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Improving prediction of phosphorus intake of cattle grazing tropical pastures

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Abstract

Nutritional deficiencies of phosphorus (P) commonly occur in northern Australian rangelands, but estimation in grazing cattle of the diet P concentration and the adequacy of P intake is difficult. Relationships to estimate diet P concentration from measurements of constituents of faeces (total P, acid insoluble P and near infrared spectroscopy of faeces (F.NIRS) to measure diet constituents) in cattle ingesting tropical forage diets were examined using multiple regression models. A data set representing 167 forages and 601 diet-faecal pairs of samples (usually 3-4, range 1-12 animals per diet) from pen feeding experiments with young *Bos indicus* cross cattle was analysed. Separate regression models were necessary for grass and grass-legume *versus* legume diets. Diet P concentration was poorly predicted from faecal P concentration (R^2=0.53). Inclusion of measurements of the concentrations of faecal total N (F-TN) and diet total N (D-TN) improved the prediction (R^2=0.67). Diet P concentration predicted from faeces was also examined in cattle grazing tropical pastures where there was other information of P status. Diet P in cattle grazing tropical forages could be estimated from faecal P concentration and F.NIRS with accuracy sufficient to improve field estimation of the diet P concentration.

Executive summary

Diet deficiencies of phosphorus (P) often occur in cattle grazing northern Australian rangelands, and arguably constitute the most important nutritional constraint. P supplementation of P-deficient cattle during the growing season often leads to large increases in annual liveweight (LW) gain of cattle and breeder herd output and is often cost-effective. However, there has been generally poor industry adoption of P supplementation or other strategies to address P deficiencies. A major constraint is the reliable identification of P status, particularly of sub-clinical P deficiencies. The most reliable test ("P-Screen") requires sampling of blood and faeces from growing cattle during the late wet season. However, implementation of this diagnostic test on remote cattle stations involves challenges and most cattle managers consider its use "too difficult". There are also uncertainties with extrapolating the information obtained from growing cattle to breeder herds. Due to such difficulties there has been little use of the P-screen test across the northern industry.

Faecal P concentration has been investigated as an indicator of diet P deficiency but the conclusions on its reliability and accuracy have varied widely. In some studies with cattle grazing tropical pastures diet P concentration was closely correlated with faecal P concentration (R^2 = 0.76 - 0.95) (Moir 1960; Dixon and Coates 2010, 2011). In contrast Wadsworth (1990) concluded from several major studies across northern Australia that the relationships were inadequate to estimate diet P concentration from faecal P. The present study used an archival sample set comprising forage diets (n = 167) and corresponding faecal samples (n = 601) to examine the relationships between the concentrations of P in the diet and faeces of tropical *Bos indicus* cross cattle ingesting tropical forages in pens. Laboratory measurements were made of the concentrations of total P (D-TP, F-TP) and calcium (D-TCa, F-TCa), and of the acid insoluble P (D-AIP, F-AIP) fraction, in forages and faeces. In addition the concentrations of total N (D-TN) in the diet, diet DM digestibility (D-DMD), the proportion of legume in the diet (D-NG), and the total N concentration in faeces (F-TN), and the NDF and ADF contents of some diets (D-NDF, D-ADF) had been measured.

The contribution of the various faecal constituents, and diet attributes measured by F.NIRS, to estimate D-TP, D-TCa and diet Ca/P ratio (D-TCa/D-TP) were examined using multiple regression models. Only diets with D-TP ≤ 4 g P/kg DM were considered since diets of higher P content would provide diet P in excess of requirements for beef cattle. Separate regression models were required for grass and grass-legume diets (with ≤ 40% legume) *versus* pure legume diets. In a simple linear regression model D-TP and F-TP were related with an R^2 = 0.53, a much lower R^2 than in the most previous studies. Likely reasons were that the sample set was very diverse (in plant species, growing environments and plant maturity), many of the diet-faecal pair samples were obtained after brief adapation to the diet, and samples were from experiments conducted in a number of laboratories and using a variety of experiment procedures. A series of multiple regression models were examined to predict D-TP using groups of faecal measurements as explanatory variables. Group 1 consisted of faecal attributes which could be routinely measured by conventional laboratory procedures (F-TP, F-TCa, F-AIP, F-AICa, F-TN) and diet non-grass (D-NG) which can be estimated from paddock observation or from $^{13}\delta C$ ratio in faeces. Group 2 consisted of F-TP and some F.NIRS measurements of diet attributes (D-TN, diet DM digestibility (D-DMD), D-

NG, D-NDF and D-ADF). Group 3 comprised all of the faecal measurements of Group 1 and the F.NIRS measurements in Group 2.

The form of the regression models was: $Y = a + bX_1 + cX_2 + dX_3 + eX_4$. The grass-legume *versus* pure legumes classes of diets were different only in the constant. The models for the grass diets were as follows (units g/kg DM):
D-TP = +0.352 + 0.3865*F-TP [R^2 0.53]
The best prediction model using the Group 1 variables was as follows:
D-TP = -0.376 + 0.2780*F-TP + 0.0936*F-TN [R^2 0.60]
D-TP = -0.077 + 0.3755*F-TP + 0.1145*F-TN – 0.6313*F-AIP [R^2 0.63]
The best prediction model using the Group 2 variables was as follows:
D-TP = 0.404 + 0.3037*F-TP - 0.0532*F-TN + 0.0837*D-TN [R^2 0.67]
The best prediction model using the Group 3 variables was as follows:
D-TP = 0.548 + 0.3842*F-TP - 0.0285*F-TN + 0.08063*D-TN – 0.485*F-AIP [R^2 0.69]
Thus D-TP could be estimated from wet chemistry analyses of faeces with an $R^2 = 0.63$, or if F.NIRS measurements of diet attributes were also available then with an $R^2 = 0.69$.

The data set used was from young, growing animals ingesting metabolisable energy (ME) intakes which provided for slow LW loss through to moderate LW gain. A difficulty is that net mobilization of body tissues (i.e. LW loss) would make additional P available and increase F-TP. Conversely, accretion of tissues (LW gain as soft tissues and bone, conceptus growth, milk secretion) will remove P and thus reduce F-TP. To extrapolate to animals in various physiological states estimation of D-TP from faecal measurements should adjust for these effects. The amounts of P mobilized or required for most of these changes can be calculated from nutritional standards (CSIRO 2007). However, there is currently no means to diagnose and estimate net mobilization or deposition of bone P. The only current option is to apply knowledge of the physiological state; that cows in early lactation are likely to have a net mobilization of up to 5 g P/day (Dixon and Coates 2010), and growing animals in P repletion depositing up to 5 g P/day (Bortolussi *et al*. 1999).

In the present project D-TP was predicted much more satisfactorily from a number of faecal attributes rather than from F-TP alone. Even though the poor correlation between F-TP and D-TP may have been at least partly due to the limitations of the sample set it must be concluded that D-TP cannot be reliably and consistently estimated from F-TP. Also if this poor correlation occurs generally then indicator ratios which include F-TP (F-TP/diet ME and F-TP/D-TN) will provide erroneous estimates of D-TP in some circumstances. A more reliable approach to estimate adequacy of D-TP and diet P intake should be to estimate D-TP concentration and then to calculate the amounts of P and ME required for cattle in various physiological states. Diet ME content, the amounts of P and ME required, and the intake of forage will need to be estimated. A problem remains that the D-TP, or the D-TP/ME ratio, required will be affected by net mobilization or deposition of bone P. An intention of the present project was to evaluate the F-TP/ME ratio but the study indicated that this ratio may not not be a robust indicator of diet P. The more useful indicators of diet P adequacy is the D-TP concentration, and possibly also the D-TP/ME ratio, required by the specific class of animal.

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1 Background

Nutritional deficiencies of phosphorus (P) are widespread in cattle grazing the seasonally dry tropical rangelands of northern Australia, Africa and South America. In many such rangeland regions P and nitrogen (N) are the first-limiting nutrients to cattle production during the rainy season and the dry seasons, respectively (Winks 1984, 1990; McDowell 1996). N supplements, usually based on non-protein N, are often provided during the dry season to alleviate such N deficiency. Supplementation to alleviate P deficiencies is generally less common. For example, Niethe (2011) suggested that perhaps only 10% of the cattle in northern Australia grazing landscapes with low P soils, and where cattle are likely to be P deficient, are effectively managed and supplemented with P. A number of reasons have been suggested for this low adoption of nutritional technology by the northern cattle industry (Niethe 2011; Dixon *et al.* 2011), but one important reason is unequivocally the difficulty to diagnose on-farm the P status of cattle grazing extensive rangelands.

Knowledge of the nutrient content of the diet selected by grazing cattle is an obvious important step for identification of deficiencies of specific nutrients, especially where the deficiency is sub-clinical. The development of near infrared spectroscopy of faeces (F.NIRS) to estimate diet constituents, including the contents of N and metabolisable energy (ME), makes possible the routine estimation of the contents of N and energy in the diet selected by grazing cattle. However, F.NIRS cannot directly measure the P concentration of the diet. A number of studies have examined the relationships between the concentrations of total P in the diet (D-TP) and in faeces (F-TP). Some studies have reported close relationships for cattle grazing tropical forage diets (Moir 1960) and in sheep fed temperate forage diets (Dove and Charmley 2004). However, other studies have reported poor relationships with forage diets (Cohen 1974; Wadsworth *et al*. 1990) and with diets containing concentrates and / or P in inorganic forms such as calcium phosphates (Cohen *et al.* 1974). Dixon and Coates (2010) collated information on the correlation relationships between diet P concentration and faecal P concentration in a group of linked experiments with cattle grazing grass and grass-legume pastures in tropical Australia, and reported useful linear regression relationships (e.g. R^2= 0.81) (Dixon and Coates 2011).

Given the variation and inconsistencies among studies in the efficacy of F-TP to diagnose D-TP and P status of cattle, the current recommended field diagnosis of the diet P adequacy depends on blood P concentration in conjunction with an estimate of the metabolisable energy intake during the late wet season (McCosker and Winks 1994). This knowledge was developed into a “P-Screen” diagnostic test. However, although sampling kits and service laboratories have been available for some 30 years at modest cost to apply this “P-Screen” diagnostic test on commercial cattle properties, its adoption and use by the industry has been negligible. The most common reason given appears to be “the difficulty” for most cattle managers to organize necessary groups of sentinel animals (young growing cattle) for sampling at the specified time of the year and the paucity of skills to blood sample designated animals. An additional limitation of the “P-Screen” test is that there has been uncertainty on how the results from growing cattle should be applied as an indicator of the P-status of breeder cows. There is considerable evidence from research (Coates and Ternouth 1992; Coates *et al.* 1996; Ternouth and Coates 1997), and also observational evidence, that breeder cows can maintain acceptably high reproductive efficiency for indicators such as mortalityand weaner liveweights even when the P-Screen test indicates that they would be P deficient for liveweight gain.

The present study was undertaken to evaluate the accuracy and the robustness of the prediction of diet P from a variety of faecal measurements. In previous studies only faecal total P (F-TP) concentration has been examined as a predictor of diet total P (D-TP) concentration. In the present study additional fractions in faeces, and diet attributes which can be measured by F.NIRS, were also considered.

