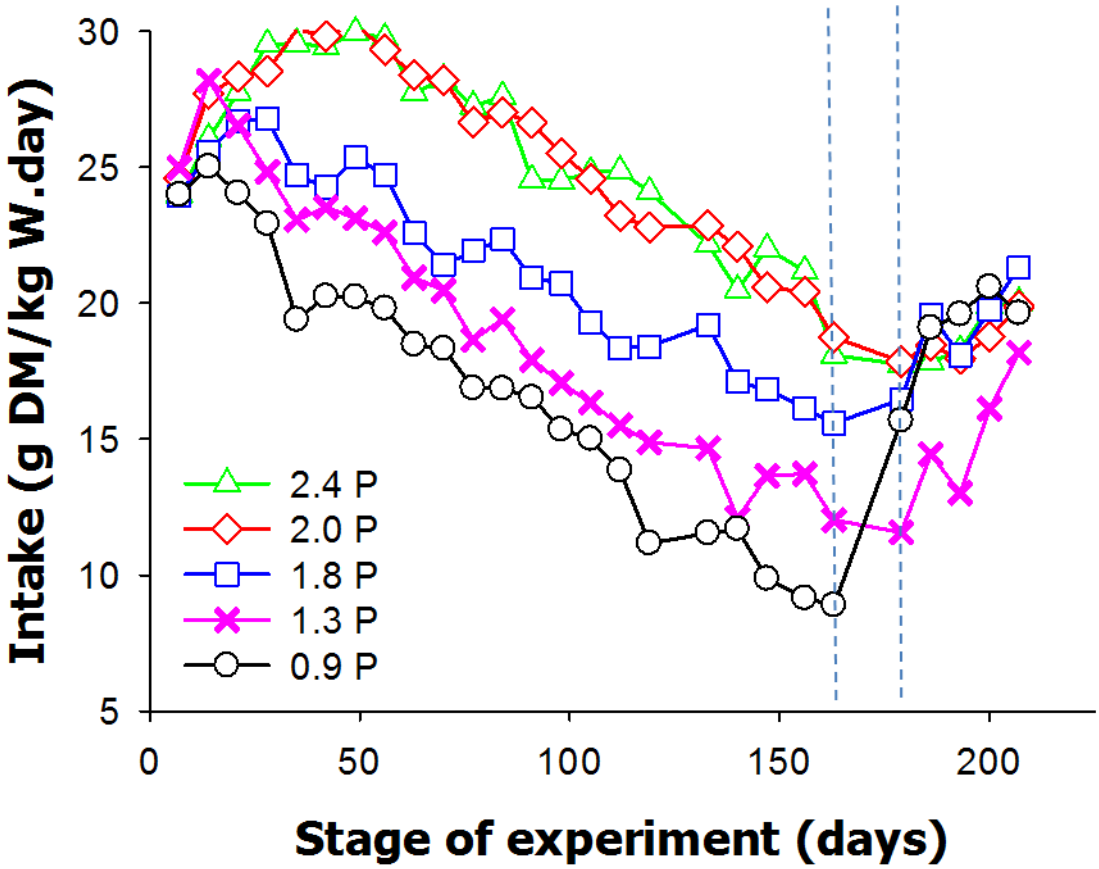


Figure 8.7.2. Change in dry matter (DM) intake of steers offered a diet with similar metabolisable energy (ME) and crude protein (CP) content with increasing phosphorus (P) content (0.9, 1.3, 1.8, 2.0 and 2.4 g P/kg DM) (*Phase 1*) followed by a high P diet (2.4 g P/kg DM) with similar ME and CP content (*Phase 2*). *Dashed line indicate end of treatment feeding in Phase 1 and commencement of full treatment feeding in Phase 2.*

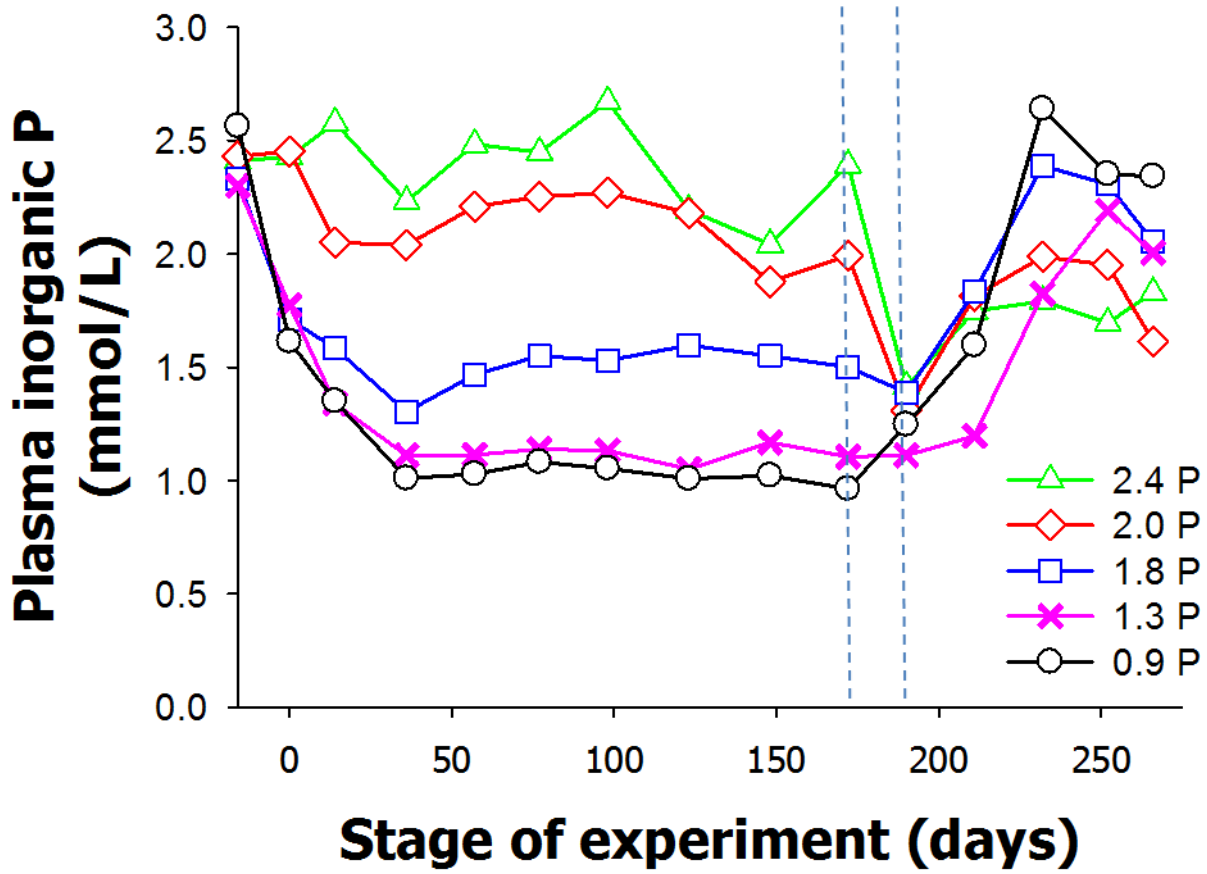


Figure 8.7.3. Change in the concentration of inorganic phosphorus (P) in the plasma of steers offered a diet with similar metabolisable energy (ME) and crude protein (CP) content with increasing P content (0.9, 1.3, 1.8, 2.0 and 2.4 g P/kg DM) (*Phase 1*) followed by a high P diet (2.4 g P/kg DM) with similar ME and CP content (*Phase 2*). *Dashed line indicate end of treatment feeding in Phase 1 and commencement of full treatment feeding in Phase 2.*