2 Project objectives

2.1 To have analysed archival forage and faecal samples from the past F.NIRS calibration experimentation to improve the reliability of the prediction of diet P concentration from faecal total P concentration, and to have examined the efficacy of using acid-insoluble P concentration in faeces as a predictor of diet P concentration. This will involve analyses of forage and faeces from approximately 150 forage diets fed to cattle in individual pens

2.2 To have analysed archival faecal samples from 3 past experiments with breeders grazing 'marginal' P pastures, and used this information to estimate the extent of bone P mobilization in breeders in late pregnancy and lactation grazing these pastures.

2.3 To have obtained and analysed faecal samples from the current major P project (B.NBP.0537) to estimate the effects of P supplements on the relationship to predict diet P concentration from faecal total P concentration and acid-insoluble P concentration.

2.4 To have obtained field samples of faeces from cattle grazing a variety of pasture systems (some fed P supplements) expected to range in P status, and to use this information to indicate whether the calculations of threshold values of faecal P indicating dietary P adequacy are valid.

3 Methodology

3.1 Animals and procedures

Diet-faecal pairs of samples were obtained from an archival sample set which had been collected during a major program to develop near infrared spectroscopy of faeces to measure diet attributes (F.NIRS). These samples had been obtained from experiments where young cattle had been fed forage diets in pens and faecal samples obtained after at least 5 days when the faeces were expected to be representative of the forage fed after digestion and passage through the animal. The number of animals fed each diet was usually 3-4. Many of these experiments were done specifically to provide the diet-faecal pairs necessary for the development of F.NIRS calibration equations. This set of diet-faecal pairs has been previously described (Coates 2004; Coates and Dixon 2011) although only the diet-faecal samples obtained from pen experiments were used for the present study. Those obtained as oesophageal extrusa samples were not included due to the error expected from contamination by salivary phosphorus. In addition diet-faecal pairs of tropical forages derived from more recent experiments reported by Kennedy and Charmley (2013) and S. McLennan at Brian Pastures Research Station (unpublished results) were included in the sample set. All of these diets consisted of forage fed alone and without concentrate or mineral supplements. In each of these experiments forages were fed *ad libitum*, or approaching *ad libitum* (>90% of previously measured *ad libitum* intake). Most forages comprised sun-dried hays made from tropical C4 grass swards representing swards dominated by (or monocultures of) common introduced pasture species, or were mixtures of native grass species, or legume hays of common introduced species. Some temperate grass and legume species were also used (hays of ryegrass, oat, wheat and barley and lucerne). Animals were usually *Bos indicus* cross, and usually steers which ranged from large weaners (>200 kg) though to about 2 year old.

3.2 Laboratory procedures

DM contents of the forages and faecal samples were generally determined as part of the animal experimentation. Total N concentration was determined by Kjeldahl digestion or microcombustion (Leico autoanalyser) depending on the laboratory. Concentrations of Ca and P were determined by inductively coupled plasma spectrometer (ICP; Optima 7300 DV, Perkin Elmer; Welleslay, MA, USA). To determine acid insoluble P (AIP) and acid insoluble Ca (AICa) samples were incubated with 0.4% hydrochloric acid for two hours at ambient temperature. The P and Ca concentrations of the supernatant were measured and the fraction of insoluble P and Ca was calculated by difference. Established procedures and F.NIRS calibration equations (Coates 2004; Coates and Dixon 2008, 2011; Dixon and Coates 2009) were used to measure the N concentration, DM digestibility and non-grass content of the diet from faeces in the grazing trials.

3.3 Calculations and biometrical modelling

All forage diets with P greater than 4 g/kg DM were excluded from analyses on the basis that these diets would be adequate in P for beef cattle. It was also anticipated that additional variables (such as excretion of P in urine) were likely to become important pathways of P excretion at such high P intakes, and this would decrease the capacity of the regression models to predict diet P concentration in forage diets containing low to medium concentrations of P which was the range of primary interest for the present study.

The following were considered as potential explanatory independent variables to predict diet total P concentration [D-TP]. The units are g/kg DM:

a) Total P concentration in faeces [F-TP],
b) Acid-insoluble P concentration in faeces [F-AIP],
c) The ratio of acid-insoluble P to total P in faeces [F-AIP/F-TP],
d) Diet DM digestibility [D-DMD],
e) Diet total N concentration [D-TN],
f) Faecal total N concentration [F-TN],
g) Diet neutral detergent fibre (NDF) concentration [D-NDF],
h) Diet acid detergent fibre (ADF) concentration [D-ADF],
i) Faecal total calcium (Ca) concentration [F-TCa],
j) Faecal acid-insoluble Ca concentration [F-AICa],
k) Faecal total Ca to total P ratio [F-TCa/F-TP],
l) Faecal acid insoluble Ca to acid insoluble P ratio [F-AICa/F-AIP].
m) Diet non-grass as can be measured using $^{13}C/^{12}C$ (delta C) ratio [D-NG].

Five groups of explanatory variables were examined. These groups were selected to comprise the various arrays of analytical measurements likely to be available in a laboratory, and with the considerations that: (i) some measurements are expected to be lower in cost, and (ii) only some laboratories will have access to the instrumentation and calibration equations for F.NIRS to measure diet attributes. The groups were as follows:

a) Group 1 comprised the following constituents of faeces measured by conventional laboratory analyses: total P concentration in faeces (F-TP), total N concentration in faeces (F-TN), total calcium concentration in faeces (F-TCa), acid insoluble P concentration in faeces (F-AIP), and acid insoluble Ca concentration in faeces (F-AICa).
b) Group 2 comprised the Group 1 variables F-TP and F-TN, plus the following variables which can be measured by F.NIRS: total N concentration in the diet (D-TN) and DM digestibility of the diet (D-DMD).
c) Group 3 comprised the Group 2 variables plus the Group 1 variables not already present (i.e. F-TCa, F-AIP and F-AICa).
d) Group 4 This comprised the Group 3 variables and also include all the remaining diet F.NIRS measurements diet NDF concentration (D-NDF) and diet ADF concentration (D-ADF).
e) Group 5 consisted of the Group 2 variables and all the diet F.NIRS measurements described above.

Models predicting D-TP from measurements in faeces (both constituents of faeces measured by conventional laboratory analyses and diet attributes measured by F.NIRS) were developed using multiple linear regression and applied to various subsets of the explanatory variables. Models were developed by sequentially adding terms from the subset of explanatory variables. Terms were added to the model in the order which considered their likely explanatory value (e.g. all models included F-TP as the first explanatory variable). Preliminary examination of the data set indicated that the classification of the diets into either (i) diets of grass and grass-legume mixtures containing up to 40% legume (G, G/L), and (ii) diets consisting entirely of legume hays (Leg) influenced important linear regressions such as between diet D-TP and faecal F-TP. Thus this classification of diet into 2 categories was included as a factor in each of the models (Leg). The interaction between legume forage (Leg) and an added term was tested and, if not significant ($P>0.05$), was removed from the model. Also, if an added term was not significant at $P<0.05$ it was removed from the model before the next term was added.

3.4 Effects of time of sampling through the daily cycle on faecal P concentrations

Steers (6 per diet treatment) were housed at QASP at UQ, Gatton, in individual pens and were fed a semi-purified diet either deficient in P or with calcium phosphate mixed into the diet so that it was adequate in P for rapid liveweight gain. Faeces were collected at approximately equal intervals 6 times through a 24-hour cycle. The P concentration in the faeces was measured to determine the extent of the variation in faecal P concentration through the daily cycle.

3.5 Examination of the effects of supplementary inorganic P in the diet on the prediction of diet P concentrations

Results were drawn from an experiment where breeders in the various treatments grazed pastures on Springmount Station (near Mareeba) with a range of low soil P (and thus pasture P) concentrations and / or were given P supplements through the drinking water (Miller *et al.* 1998; D B Coates and C P Miller, unpublished results). The linear regression relationships between F-TP and D-TP in animals obtaining all of their P from pasture, or obtaining a substantial proportion of their diet P from P supplement.

3.6 Prediction of diet P concentration from faecal P concentration in cattle fed semi-purified diets

In an experiment at Brian Pastures Research Facility (Task 1, NBP.0689 phosphorus project) heifers were fed P deficient diets based on wheat straw, wheat flour and sugar. Diet and faeces were sampled at intervals over 6 months as the heifers developed P deficiency. The P concentration was measured so that the prediction of diet P concentration from the regression relationships developed for forage alone diets could be examined.

In a second experiment at QASP UQ, Gatton, steers (described in section 3.5 above) steers were fed a semi-purified diet similar to that described at Brian Pastures (Quigley et al. 2015). Results from this project (B.NBP.0537, "Validation and demonstration of a diagnostic tool for P status of beef cattle") were examined to indicate whether the relationships between faecal P concentration and diet P concentration in steers fed the semi-purified diets and various concentrations of P were consistent with the regression relationships developed in the present study.

4 Results

4.1 Structure of the diet-faecal pair sample set

The composition of the selected data set of forages fed to cattle and where the total P concentration was < 4 g P/kg DM is given in Table 1. Because some diets consisted of mixtures of forages, and for some experiments the same nominal forage was fed during several experimental periods, the number of diets does not correspond exactly to the number of forages. Mean concentrations of total P (D-TP) and total Ca (T-Ca) were 1.77 g P/kg DM and 5.28 g Ca/kg DM; thus mean Ca/P ratio was 3.68. Mean acid insoluble P (D-AIP was 0.39 g P/kg DM or 0.249 of total P (D-TP). There were linear relationships between the acid insoluble P fraction (D-AIP) and the total P (D-TP) concentrations (Figure 1A), and also between these fractions of Ca (Figure 1B), but with low R^2 (R^2=0.33 and R^2=0.44, respectively). Mean acid insoluble Ca (D-AICa) was 0.068 of total Ca (D-TCa). The range in total N concentration (1.0 – 42.9 g N/kg DM, in DM digestibility (453-704 g/kg), and in NDF concentration (290 – 818 g/kg) indicated that the sample population of forages encompassed the range of N and digestibility generally observed in the diet of cattle grazing tropical pastures. The composition of the faeces derived from the selected forages is given in Table 2.

Table 1. The population of forages fed to animals during the pen trials and where forage contained < 4 g P/kg DM. Concentrations of constituents of phosphorus (P) and calcium (Ca) (Total P, Acid-Insoluble P, ratio of acid-insoluble P to Total P, Total Ca, Acid-insoluble Ca, and ratio of Total Ca to total P) in the forage samples (n = 167).

	D-TP (g/kg DM)	D-AIP (g/kg DM)	D-AIP /D-TP (g/kg)	D-TCa (g/kg DM)	D-AICa (g/kg DM)	D-TCa /D-TP (g/g)	D-TN (g/kg DM)	D-DMD (g/kg DM)[a]	D-NDF (g/kg DM)	F-TN (g/kg DM)[a]
Mean	1.77	0.39	249	5.28	0.36	3.68	11.79	540	681	12.24
SD	0.88	0.18	105	3.81	0.50	3.03	7.84	47.0	110	3.65
MIN	0.30	0.08	71.7	1.87	0	0.70	1.00	453	290	6.83
MAX	3.96	1.07	609	33.89	3.80	21.64	42.94	704	818	27.3

a, Values predicted by NIRS calibrations (Coates 2009).

Table 2. The population of faecal samples derived from the forages fed to animals during the pen trials and where forage contained < 4 g P/kg DM. Concentrations of constituents of phosphorus (P) and calcium (Ca) (Total P, Acid-Insoluble P, ratio of acid-insoluble P to Total P, Total Ca, Acid-insoluble Ca, and ratio of Total Ca to total P) in the faeces samples (n = 601).

	F-TP (g/kg DM)	F-AIP (g/kg DM)	F-AIP/F-TP (g/kg)	F-TCa (g/kg DM)	F-AICa (g/kg DM)	F-TCa/F-TP (g/g)	F-TN (g/kg DM)[a]
Mean	3.28	1.33	451	9.43	0.75	3.18	12.17
SD	1.60	0.43	134	6.45	1.91	1.99	3.68
MIN	0.94	0.00	0.00	3.17	0	0.64	6.24
MAX	9.28	3.86	743	41.76	24.08	13.30	29.61

a, Values predicted by NIRS calibrations (Coates 2009).

4.2 Determination of acid insoluble P and acid insoluble Ca and their relationship with total P and total Ca

The proportion of total P which was solubilised by the hydrochloric acid was not affected by the extraction time and there was good repeatability in extraction of a soluble P (Table 3). The proportion extracted by 0.1% hydrochloric acid was 0.95 of that extracted with 0.4% hydrochloric acid. The pH of the solution at the end of the extraction interval was elevated for some samples when the 0.1% hydrochloric acid rather than the 0.4% hydrochloric acid extraction procedure was used. This occurred for lucerne forage and faeces derived from lucerne, and also to a lesser extent for *Dolichos lablab* forage and faeces derived from *Dolichos lablab*.

Table 3. The concentrations in selected forage and faeces samples of total phosphorus (P) and the proportions (g/kg) of P solubilised following 4 procedures involving 0.1% or 0.4% hydrochloric acid each for 1 or 2 hours extraction time.

		Acid extraction procedure			
	Total P (g/kg DM)	0.1% HCl 1h	0.1% HCl 2h	0.4% HCl 1h	0.4% HCl 2h
Forage samples					
Buffel_A					
Buffel_B	3.95	810	814	841	841
Dolichos	2.54	588	591	647	662
Lucerne	2.45	522	504	537	544
Speargrass	1.59	757	766	781	780
Stylo	1.77	698	706	737	712
mean	2.36	675	676	709	708
SD	0.86	119	128	119	114
Faecal samples					
Buffel_A	9.93	730	727	776	761
Buffel_B	5.51	685	699	709	697
Dolichos	4.79	651	669	700	709
Lucerne	4.49	609	592	661	644
Speargrass	2.81	551	554	568	577
Stylo	2.67	628	611	655	668
Gatton_diet	5.06	456	442	492	474
Gatton_diet	4.98	458	440	495	484
mean	5.03	596	592	632	627
SD	2.24	100	109	103	105

4.3. Prediction of diet P and Ca using faecal measurements as explanatory variables

The simple linear regression for the entire data set and before removal of diet samples with a diet P concentration > 4 g P/kg DM is shown in Figure 2. Since this relationship had an R^2 = 0.53 it indicated that faecal P concentration would provide poor prediction of diet P concentration. Thus additional explanatory variables were considered in more complex models. Multiple regression models were used to evaluate the contribution of Group 1, then Group 2, and then Group 3 (i.e. 1+2) measurements derived from faeces to predict the D-TP. There was some colinearity among the explanatory variables. The concentrations of total P and acid insoluble P, and between total Ca and acid insoluble Ca, in faeces were correlated. Since the relationships were different ($P<0.05$) for the two classes based on botanical composition (grasses and grass-legume mixtures (G, G/L) *versus* pure legumes (Leg)) separate models were developed for each of these classes; thus two prediction equations are presented for each combination of independent variables. The regression models which predicted D-TP were as follows:

Group 1.

D-TP = +0.352 + 0.3865*F-TP [R^2 0.53] G, G/L (Eqn 1A)

D-TP = +0.714 + 0.3865*F-TP [R^2 0.53] Leg (Eqn 1B)

D-TP = -0.376 + 0.2780*F-TP + 0.0936*F-TN [R^2 0.60] G, G/L (Eqn 2A)

D-TP = -0.441 + 0.2780*F-TP + 0.0936*F-TN [R^2 0.60] Leg (Eqn 2B)

D-TP = -0.077 + 0.3755*F-TP + 0.1145*F-TN – 0.6313*F-AIP [R^2 0.63] G, G/L (Eqn 3A)

D-TP = -0.398 + 0.3755*F-TP + 0.1145*F-TN – 0.6313*F-AIP [R^2 0.63] Leg (Eqn 3B)

Group 2.

D-TP = 0.404 + 0.3037*F-TP - 0.0532*F-TN + 0.0837*D-TN [R^2 0.67] G, G/L (Eqn 4A)

D-TP = -0.031 +0.3037*F-TP - 0.0532*F-TN + 0.0837*D-TN [R^2 0.67] Leg (Eqn 4B)

Group 3.

D-TP = 0.412 + 0.380*F-TP + 0.0692*D-TN – 0.533*F-AIP [R^2 0.69] G, G/L (Eqn 5A)

D-TP = 0.736 +0.380*F-TP + 0.0692*D-TN – 1.319*F-AIP [R^2 0.69] Leg (Eqn 5B)

Although F-TN), F-AICa and D-NDF were all statistically significant if added as additional independent variables into Equation 5, the increase in the R^2 value in each case was small (<0.004) compared with the increased complexity of the model. Thus it was considered inappropriate to include them.

D-TP concentration was consequently predicted from F-TP concentration with an $R^2 = 0.53$. Inclusion of F-TN in the regression model increased the R^2 to 0.60, and further inclusion of F-AIP increased the R^2 to 0.63. When F.NIRS measurements are available the inclusion of D-TN with F-TP and F-TN increased the R^2 to 0.67. Although the relationships for the grass and grass-legume diets differed from those of the pure legume diets the relationships were parallel so the regression models differed only in the constant coefficient, and not in the coefficients for the independent variables. The exception was for Equations 5A and 5B where there was an interaction between the botanical category and the concentration of AIP in faeces (F-AIP); thus the coefficient for F-AIP differed between Equation 5A and 5B. It follows from the above regression models that, in the absence of F.NIRS estimates of the diet, the D-TP can be predicted from the concentrations of F-TP and F-TN with moderate confidence. Although the concentration of F-AIP made a statistically significant ($P<0.05$) improvement in the regression model there was little improvement in the R^2; thus the additional laboratory analyses to measure F-AIP may be difficult to justify. When F.NIRS estimates of diet are available, the inclusion of D-TN concentration improved the prediction. Inclusion of F-AIP led to a further small increase in the R^2 for prediction and would arguably be of value. The concentrations of F-TCa or F-AICa from analyses of faeces, or of the diet digestibility or NDF or ADF fibre constituents, did not contribute to the prediction. There are however other advantages, as discussed below, to the F.NIRS measurements of diet attributes which contribute to adequacy of the prediction of diet P and of the P status of the animals.

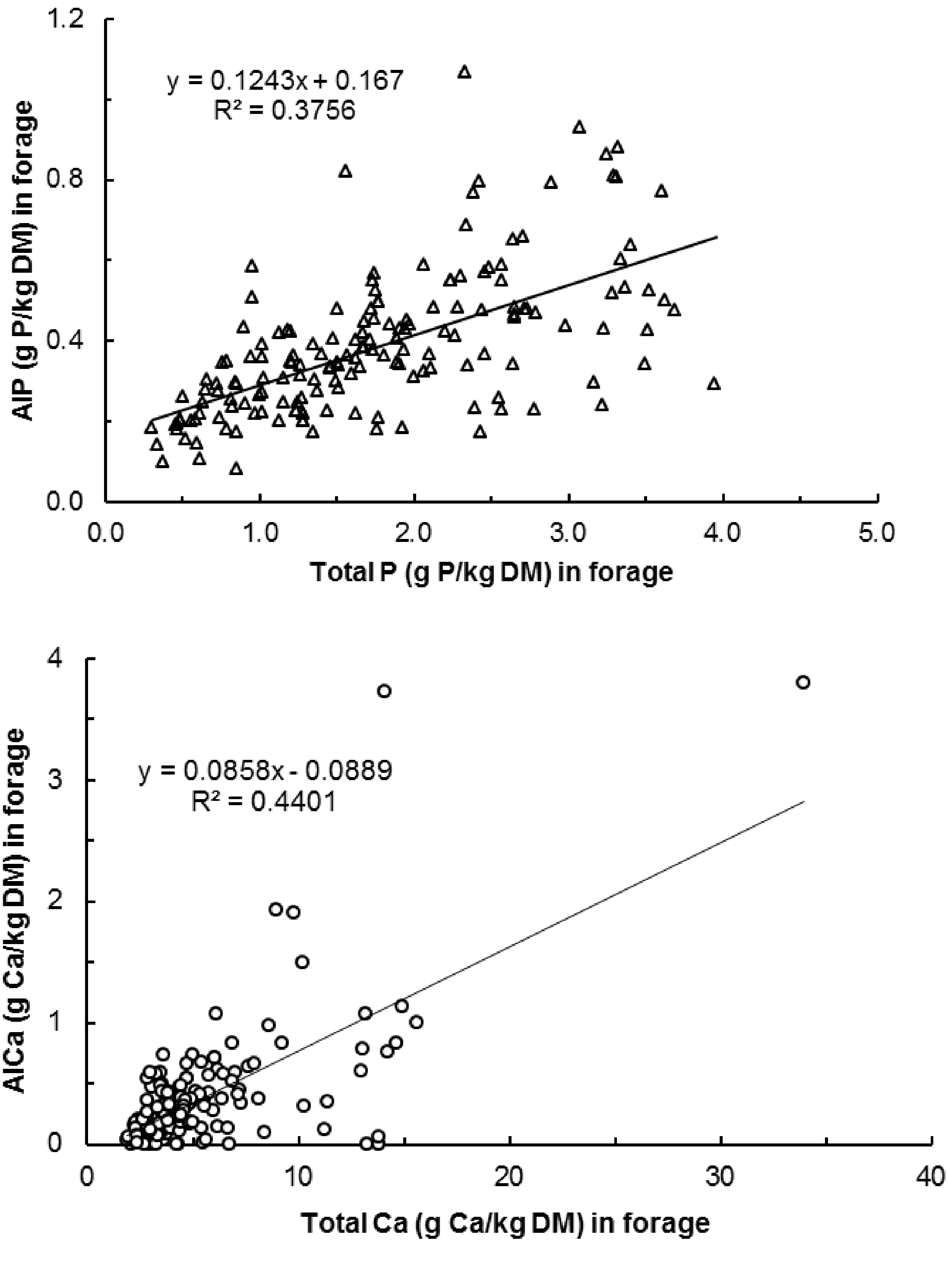

Figure 1A and 1B. Relationships in forages (A) between total P (D-TP) and acid-insoluble P (D-AIP) (R^2 = 0.38), and (B) between total Ca (D-TCa) and acid-insoluble Ca (D-AICa) (R^2 = 0.44). The 2 outlier points in Fig. 1B comprised Cavalcade and Leucaena legume.