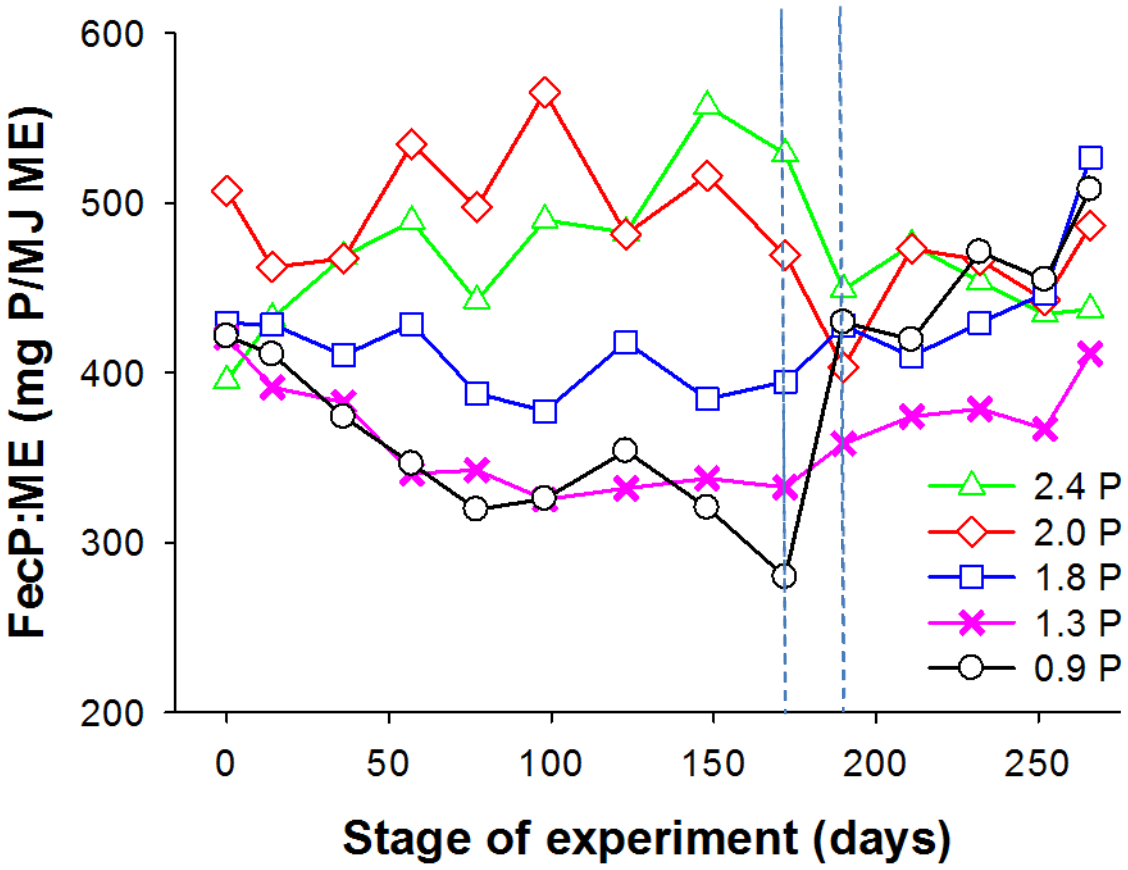


Figure 8.7.4. Change in the concentration of phosphorus (P) in the faeces relative to dietary metabolisable energy (ME) content of steers offered a diet with similar ME and crude protein (CP) content with increasing P content (0.9, 1.3, 1.8, 2.0 and 2.4 g P/kg DM) (*Phase 1*) followed by a high P diet (2.4 g P/kg DM) with similar ME and CP content (*Phase 2*). *Dashed line indicate end of treatment feeding in Phase 1 and commencement of full treatment feeding in Phase 2.*

8.8 Appendix 8. Bone histology

Background

Historically the rib biopsy method has been used to assess the P status of ruminants. The method was originally developed by Little (1972) and was based on P content of the bone (% dry bone weight) and later on the thickness or density of the cortical bone (Little, 1984). While there are logistical issues with this technique under field conditions, under research conditions it provides perhaps the best method to describe the long-term P status, or P reserves, of an animal as opposed to other measures which largely reflect P intake (PiP and FecP). The most commonly reported method describes the collection of a bone core from the rib which is then cleaned of trabecular bone, leaving a small disc of the outer cortical bone (CB) on which CBT, SG and either fresh or fat-free (after soxhelt extraction) ash and mineral content are determined. These indices are fine for comparing animals that have been subjected to a long-term P deficiency at a single point in time however they are not sensitive enough to give an accurate description of dynamic bone growth and completely ignore the role of trabecular bone in bone growth, strength and structure in response to P. Dr L Kidd and Dr D McNeill developed an alternative bone biopsy method in Experiment 1 involving the collection of biopsies from the tuber coxae bone which was considered to be less invasive, provide access to a larger sample of trabecular bone (albeit associated with a smaller amount of CB), be more sensitive to nutrient supply and of more relevance to LWG than a rib bone biopsy, on which more detailed information around the effect of P on dynamic bone growth, strength and structure could be generated.

Materials and methods

As described in the materials and methods (section 3.1), steers were treated with oxytetracycline intravenously, 10 days apart, with the final dose applied 10 days prior to bone biopsies. Biopsies, 16 mm in diameter, were collected from the 12th rib and tuber coxae (Figures 8.8.1 and 8.8.2). The biopsies were split, with one section of each placed in 70% ethanol before embedding in methylmethacrylate un-decalcified. Sections were cut using a specialised bone microtome (Leica 1600) with a circular, diamond-edged cutting saw. Mounted sections were examined and photographed using fluorescence microscopy. Another portion of each biopsy was fixed in formalin, decalcified in EDTA, embedded in paraffin and sectioned and photographed in a routine manner. All images were analysed using the software ImageJ and BoneJ. The primary and derived measurements obtained are summarised in Table 8.8.1.