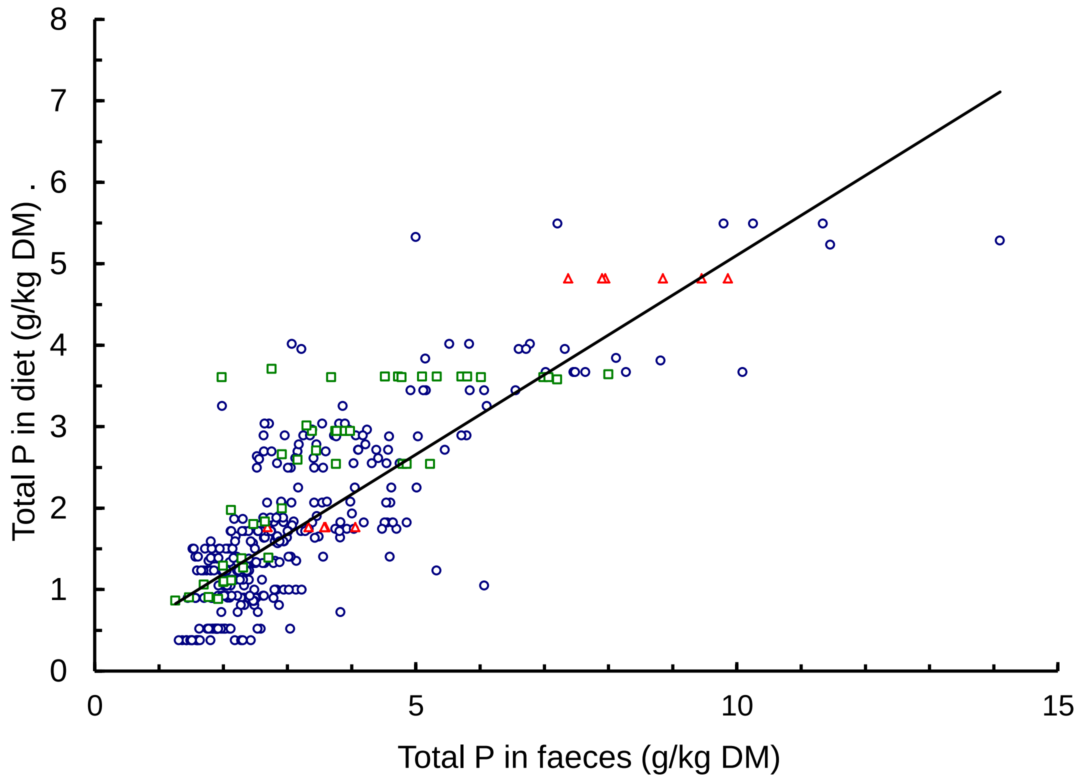

Figure 2. The relationship between the measured concentration of total P in faeces (F-TP) and the measured concentration of total P in the diet (D-TP) (g P/kg DM) before selection of the sample set with P < 4 g/kg DM. Classes of forages were as follows: o, C_4 grasses fed alone or with tropical legumes (n = 334); Δ, C_3 grasses (n = 13), and □, diets of lucerne or C_4 grass - lucerne mixtures (n = 44).

Multiple regression models were also examined to predict the diet acid insoluble P (D-AIP) concentration and the diet total Ca concentration (D-TCa) from faecal measurements. The prediction of D-AIP (or diet acid soluble P) was similar to, or not as good as, the prediction of D-TP. Given that the acid soluble P fraction of the diet is likely to be similar to, or no better than, the total P fraction as a measure of the P in the forage available to cattle, there does not appear to be any justification for the prediction of diet acid soluble P rather than D-TP. D-TCa was well predicted by the F-TCa (R^2 = 0.91) and when other variables were included the accuracy of the prediction was even higher (R^2 = 0.94).

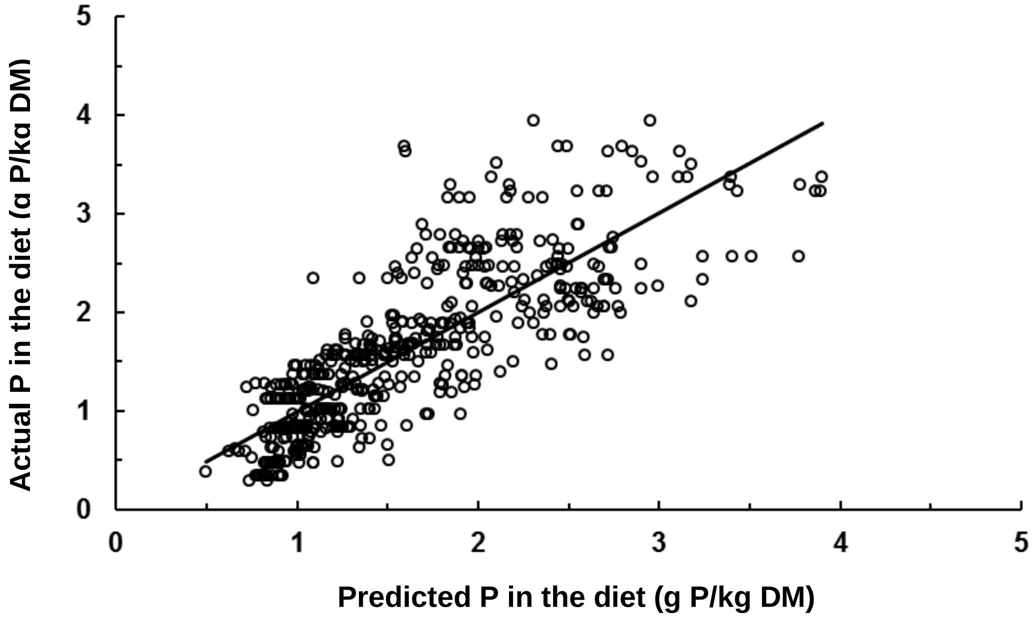

Figure 3. The relationship between the actual measured concentration of P in the diet (g P/kg DM) and the diet P concentration (g P/kg DM) predicted from three attributes measured in faeces and using by a multiple regression model (Equation 4A and 4B above) in tropical forages containing < 4 gP/kg DM. The attributes measured in faeces were the concentration of total P, the concentration of total N, and diet N as measured by F.NIRS. The equation was as follows:
$Y = 1.0086X – 0.0125$ (n = 523, $R^2 = 0.67$).

4.4. Effects of time of sampling through the daily cycle on faecal P concentrations

There was only a small change in concentrations of both the TP and AIP fractions in faeces during the daily cycle in steers fed once daily and where the P was mixed through the diet (Table 4), even in the high P diet where most of the P was in the form of dicalcium phosphate. These results indicated that in these steers fed *ad libitum* in pens the time of day when faecal samples are obtained had only a small effect on the faecal P concentration and thus on the estimate of dietary P concentration calculated from faecal P concentration. This experiment has been published (Dixon *et al.* 2016) (Appendix 1).

4.5. Prediction of diet P and Ca in diets containing inorganic P as a supplement to forage diets.

In this experiment there was no effect on the slope of the simple linear regression relationship between the concentrations of total P in faeces and in the diet (Figure 3). However there was a large difference in the elevation so that at a faecal P concentration of 2 g total P/kg DM (when diet P would be marginal in a growing animal) the diet P concentration was predicted to be 67% greater than in the diet of forage alone. Thus major error was introduced if the regression model developed for forage diets was used to predict diet P concentration from the faecal P concentration, or an underestimation of diet P by about 60%. The data set was considered not to be adequate to explore the error in the prediction of diet P when the multiple regression models were applied.

On the basis of this information we conclude that the the multiple regression models developed in the present study to predict diet P from measurements in faeces are not appropriate when P supplements are being fed.

Table 4. The concentrations of total phosphorus (F-TP) and acid insoluble P(F-AIP) in the faeces of steers fed a low P semi-purified diet (LowP) or of six steers fed the same diet with addition of calcium phosphate (HighP). Faeces were sampled during six intervals during the 22 hours after feeding

Measurement	Diet	Interval after feeding during the 24 hour cycle (h)					s.e.m. (DxT)	Probability		
		0-4	4-8	8-12	12-16	16-22		D	T	DxT
Percent of intake ingested during each interval[#]	LowP	45	33[a]	13	5[a]	4	4.1	n.d.	***	n.s.
	HighP	38	27[b]	18	7[b]	10				
F-TP (g/kg DM)	LowP	3.35	3.38	3.36	3.31	3.43	0.19	***	n.s.	n.s.
	HighP	4.90	4.83	5.26	5.24	4.77				
F-AIP (g/kg DM)	LowP	1.88	1.89	1.90	1.91	1.93	0.069	***	n.s.	n.s.
	HighP	2.39	2.24	2.27	2.32	2.28				

[#]Percent of total daily feed intake which was ingested during the interval indicated. D, diet; T, time interval. n.d., not determined; n.s., not significant; ***, $P<0.001$. s.e.m., standard error of the mean. Superscripts within columns indicate that the percent of intake ingested during interval was different between diets ($P<0.05$).

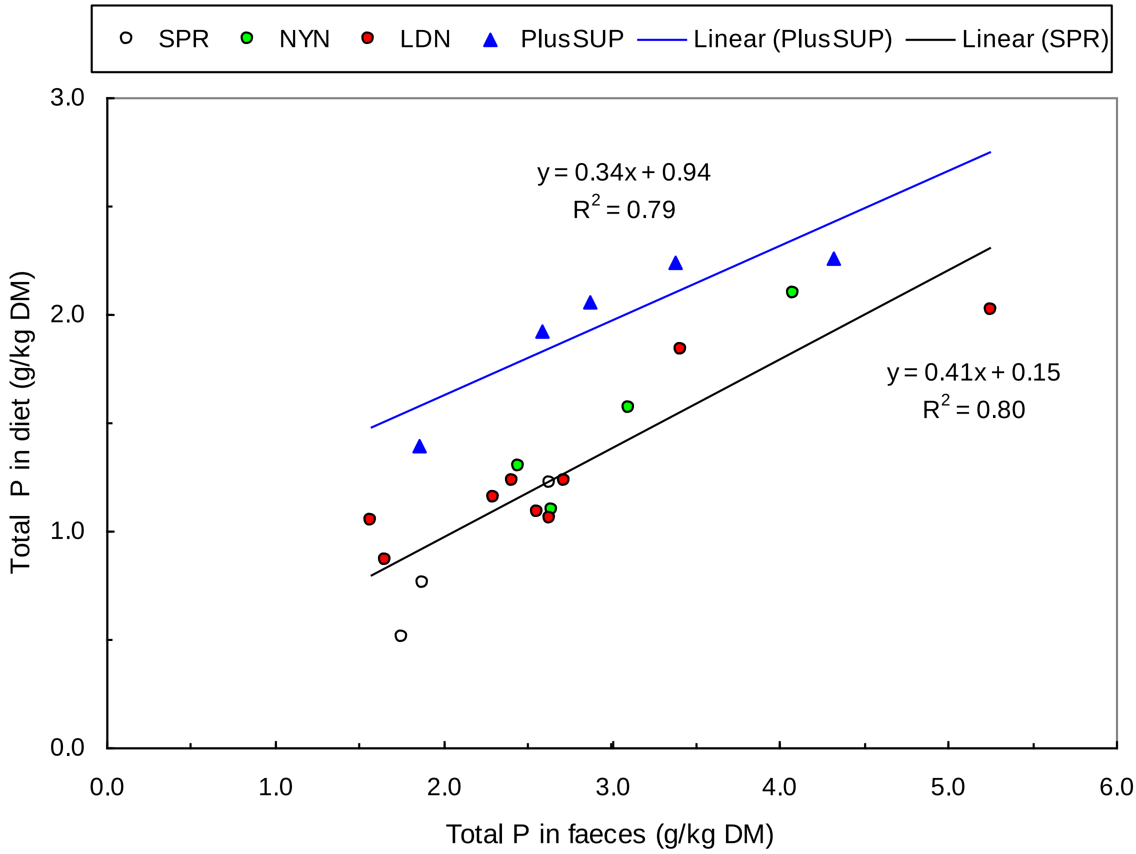

Figure 4. The relationships between the measured concentration of total P in faeces and measured total P in the diet ingested by grazing cattle without P supplements at Springmount (SPR, ○), Narayen (NYN, ●) and Lansdown (LDN. ●), or with P supplements (▲). (D B Coates, unpublished data).