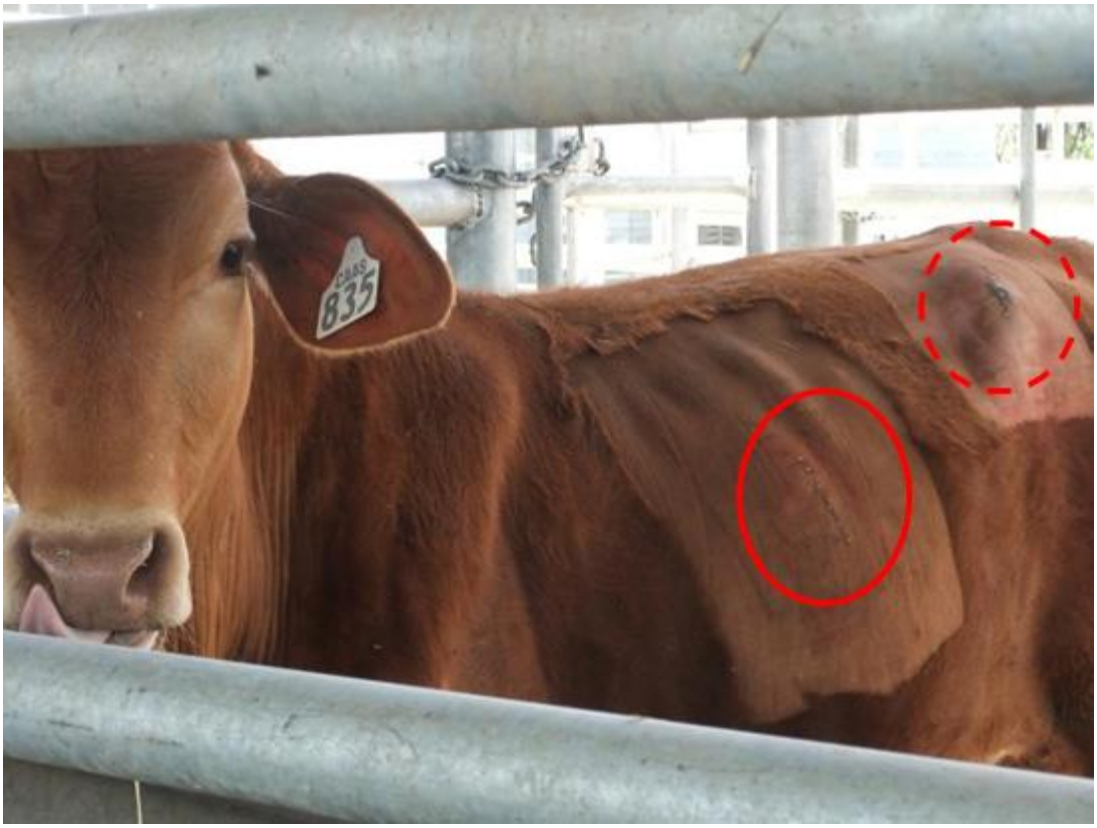
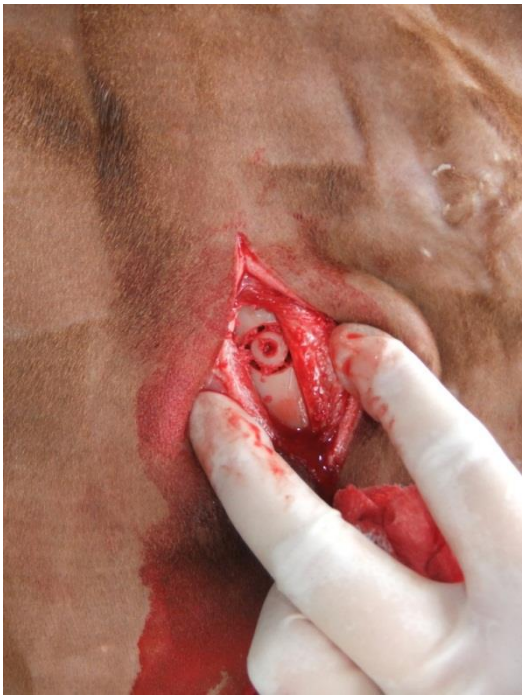


Figure 8.8.1. Anatomical sites for the rib (solid circle) and tuber coxae (dotted circle) biopsies. (Source: L Kidd)

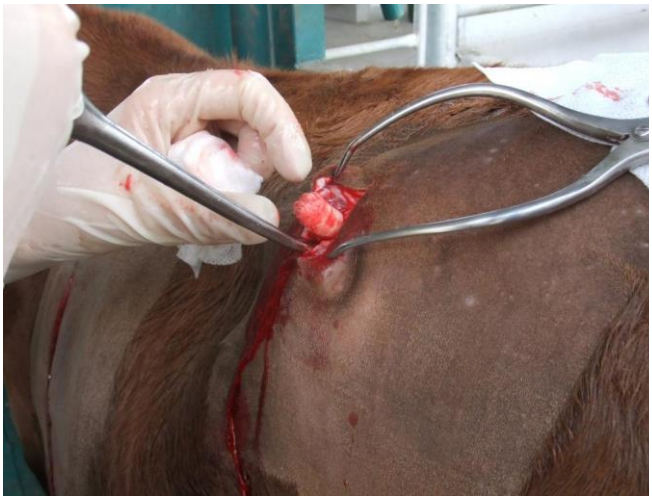
Table 8.8.1. Description of variables measured in the decalcified and un-decalcified sections of the tuber coxae bone biopsies collected from steers in Experiment 1

Parameter			
	Measured in decalcified sections - trabecular bone volume and structure		
Bone volume	%	B.V/T.V	How much bone tissue is there in a given area
Trabecular thickness	µm	Tb.Th	The average thickness of trabecular struts
Trabecular separation	µm	Tb.Sp	The average distance between trabecular struts
	Measured in un-decalcified sections - mineralisation and bone formation		
Mineral apposition rate	µm/day	MAR	How quickly osteoblasts (bone forming cells) are forming and mineralising new bone
Mineralizing perimeter	%	M.Pm/B.Pm	Proportion of the bone surface that is mineralising
Bone formation rate	$\mu m^2/\mu m.day$	BFR/B.Pm	The rate of bone formation per unit of bone surface

a.



b.



c.

d.

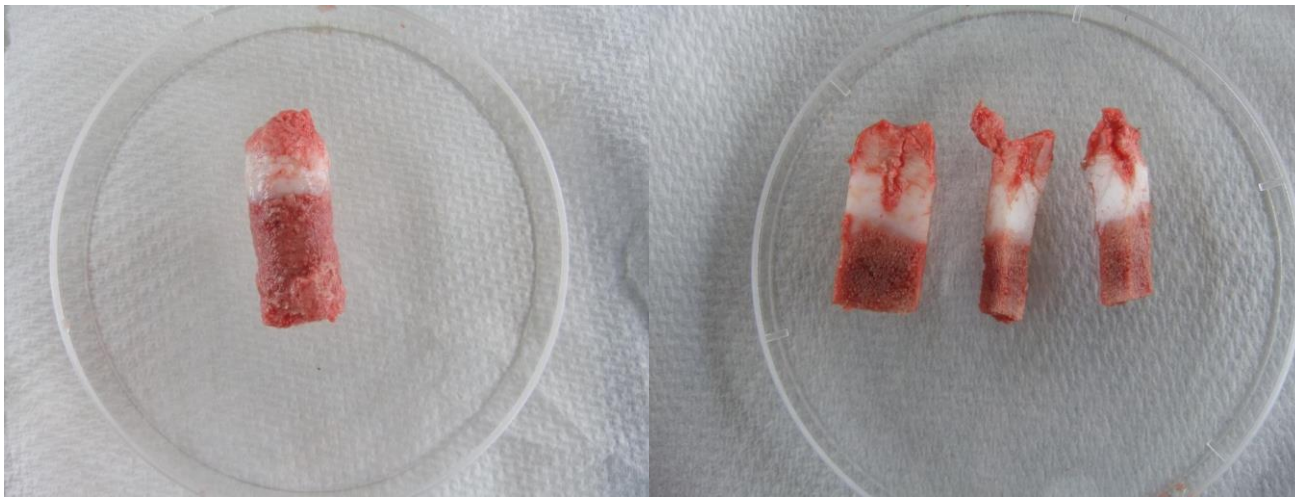


Figure 8.8.2. Collection of biopsy from the rib (a.) and tuber coxae (b.) bones, and full (c.) and divided (d.) tuber coxae baseline biopsies collected from steers prior to the commencement of Experiment 1. (Source: L Kidd and S Quigley)

Results

The surgical procedures required for the collection of bone biospies from the tuber coxae site was less invasive than the collection of biopsies from the 12th rib. However, there was little difference in the time taken to collect samples from either site. There were some difficulties with extracting the actual biopsy from the tuber coxae site, as it was a long biopsy (30 to 50 mm in length) and was subject to breaking. Repeat biopsies near the same site would also be difficult using this site. However, data around dynamic bone growth and structure was revealed from biopsies collected at this site, as this is a site of rapid bone growth (compared with the rib bone) and is structurally more important in influencing LWG of cattle than the rib bone. The preliminary results from the bone histology work (Figure 8.8.3) suggest an increase in bone formation, bone

volume, mineral apposition rate, mineralising perimeter, bone volume and trabecular thickness and a decrease in trabecular separation of the tuber coxae bone of steers in response to increasing dietary P content and DM intake (Figures 8.8.4 and 8.8.5). The results demonstrate that the tuber coxae site is sensitive to nutrient supply and this is a function of anatomical location and importance of the bone and large amounts, and nutrient sensitivity, of trabecular bone that was present at the site.

Conclusions

The tuber coxae is a suitable alternate site for bone biopsies in cattle when dynamic changes in bone growth and structure are of primary interest. The traditionally used CBT as an indicator of P status would still require a rib bone biopsy to facilitate comparisons with earlier studies. Bone formation and density of the tuber coxae, a relatively rapidly growing bone which may be of economic importance given its likely relationship with W, was influenced by dietary P intake and was sensitive to nutrient intake by steers.