4.6. Prediction of diet P and Ca in diets containing concentrates with forage

Two sets of recent experimental results where cattle have been fed semi-purified P-deficient diets containing a high proportion of readily fermentable carbohydrate were available for comparison of actual diet P concentrations with those predicted by the multiple regression models developed in the present study. In both of these studies the P-deficient diets were semi-purified diets based on cereal straw, wheat flour, sugar, and in one of the studies, gluten. In the first of these studies (Project B.NBP.0689, Task 1, R M Dixon unpublished results) heifers were highly P deficient (plasma inorganic phosphorus <1 mmol/L) as a consequence of being fed a P-deficient diet for > 3 months. In these heifers the concentration of total P in the diet predicted from the total P in faeces for 9 diet-faecal pairs of samples and using Equation 1A (above) was on average 220% (SD = 40%) of the actual measured diet P concentration. In the second of these studies (Quigley *et al.* 2015) steers were fed a semi-purified diet of high ME content and a range of diet P concentrations was achieved by addition of dicalcium phosphate. The study indicated that the concentration of P in the faeces was about 3 g P/kg DM between 50 and 175 days of feeding of the most P-deficient diet containing 0.91 g P/kg DM. Equation 1A (above) predicted a diet P concentration of 1.5 g P/kg DM which was 166% of the actual measured diet P concentration. In these two independent studies the regression models developed in the present study from tropical forage diets seriously overestimated the diet P concentration in cattle fed semi-purified diets containing a high proportion of high readily fermentable carbohydrate as starch and sugar.

On the basis of this information it is concluded that the the multiple regression models developed with forage diets in the present study to predict diet P from measurements in faeces are not appropriate when concentrate supplements are being fed.

5 Discussion

5.1. Limitations of the sample set and the multiple regression models

All of the diets included in the data set used to develop the multiple regression models of diet P concentration were tropical grass or grass-legume forages fed without additional P or concentrates. The N concentration and DM digestibility (Table 1) were (mean ±one SD unit and encompassing about 67% of the sample population) were 11.8 (4 - 20) gN/kg DM [7.4 (3-12) crude protein %), and 540 (490 – 590) g/kg, respectively. Thus this population of samples should have represented forage quality across the annual range for mainstream pastures; wet season grass or grass-dominant pastures seldom exceed about 18 gN/kg DM or 600 g/kg DM digestibility except for a few weeks at the peak feed quality during the wet season. Hence the models to predict diet P concentration should be generally applicable to grazing cattle including when grazing wet season pastures.

In the present sample set measurements were not available of the concentrations of neutral detergent fibre (NDF) or acid detergent fibre (ADF) in faeces. Therefore it was not possible to examine the inclusion of these faecal constituents into the multiple regression models to estimate diet P. Correlation relationships between P concentration and fibre concentration could be expected if only because as grasses senesce the P concentration usually decreases as the proportions of the fibre constituents increase. Since many laboratories can routinely measure these constituents in faeces using wet chemistry their contribution to the regression models is of interest, especially for laboratories without access to NIRS instrumentation and F.NIRS calibrations. Since it was expected that concentrations of NDF and ADF in faeces would be correlated with the respective concentrations in the diet, the latter were examined in the models as indicative of whether they would contribute to the regression models to predict diet P concentration.

There are two reasons the regression relationships between faeces and diet P concentration can be expected to differ in cattle ingesting high quality forage diets. First, with higher DM digestibility there will be a smaller amount of faecal DM excreted per kg diet intake, so the same amount of P excreted in faeces will be associated with a higher concentration of P in the faeces. Second, if cattle are consuming high quality forage *ad libitum* as usually occurs during the wet season then the growing animal should be gaining LW rapidly and the breeder cow should be in late pregnancy or early lactation. The deposition of both soft tissue and bone during LW gain, and the growth of conceptus in late pregnancy or milk synthesis, will create sinks for net deposition of P. If P is deposited it is clearly not available to be excreted in faeces and the concentration of P in faeces must be decreased. Since P deficiency in northern Australia most often occurs in growing cattle grazing high quality forages during wet season this becomes a important consideration for the interpretation and application of faecal P measurements to estimate diet P concentration.

5.2. Fractions of P in forages and faeces

The measurement of acid soluble and insoluble fractions of the total P in cattle faeces has been reported in the context of temperate agricultural systems (Kebreab *et al. 2005;* Dou *et al.* 2007; Nordqvist 2012) and is comparable with other *in vitro* solubilisation procedures to estimate the availability of P in feedstuffs for livestock. Thus a similar approach and extraction procedure was used as a measure of the acid soluble and insoluble fractions of phosphorus in the forages and the faeces of cattle grazing tropical pasture systems. Since this approach has not been reported for tropical forages or for the faeces derived from such diets an examination of the solubilization procedures was examined in forages and faeces representing the range of tropical forages, and also in two samples of faeces from steers fed semi-purified diets low or high in P content (Quigley *et al.* 2015).

The solubilisation in 0.4% hydrochloric acid for one hour was selected as an optimal and repeatable procedure. In the sample set considered in the present project to develop an appropriate laboratory procedure to estimate a P fraction soluble in hydrochloric acid the proportion of total P which was solubilised by the hydrochloric acid was not affected by the extraction time and the repeatability in extraction of a soluble P fraction and between duplicate analyses was excellent (Table 3). The proportion extracted by 0.1% hydrochloric acid was 0.95 of that extracted with 0.4% hydrochloric acid. The pH of the solution at the end of the extraction interval was elevated for some samples when the 0.1% hydrochloric acid rather than the 0.4% hydrochloric acid extraction procedure was used. This occurred for lucerne forage and faeces derived from lucerne, and also to a lesser extent for *Dolichos lablab* forage and faeces derived from *Dolichos lablab*. This was of concern during development of the laboratory procedure even though there was little effect on the proportion of P solubilised in the present group of samples. It suggested that under some circumstances and for some classes of samples insufficient acid would be present when the 0.1% hydrochloric acid extraction solution was used. This effect could be expected to be larger and even more important when there is contamination of the forage or faecal samples with calcarious soils such as commonly occur in northern Australia. The conclusion drawn was that the 0.4% hydrochloric acid solution and a 1 hour extraction interval were most appropriate procedure and was therefore used. The solubility of P in the forage and faecal samples incubated in a solution of 0.4% hydrochloric acid for 2 h was measured following a procedure similar to that used by Quigley *et al.* (2015) to estimate the availability of P in various sources of phosphorus as feedstuffs, and was designed to mimic conditions of abomasal digestion. Consequently the observation in the present study that about 80% of the total P in the forages was soluble in hydrochloric acid (Figure 1A) indicated that this same proportion of P in the forages was available to cattle.

5.3. Multiple regression models

The simple linear regression between the concentrations of total P in faeces and in the diet with an R^2 of only 0.53 was clearly not satisfactory for prediction of diet P from this measurement in faeces. This is in contrast with the good relationships reported for sample sets within experiments, or within groups of experiments, collated by Dixon and Coates

(2010, 2011). A number of factors may explain the poor relationship in the present study. First, the sample set used in the present study was very large and represented a very diverse range of tropical forage species, growing environments and plant maturity compared with the sample sets used in the previously reported experiments. Second, samples were from experiments conducted in a number of laboratories and using a variety of animals and experiment procedures. Third, many of the diet-faecal pair samples were obtained after an adapation interval to a new diet of 5-10 days; this diet adaptation interval was appropriate to provide diet-faecal pairs for the faecal NIRS calibrations for which the experiments were designed, but was much shorter than is generally used in ruminant nutrition. Furthermore it is known that in ruminants phosphorus metabolism may require many weeks (e.g. Quigley *et al.* 2015) or months (e.g. Gartner *et al.* 1982) to reach a new equilibrium and stable concentrations of blood P or faecal P concentration following a change of diet. Also, it is likely that in some of the experiments to develop diet-faecal pairs animals in P-deficient status were introduced to high P diets, or animals in high P status introduced to low P diets, and the diet-faeces diet pairs obtained without time for adaptation and establishment of a new equilibrium. It is expected that deposition of P to tissues during LW gain or to bone during net bone P deposition, or mobilization of P from tissues during LW loss or net bone mobilization, would introduce a deviation into the relationship between the concentrations of P in the diet and in faeces and thus reduce the R^2 of the relationship. The equations developed in the present study were limited to diets of forage alone, and are not appropriate for diets containing concentrates or inorganic P supplements such as dicalcium phosphate.

The capacity of the multiple regression models to predict diet total P concentration (D-TP) from faecal measurements, with R^2 up to 0.69, was considered sufficiently reliable for this approach to be used as a guide to indicate diet P adequacy in grazing growing cattle at about maintenance energy intake or slow LW gain. In laboratories not able to provide F.NIRS measurements of diet quality due to lack of instrumentation or of calibration equations (i.e. restricted to Group 1 measurements of faeces) it was clear that the total N concentration in faeces (F-TN) as well as the total P concentration of faeces (F-TP) should be measured and used to improve the prediction of diet P (D-TP). The additional measurement of acid insoluble P (F-AIP) involves only simple laboratory procedures and led to a small improvement in the R^2 of prediction of diet P ($P<0.05$) from R^2 = 0.60 to an R^2 = 0.63. However for laboratories also able to provide F.NIRS measurements of diet quality (Group 2 measurements of faeces) the inclusion of the diet N concentration improved the prediction, and inclusion of the acid insoluble P concentration in faeces increased the R^2 to 0.69. Clearly the identification of faecal samples into the classes of grass and grass-legume mixtures *versus* diets consisting entirely of legume substantially improved the accuracy of the prediction of diet P. Since this information could usually be obtained by observation of the pasture system at the time of sampling faeces, and is information which will usually be known and also required for other aspects of grazing animal management, the use of the separate equations for the 2 classes of pasture appears warranted. If this information is not available from field observation at sampling of faeces then it could be evaluated in most tropical pasture circumstances by determination of the $^{13}\delta C$ ratio of faeces by mass spectroscopy. Neither the digestibility of the diet (D-DMD) nor the fibre concentration (D-NDF or D-ADF) of the diet contributed ($P>0.05$) to the multiple regression models; possibly this was because the diet N concentration (D-TN) was already included in the regression model and was correlated with digestibility and fibre concentration.

It was also possible to develop equations to predict diet total Ca concentration (D-TCa) for the sample set examined. D-TCa concentration was well predicted (R^2 = 0.91) simply from the concentration of total Ca in faeces, although the prediction was improved slightly by inclusion of additional explanarory variables.

Apart from the estimates of F-TP and D-TP, related predictors such as the ratios of F-TP concentration to diet ME concentration (F-TP/ME), and the ratio of diet total N concentration to F-TP, have been proposed as useful field indicators of the adequacy of diet P in the diet selected by various classes grazing cattle (Jackson *et al.* 2012; D B Coates, unpublished data). In the Phosphorus Producer Manual (Jackson *et al.* 2012) the ratio faecal P / diet ME was presented as an index of the adequacy of P in the diet of grazing cattle. The advantage is that such ratios change through a much smaller range than does diet P or faecal P concentration as the ME intake and the productivity of the animal increases. However the calculations and FP/ME ratios presented in the P Producer Manual (Jackson *et al.* 2012) were calculated on the assumption that the simple linear regression between faecal P and diet P reported by Dixon and Coates (2011) could be applied across northern Australian pasture systems. The results of the present study in which a much larger data set was analysed clearly indicated that in many circumstances diet P is not well correlated with faecal P, and this will lead to large error in use of the FP/ME ratio or the diet N/FP ratio. Furthermore observations from an experiment at Brunchilly (Quigley *et al.* 2015), and some anecdotal reports from commercial cattle properties, have also provided evidence that under some circumstances the ratio faecal P / diet ME does not provide reliable estimates of the diet P intake.