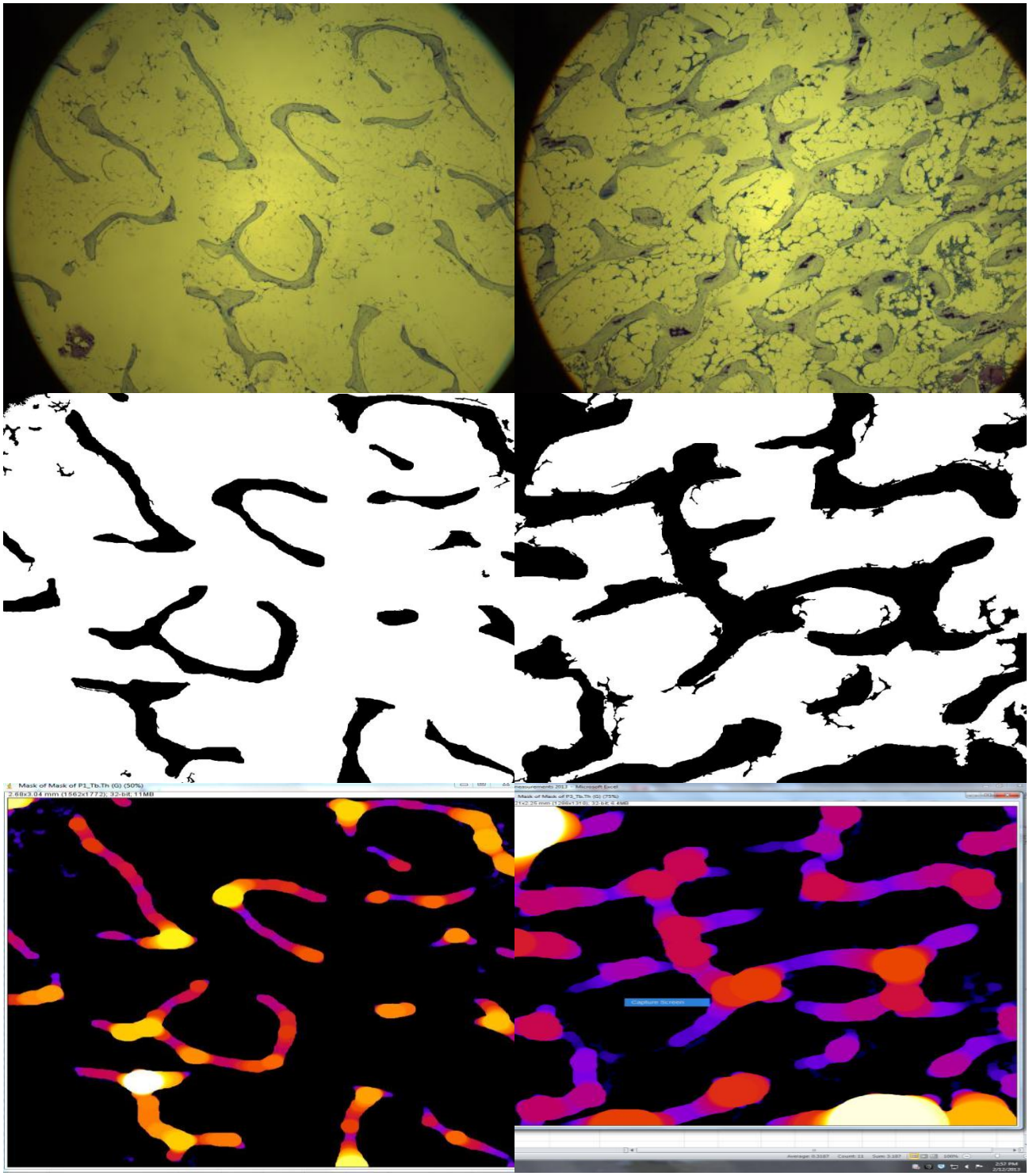
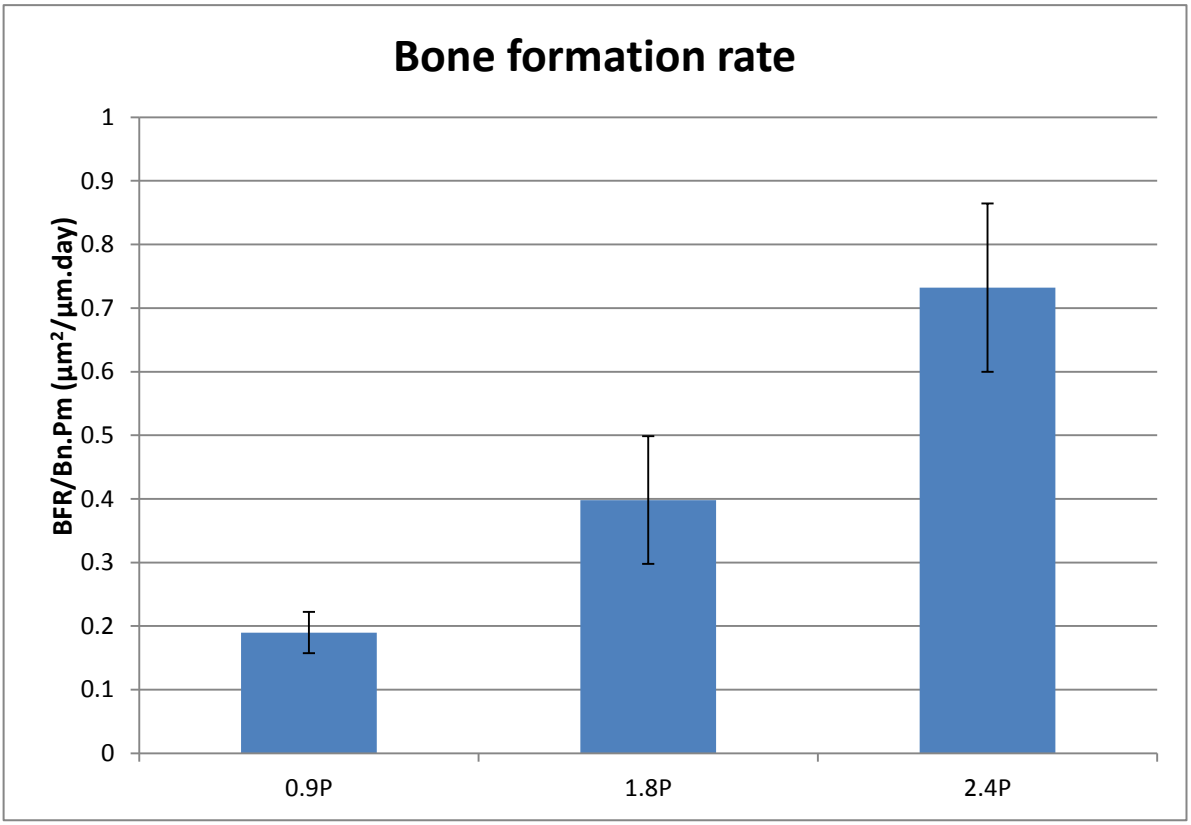
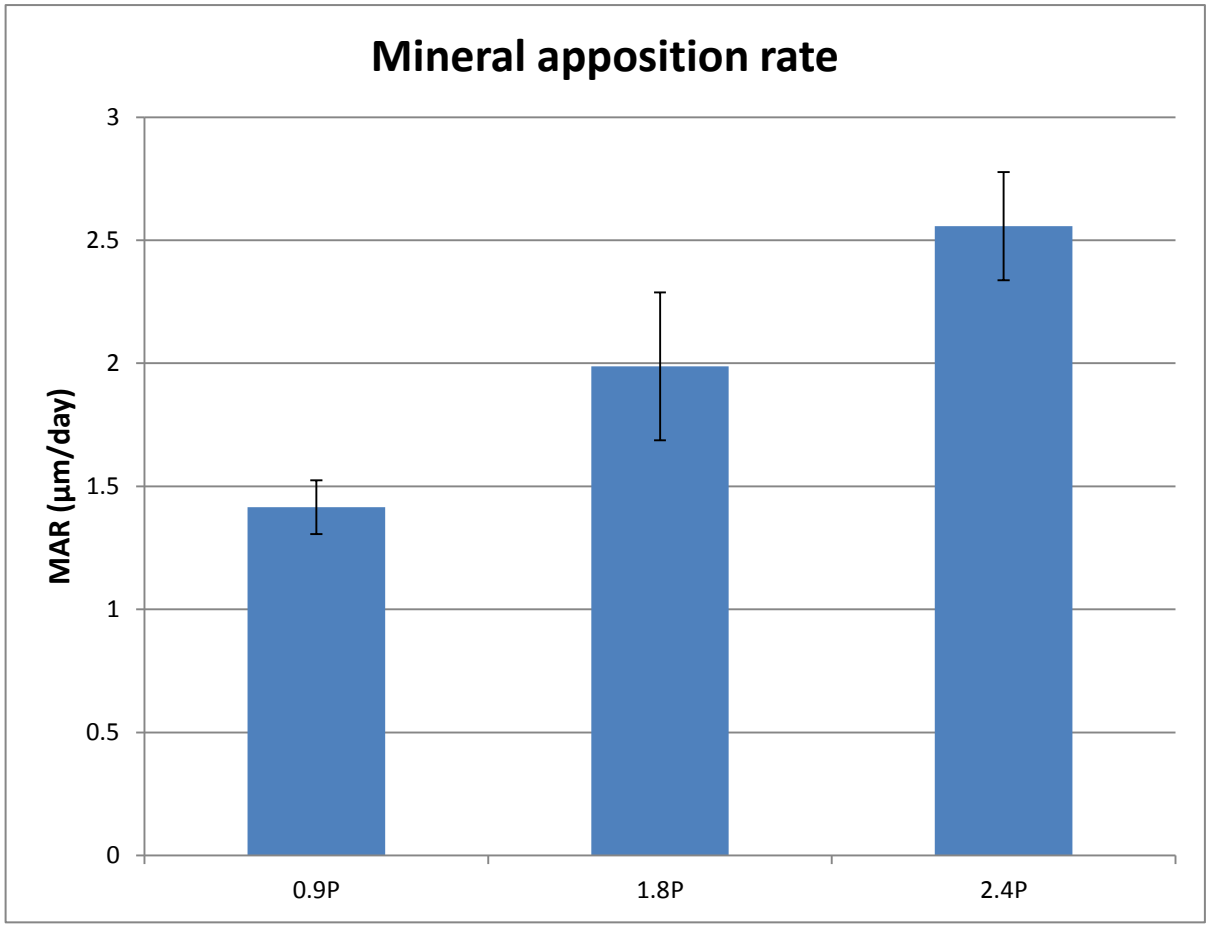


Figure 8.8.3. Typical histology of the trabecular bone from the tuber coxae bone of steers offered the 0.9P (column 1) and 2.4P (column 2) diets for 172 days in Experiment 1. *Top row = raw image; Middle row = middle masks; Bottom row = imagine analysis of trabecular thickening.*

a.


Bone formation rate
BFR/Bn.Pm (µm²/µm.day)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0.9P
1.8P
2.4P

b.


Mineral apposition rate
MAR (µm/day)
3
2.5
2
1.5
1
0.5
0
0.9P
1.8P
2.4P

c.

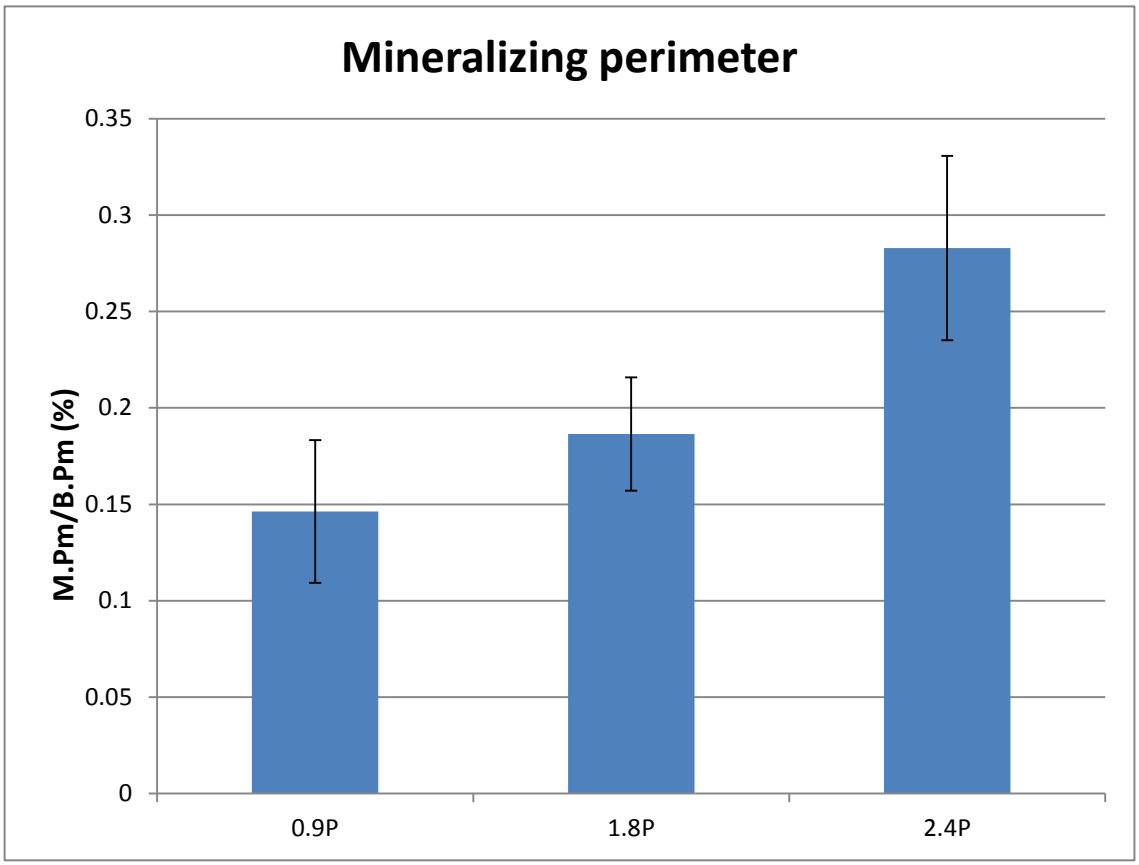
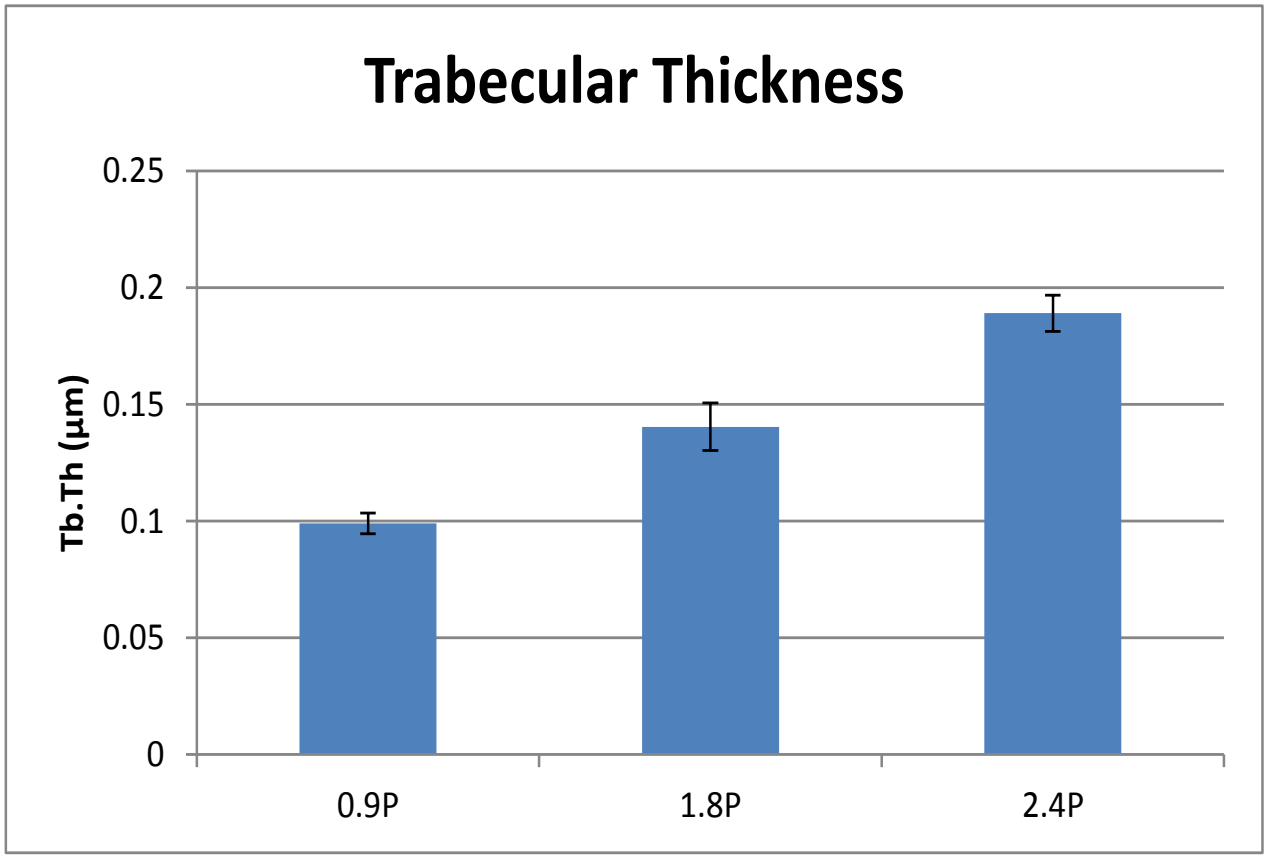
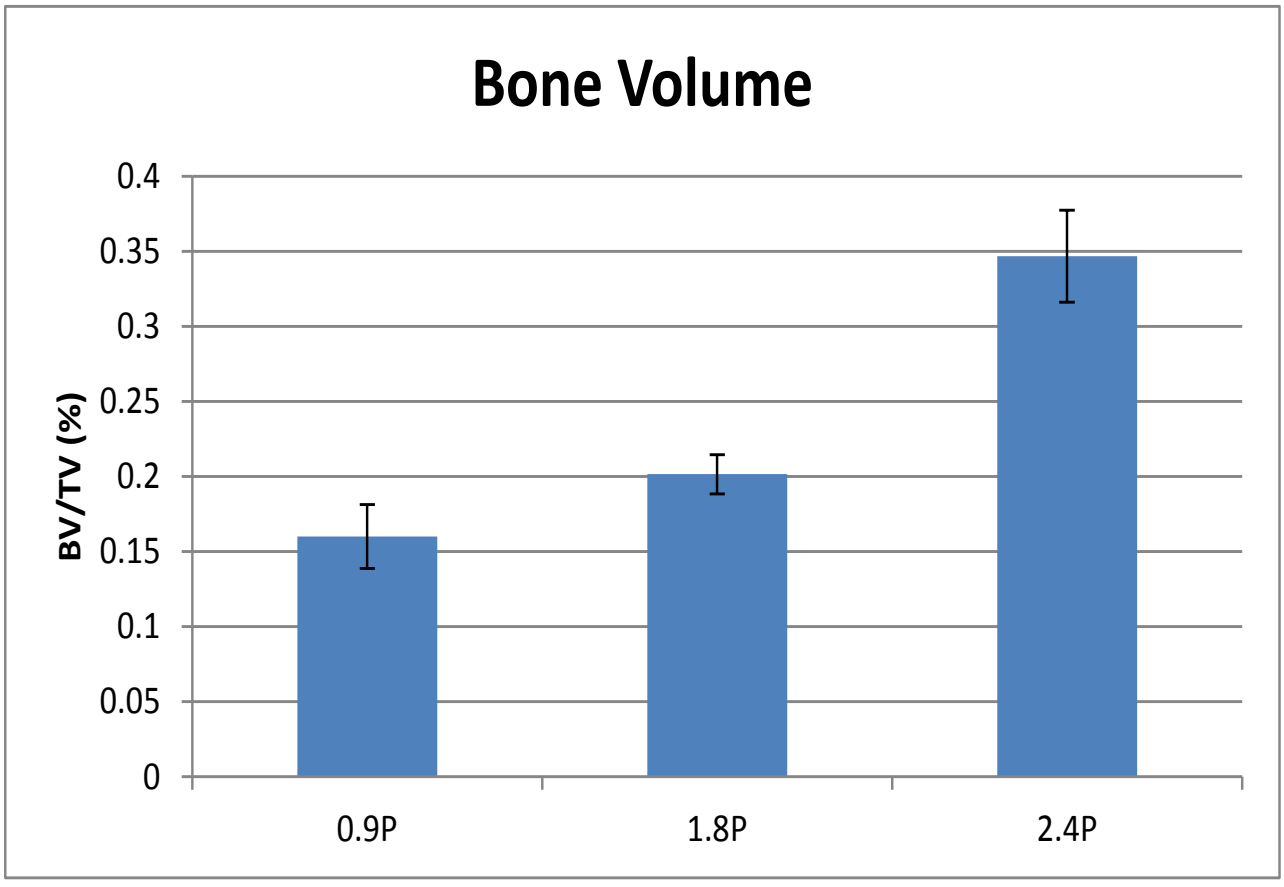