In conclusion it appears that the FP concentration, or ratios of FP with diet N or diet ME, do not provide acceptably reliable estimates of the diet P concentration or intake. The most appropriate alternative appears to be to use the measurements in faeces and other available information (faecal total P concentration, faecal total N concentration, diet N concentration, faecal acid insoluble P concentration, botanical class of the diet) in the multiple regression models described above to estimate diet P concentration in the young animal at about maintenance metabolisable energy (ME) intake. This can be used in conjunction with other information about the diet (e.g. the ME and N contents of the diet from F.NIRS) and estimates of the physiological state and productivity of the animal, to indicate whether the diet selected is likely to be adequate in P.

5.4. Effects of time of sampling through the daily cycle on faecal P concentrations

There were only small changes in the faecal concentrations of both total P (F-TP) and acid insoluble P (F-AIP) during the daily cycle, even in the high P diet where most of the P was in the form of dicalcium phosphate. These results indicated that in these steers fed *ad libitum* in pens the time of day when faecal samples are obtained had only a small effect on the faecal P concentration and thus on the estimate of dietary P concentration calculated from faecal P concentration. However, in this experiment the cattle were fed a diet with the P (as constituents of the feedstuffs or as dicalcium phosphate) mixed through the diet. These results do not establish the variation in faecal P concentration in cattle ingesting a P supplement during a brief interval during the daily cycle, or on one occasion each several days. Thus further information is needed for grazing cattle and where a P supplement is effectively available to be ingested during only an interval during the daily cycle. This experiment has been been published (Dixon *et al*. 2016).

From the studies of Kebreab *et al.* (2005), Dou *et al.* (2007) and Nordqvist (2012) it appeared likely that the acid-insoluble P fraction in faeces (F-AIP) would primarily comprise P bound with indigestible fibre. The observation that the concentrations of insoluble P in faeces was 1.90 and 2.30 g P/kg DM, and 565 and 460 mg P/g total P, in the low P and high P diets, respectively, indicated that some P in undigested microbial debris in faeces and/or in undigested dietary inorganic P was not solubilised by the acid treatment of the samples. These results indicated that the acid-insoluble P concentration in faeces (F-AIP) does not provide a simple and direct measurement of the diet P bound with indigestible fibre and does not provide a simple analysis to circumvent the difficulties of estimating diet P concentration in P-supplemented diets.

5.5. Considerations of the diet and animal factors likely to influence the relationship between the concentrations of P fractions in faeces and in the diet

The relationship between the concentration of P in the diet and the concentration of P in faeces is potentially influenced by numerous factors. In a simple equilibrium model if there is no net deposition or mobilization of P into or from body tissues or a foetus, and if there is negligible excretion of P in urine or milk, then the amount of P excreted in faeces must equal the intake of P. Since the amount of faecal DM excreted is a simple function of diet DM digestibility and daily DM intake, then the concentration of P in faeces must in these circumstance be a function of the concentration of P in the diet and DM digestibility. For example if DM digestibility is 500 g/kg then faecal P concentration would be 2 times diet P concentration.

In many situations there will be net deposition of diet P into sinks in the form of soft tisse and bone growth, milk secretion and conceptus growth. Alternatively during liveweight loss there will be net mobilization of P from both soft tissues and bone, and there may be substantial mobilization of bone P to meet short-term deficits of P. The amounts of P deposited or mobilized during soft tissue growth or mobilization, during milk synthesis, and for conceptus growth, can be estimated with confidence from feeding standards (e.g. ARC 1980; CSIRO 2007) if the amounts of tissue gained or lost, milk production, and stage of gestation, are known. The potential rate of gain or loss of bone P in addition to that gained or lost concurrently with soft tissues is more difficult to estimate and cannot be measured routinely. However extrapolation from measurements of the changes in bone P during prolonged severe P deficiency suggest that 3-5 g bone P per day may be mobilized in mature breeder cattle, or in P deficient growing cattle the deposition of P into bone may be reduced by a comparable amount (Coates *et al.* 2016; Dixon *et al* 2016). A further consideration is that separate processes affecting net mobilization or deposition of P may tend to cancel in some circumstances; for example numerous studies have shown that in lactating dairy cows, goats and sheep there is net mobilization of bone P which will provide some of the additional P required for milk secretion (Braithwaite et al. 1986; Deitert and Pfeffer 1993; Rodehutscord et al. 2000; Knowlton and Herbein 2002; Valk et al 2002). However in early lactation voluntary intake usually increases, by 20-30% in the *Bos indicus* cow (Penzhorn and Meintjies 1972; Hunter and Siebert 1986), and this will increase P intake. It is clear that during replenishment of P following an interval of diet P deficiency the P retention may be much greater than can be attributed to soft tissue gain indicating that substantial net deposition of P into bone can occur (Bortolussi *et al.* 1999) and bone growth can be more rapid in previously P depleted animals (Quigley and Poppi 2013).The observation by Dixon and Coates (2011) that the diet P and faecal P concentrations in groups of steers and lactating breeders were well described by a linear regression (R^2 = 0.81) provides evidence that in northern breeders the additional P required for milk synthesis can be derived substantially from bone mobilization; the observation that diet P concentration and faecal P concentration in growing and lactating cattle could be described by a single linear regression cannot otherwise be explained.

A number of the factors which influence faecal P concentration discussed above would not have been represented or encompassed by the data set used in the present study to develop the multiple regression models to predict diet P concentration (D-TP). The animals used were young cattle with potential for growth and were fed forage diets for short intervals (generally < 3 weeks). Diet quality was usually sufficient for the cattle to be in slow LW loss through to slow LW gain and the data set did not include animals in rapid LW gain, pregnancy or lactation. It follows that there may be considerable bias error in prediction of the diet P concentration (D-TP) from the multiple regression models where cattle are in physiological states different to the animals used in the present study.

As discussed above as diet digestibility increases and there is a smaller amount of faecal DM for a specified excretion of P per day the concentration of P in faeces can be expected to increase. It was therefore unexpected that diet digestibility was less important and significant than diet total (D-TN) as an explanatory variable in the multiple regression models. Possibly this was because D-TN had an important influence than DMD on voluntary intake since in many of the diets D-TN was < 10 g N/kg. The mean D-TN was 11.8 (SD 7.84) g N/kg DM (Table 1) and tropical forage diets less than about 10 g TN/kg DM are expected to be N deficient (Minson, 1990), thus almost half of the diets were expected to be deficient in D-TN. Also D-TN concentration was correlated with diet DMD.

An additional consideration is that the multiple regression models developed to estimate diet P concentration from faeces in the present study are applicable only to tropical forage diets and in the absence of diet concentrates or P supplements. There were apparently entirely different relationships between diet P and faecal P concentrations (D-TP, F-TP) in cattle fed semi-purified diets containing substantial proportions of flour and sugar. The difference may be associated with the much greater rumen fermentation and microbial synthesis from the readily fermentable carbohydrate in the semi-purified diets, and the presence of microbial debris high in P in faeces. It has been shown (Rodehutscord *et al.* 2000) that in lactating goats fed P-deficient diets the addition to the diet of a readily fermentable carbohydrate in the form of potato starch was associated with a large increase in faecal P excretion and in concentration of P in faeces; this was likely due to increased hindgut fermentation due to the starch and excretion of the microbial material in faeces. The high ratios of faecal P concentration to diet P concentration, or of faecal P concentration to diet ME content, in the studies with semi-purified diets high in readily fermentable carbohydrate are consistent with this observation in goats, and do not challenge, the validity of the estimation of diet P concentration in forage diets as described in the present study.

In order to explore the likely consequences of differences in diet digestibility, net deposition of P into soft body tissues during growth, mobilization of P from body tissue and bone reserves during LW loss, and deposition of P into bone body reserves during repletion, calculations were made of the change in diet P concentration for various scenarios of diet DMD, LW change, milk secretion and bone P mobilization / deposition. Example scenarios are given in Table 5. A number of these scenarios would represent unusual extremes of situations of northern grazing cattle, but this was done to examine the potential of some effects. Scenarios included diet digestibility ranging from 40% to 67%, LW loss of 0.3 kg/day on a diet typical of the dry season, LW gain of 0.9 kg/day on a diet typical of the wet season, and a cow lactating during the wet season, and little consideration was given to the the expected associations between these variables in grazing cattle. For example in practice a high growth rate would only be associated with high diet digestibility. The consequences for the ratio faecal P to diet ME (F-TP/ME) for P adequacy was calculated and compared with the values estimated for the P Producer Manual (Jackson *et al.* 2012). The tabulated values show that considerable deviation from the estimated FP/ME value can be expected to occur for the animal in high LW loss or high LW gain. In the absence of experimental measurements the assumed values for deposition of P or mobilization of P are highly speculative but do indicate the potential importance of variation in the factor. For example in Scenario 7 the calculation assumes a net deposition of 10.7 g P/day; this was based on measurements of up to the equivalent of 7 g P/day in steers during P repletion and gaining 0.5 kg/day (Bortolussi *et al.* 1999), and measurements of net P deposition during late lactation in dairy cows of up to the equivalent of about 14 g P/day in a 400 kg dairy cow. There are no comparable data available for beef breeder cows. Furthermore it is important to comment that these calculations do not accommodate any capacity of the animal to use body P reserves to provide for short-term dietary deficiencies of P or to deposit surplus available P. This was also emphasized in the P Producer Phosphorus Manual (Jackson *et al.* 2012). Althought the threshold F-TP/ME ratios will change if there is mobilization / deposition of body P reserves there is presently no appropriate method to measure of the extent of this mobilization / deposition on-farm. Regardless, the calculations presented in Table 5 demonstrate how F-TP/ME ratio may be affected by animal status as well as diet factors, and the application of the F-TP/ME ratio may be constrained to specified mainstream cattle status situations. The same problem will occur with variation in the D-TP/ME ratio with differences in diet digestibility, LW loss or gain, milk secretion, and bone P deposition or mobilization. In general estimation of mobilization or deposition of body P reserves will presumably have to depend on other approaches such as the measurement of metabolic or endocrinological markers. These are not presently developed or available as diagnostic tests. In contrast, faecal NIRS technology which provides in many circumstances measurements of organic constituents of the diet, has a role to provide information about the diet and animal status in addition to the conventional wet chemistry measurements of the P fractions in faeces.

Given the number and complexity of factors determining faecal P output and faecal P concentration it is inevitable that there may be substantial error in estimation of diet P concentration (D-TP) of grazing cattle across northern Australia. The most reliable approach to evaluate diet P intake and the P status of grazing cattle in undoubtedly the P-Screen test based on blood and faecal samples from sentinel growing cattle which have grazed a paddock through the wet season and are tested late in the wet season. This circumstance with a sentinel group grazing the paddock through the wet season and sampled late in the wet season should also be appropriate to obtain most reliable results from measuremements of faeces alone. The sentinels should have exhausted any capacity to mobilize bone P and should be in moderate growth rate on a medium digestibility diet.