Figure 8.8.4. Preliminary bone formation (a.), mineral apposition rate (b.) and mineralising perimeter (c.) results from un-decalcified sections from the tuber coxae of steers fed 0.9P, 1.8P and 2.4P (0.9, 1.8 and 2.4 g P/kg DM) treatment diets for 172 days in Experiment 1. *Indices were determined at the end of Experiment 1.*

a.


Trabecular Thickness
Tb.Th (µm)
0.25
0.2
0.15
0.1
0.05
0
0.9P
1.8P
2.4P

b.


Bone Volume
BV/TV (%)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0.9P
1.8P
2.4P

c.

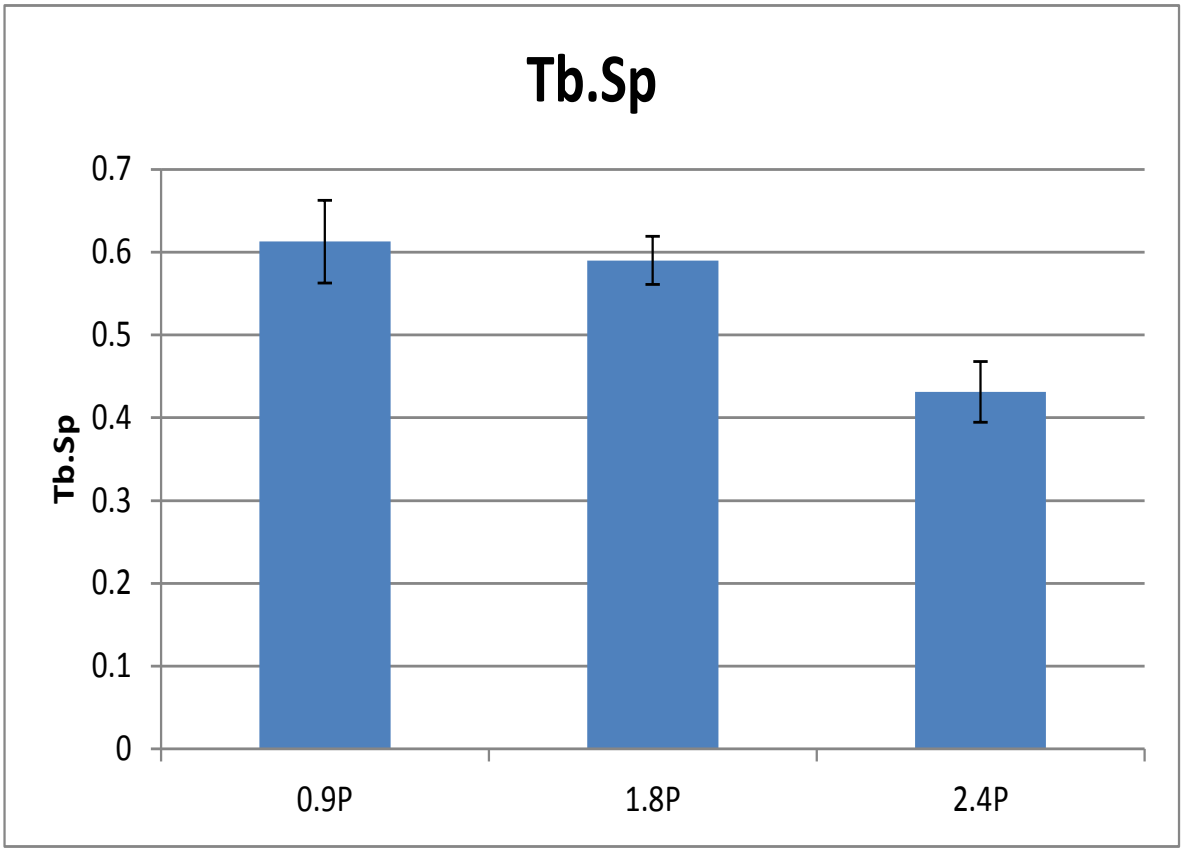


Figure 8.8.5. Preliminary trabecular thickness (a.), bone volume (b.) and Tb.Sp (c.) results from decalcified sections from the tuber coxae of steers fed 0.9P, 1.8P and 2.4P (0.9, 1.8 and 2.4 g P/kg DM) treatment diets for 172 days in Experiment 1. *Indices were determined at the end of Experiment 1.*

8.9 Appendix 9. Glucose homeostasis

The work below was conducted on a cohort of steers from Experiment 1 by Dr D McNeill. The specific work presented here was funded by The University of Queensland and Dairy Australia and was an add-on to the work described in the main sections of this report for Experiment 1 which was funded by MLA. The work was presented as a one-page paper at the Joint Meeting of the Australian and New Zealand Societies of Animal Production, Lincoln, New Zealand, July 2012.

Can Long-Term Differences in Phosphorus Nutrition Affect Glucose Homeostasis in Growing Steers?

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Bone tissue is now considered an endocrine organ following the discovery that in mice models a form of oesteocalcin (undercarboxylated osteocalcin; GluOC) directly communicates with the pancreas and adipose tissue to regulate glucose homeostasis (Lee et al. 2007). Our aim in this study was to extend the understanding of this new hormone in food producing animals, such as the steer. A practical means of manipulating bone in steers is to modify phosphorus (P) nutrition (Underwood and Suttle, 2001). So we tested the hypothesis that long term improvements in the P nutrition of steers might improve glucose homeostasis, via GluOC.

To generate groups of steers with different bone development, eighteen Droughtmaster weaners, starting at approximately 180 kg liveweight, were individually fed, *ad libitum*, a complete pelleted diets at one of three levels of P (Severely inadequate, P1, 0.9gP/KgDM; Moderate, P3, 1.8gP/KgDM; Excessive, P5, 2.4gP/KgDM), n = 6 per P level, continuously for approximately 5 months. After the 5 months an intravenous glucose tolerance test (IVGTT) was applied to each steer. The IVGTT involves a 300 mg/kg liveweight jugular infusion of glucose, with serum samples collected for glucose and insulin analysis at 2, 4, 6, 8, 10, 12 , 15, 18, 20, 23, 26, 30, 35, 40, 50, 60, 90, 120, 150, 180, 210 and 240 minutes relative to the glucose infusion. The IVGTT data were subsequently entered into MINMOD, a computer program that calculates insulin sensitivity parameters (Boston et al. 2003). Serum collected prior to the glucose infusion was also assayed for the hormonal (Glu) and non-hormonal (Gla) forms of OC. To indicate the extent of treatment effect on bone development, liveweight and hip heights were taken, and bone density on a biopsy from the 12th rib approximately two weeks after the IVGTT was gravimetrically determined.

At the time of the IVGTT, liveweights (kg ± se) were: P1, 300 ± 5; P3, 359 ± 12; P5, 417 ± 10. Rates of change in hip height (mm/100 days ± se) over the entire feeding period were: P1 = 28.8 ± 3.0; P3 = 45.7 ± 7.3; P5 = 55.9 ± 1.2. Yet despite these large differences in animal performance, the density (g/cm^2 ± se) of the outer cortical bone in rib biopsies was unaffected by P treatment (P1 = 1.63 ± 0.02; P3 = 1.59 ± 0.02; P5 = 1.67 ± 0.04), as were serum levels of both GluOC (P1 = 9.4 ± 4.3 ; P3 = 12.2 ± 3.0; P5 = 26.6 ± 10.9 ng/ml) and GlaOC (P1 = 163 ± 34 ; P3 = 151 ± 22; P5 = 175 ± 7 ng/ml). Moreover, the MINMOD software could not interpret the IVGTT data for the majority of the steers. This precluded estimation of parameters of insulin sensitivity.

The inability of MINMOD to accommodate the IVGTT data could have been due to the steers being overtly stressed at the time of sampling during the IVGTT. Retrospective measurement of serum cortisol at the beginning and end of the IVGTT confirmed a stress response in most animals that persisted throughout the IVGTT. We conclude that this stress response obscured our testing of the hypothesis that previous P nutrition influences the sensitivity of steers to insulin.

This project was funded by Meat and Livestock Australia, the University of Queensland, and Dairy Australia.

Boston et al. (2003). Diabetes Technology and Therapeutics. 5, 1003-1015.

Lee N.K., Sowa H., Hinoi E., Ferron M., Deok Ahn J., Confavreux C., Dacquin R., Mee P.J., McKee M.D., Young Jung, D., Zhang Z., Kim J.K., Mauvais-Jarvis F., Ducy P. and Karsenty G. (2007). *Cell*. **130**, 456-469.

Underwood E.J. and Suttle N.F. (2001). The Mineral Nutrition of Livestock. CABI publishing, Oxon, UK.

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