In conclusion the present study indicated that measurements of faeces will often provide a useful guide of the diet P intake of grazing cattle not fed P supplements and during the wet season when cattle are most likely to respond to P supplements. This role will be most useful where it is not possible to obtain blood samples for the P-Screen diagnostic test. The use of faecal samples also has the advantage that a sequence of samples can be easily obtained from the herd, whereas the P-screen test can generally be done only infrequently when cattle are yarded.

Table 5. The calculated effects of changes in diet DM digestibility and or changes in net deposition/ mobilization of body P reserves on the concentration of faecal TP required, and the ratio faecal TP/ diet ME required, to meet nutritional requirements of cattle. As discussed in the text the scenarios are intended to represent extremes of the range of situations likely to occur with grazing cattle in northern Australia and although some of the input values for the calculations are highly speculative indicate the variation which may be associated with variation in circumstances.

Scenario		Diet DMD%	Deposition / mobilization of body P (g/d)	Expected F-TP (g/kg DM) based on the 1:2 relationship	Expected F-TP (g/kg DM) after allowing for deposition / mobilization	Expected FP/ME ratio (mg P/MJ ME) after allowing for deposition / mobilization	FP/ME ratio (mg P/MJ ME) with no allowance for deposition / mobilization
1	Steer, 400 kg, nil LW change. Wet-dry transition season. Intake 20 gDM/kgLW.d. Diet P = 1.0 g/kgDM	50	Nil	2.0	2.0 (no difference)	290	290
2	Steer, 400 kg, nil LW change. Wet-dry transition season. Intake 20 gDM/kgLW.d. Diet P = 1.0 g/kgDM	40	Nil	2.0	1.7 (-17% difference)	323	290
3	Steer, 400 kg, nil LW change. Wet-dry transition season. Intake 20 gDM/kgLW.d. Diet P = 1.0 g/kgDM	67	Nil	2.0	3.0 (+52% difference)	309	290
4	Steer, 400 kg, Losing 0.3 kg/day LW. Dry season Intake 15 g DM/kgLW.d. Diet P = 1.0 g/kg DM	48	Mobilization of 3 g P/ day from tissues and bones with LW loss	2.0	2.9 (+44% difference)	418	220
5	Steer, 400 kg, Gaining 0.9 kg/day LW. Wet season Intake 29 g DM/kg LW.d. Diet P = 1.5 g/kg DM	60	Deposition of 5.7 g P/day into tissues and bones with LW gain	3.0	2.5 (-16% difference)	293	360
6	Lactating cow, 400 kg, 5 kg milk/day, nil LW change. Wet season Intake 24 g DM/kg LW.d. Diet P = 1.74 g/kg DM	54	Deposition of 6 g P/day into milk	3.5	2.8 (-20% difference)	364	420
7	Steer, 400 kg, Gaining 0.9 kg/day LW. Wet season. Intake 29 g DM/kg LW.d. Diet P = 1.5 g/kg DM.	60	Deposition of 10.7 g P/day into tissues and bone reserves	3.0	1.4 (-52% difference)	167	360

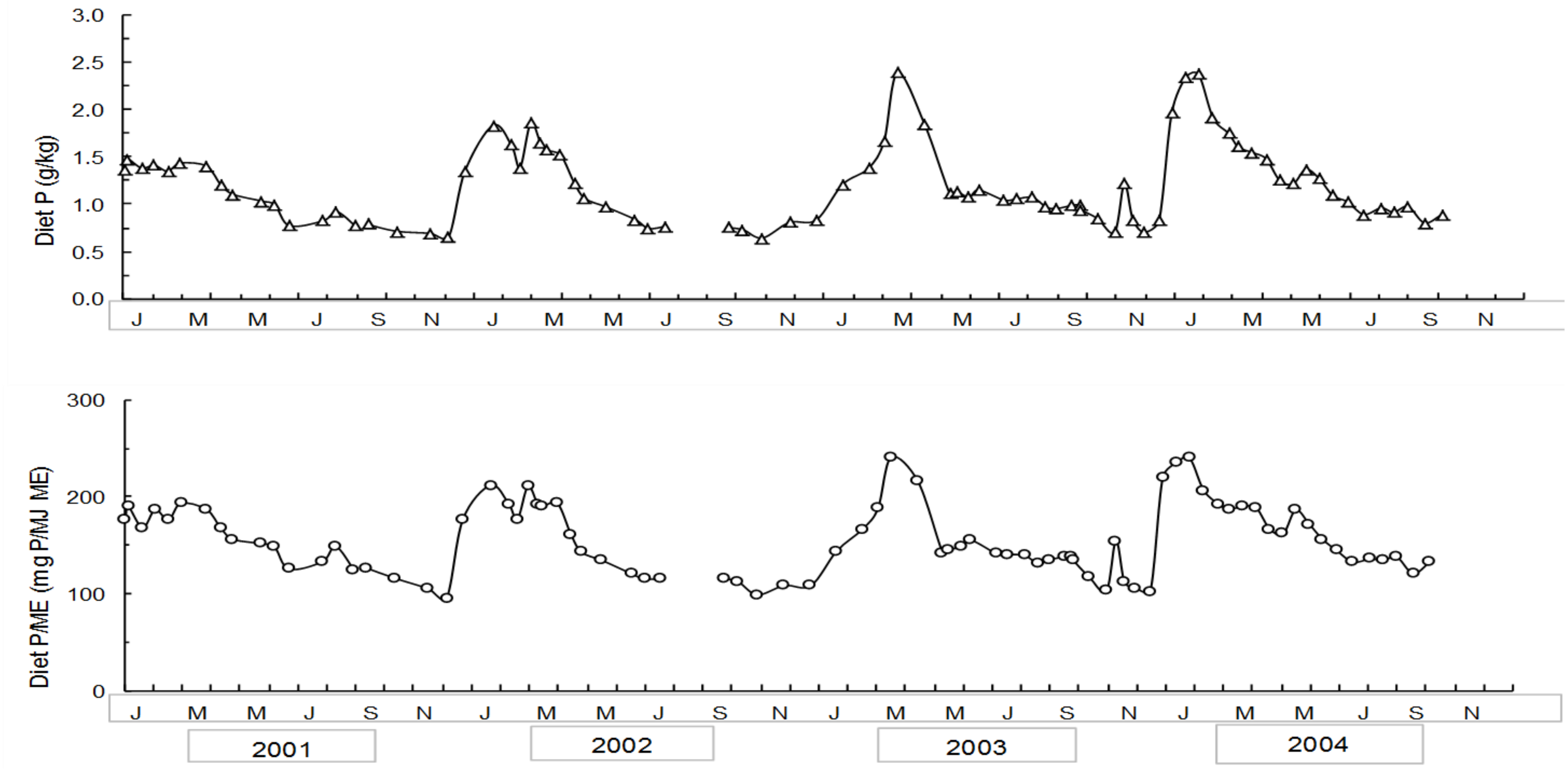

Figure 5. Estimates of the concentration of total P, and of the diet P / diet ME ratio, in the diet selected by young breeder cows (non-pregnant non-lactating, or through late pregnancy and lactation, grazing native pasture at Swans Lagoon through 4 annual cycles (Dixon et al. 2007).
P requirements for 400 kg breeder at LW maintenance: (i) dry non-pregnant 1.0 gP/kgDM, lactating 1.7 gP/kgDM, (ii) dry non-pregnant 130 mgP/MJ ME, lactating 220 mgP/MJ ME. The paddock was expected from other knowledge of this research station to be marginal in P status ie not acutely P deficient.

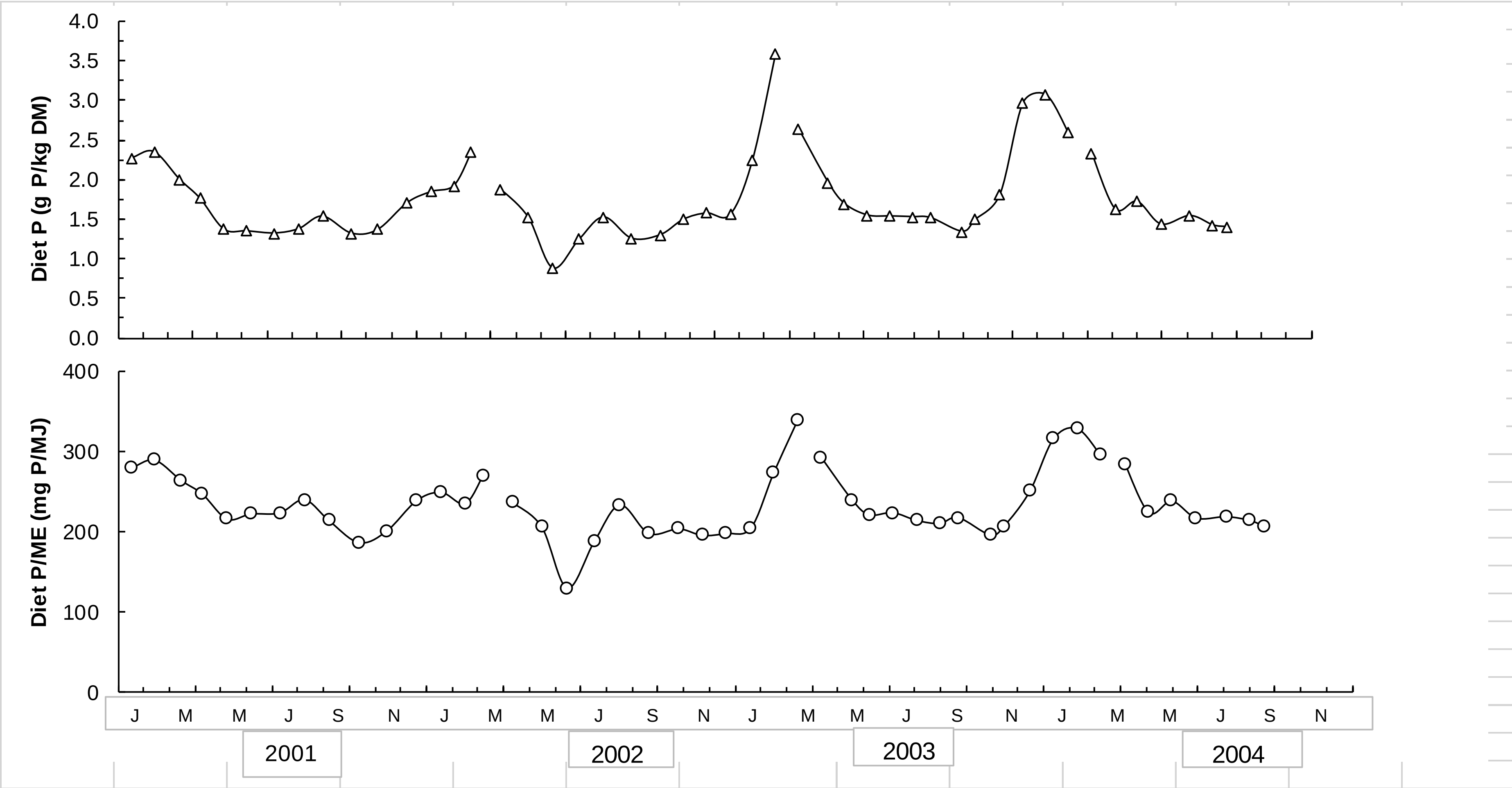

Figure 6. Estimates of the concentration of total P, and of the diet P/diet ME, in the diet selected by yearling heifers grazing native pasture at Swans Lagoon (Dixon 2008). The diet P concentration required by 200 kg growing heifers for 0.6 kg/day LW gain was 1.8 g P/kgDM or 220 mgP/MJ ME. The paddock was expected from other knowledge of this research station to be marginal in P status for grazing cattle.

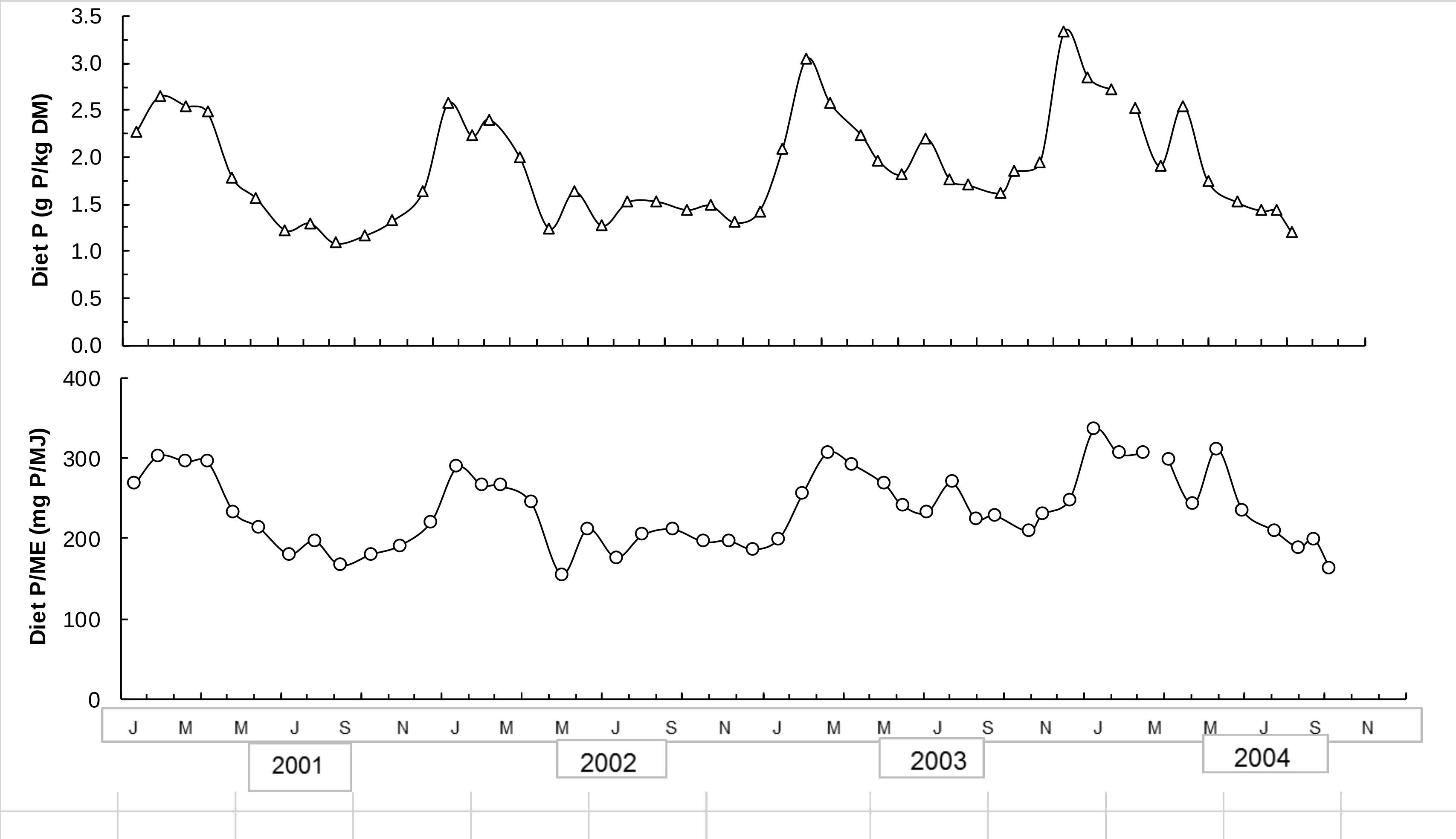

Figure 7. Estimates of the concentration of total P in the diet selected by yearling heifers grazing native grass- stylo legume pasture at Swans Lagoon (Dixon 2008). The diet P concentration required by 200 kg growing heifers for 0.6 kg/day LW gain was 1.8 g P/kgDM or 220 mgP/MJ ME. The paddock was expected from other knowledge of this research station to be marginal in P status for grazing cattle.

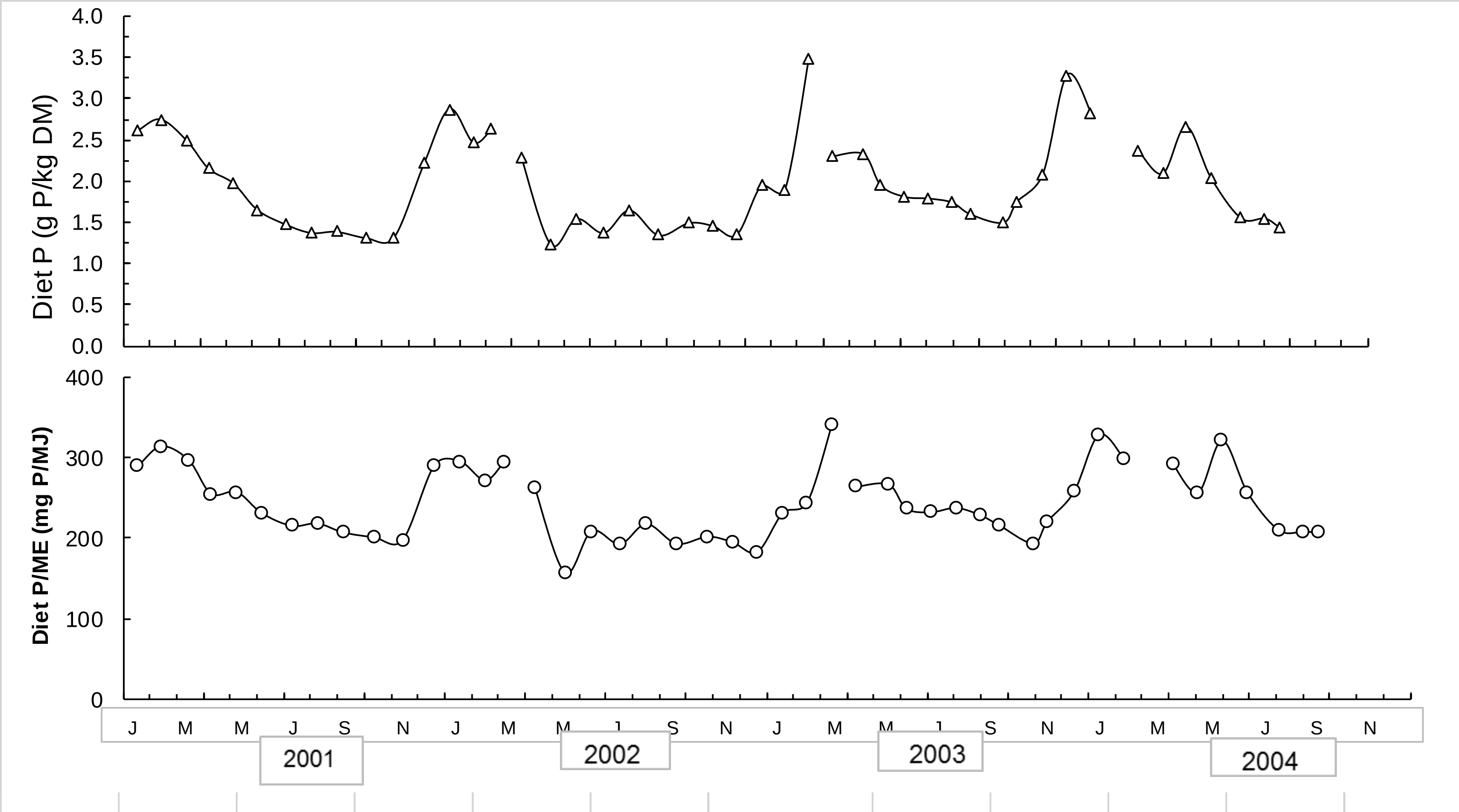

Figure 8. Estimates of the concentration of total P in the diet selected by yearling heifers grazing native grass pasture with a para graas swamp area, at Swans Lagoon (Dixon 2008). The diet P concentration required by 200 kg growing heifers for 0.6 kg/day LW gain was 1.8 g P/kgDM or 220 mgP/MJ ME. The paddock was expected from other knowledge of this research station to be marginal in P status for grazing cattle.

5.6. Prediction of diet TP concentration and the diet TP to diet ME ratio in grazing experiments

The predicted values for diet TP concentration and diet P/ME from a major experiment (Expt E863, Dixon *et al.* 2007) conducted as part of a previous project (B.NBP.302. Utilizing faecal NIRS measurements to improve prediction of grower and breeder cattle performance and supplement management) on the development of F.NIRS technology are shown in Figure 5. In brief, drafts of heifers were introduced into the experiment in the mid-dry season (August) and remained for 12 months. Most of the heifers in the herd (n 40) were pregnant, calved in November-December, and then were lactating through to early weaning (April) or late weaning (August). Some additional heifers (n 7-9) were non-pregnant at introduction and thereafter. The concentration of diet P was predicted from faecal total TP and F.NIRS measurements of faecal TN concentration and diet TN concentration using the Equation 4 (section 4.3) described above. The concentration of P in the diet was estimated to generally exceed 1.3 g TP/kg DM during the wet season and ranged from about 0.8 to 1.3 g P kg DM during the dry season. No independent measurements of the actual concentration of TP in the diet were available to provide a validation of these estimates TP concentration in the diet, or the diet TP / ME ratio. It can only be stated that the diet TP concentrations estimated from the faecal measurements were consistent with expectations for cattle grazing marginal P native pastures.

The predicted values for diet TP concentration and diet TP/ME from a second major experiment (Expt E864, also conducted as part of a previous MLA project (B.NBP.302) are shown in Figures 6, 7 and 8. Heifers, initially yearlings, grazed three northern speargrass pasture systems (native pasture, native pasture with substantial stylo legume component, and a native pasture paddock with an area of para grass swamp) through three annual cycles. The estimated diet P concentration in the heifers grazing the native pasture was comparable with those observed in the young cows. Estimated diet P concentrations in the heifers grazing the grass-stylo pasture or the paddock with the para grass swamp were substantially higher, being generally > 2 g P/kg DM during the wet seasons and between 1.2 – 2.0 g P / kg DM during the dry seasons. Again it can only be stated that the diet TP concentrations estimated from the faecal measurements were consistent with expectations for cattle grazing the pastures described.

6 Conclusions/recommendations

6.1 Laboratory analyses of samples from a large archival data set showed that in tropical cattle ingesting tropical forages the diet total P concentration (D-TP) was not well correlated with faecal total P concentration (F-TP) (R^2 = 0.53). Thus in general the diet total P concentration cannot be reliably estimated directly from measurements of the total P concentration in faeces.

6.2 Multiple regression models could be developed to estimate diet P concentration from measurements in faeces of cattle ingesting tropical forage diets. Diet P was estimated with an accuracy acceptable for many field situations (R^2 = 0.60) from measurements of total P and total N in faeces and knowledge of whether the pasture was a grass/legume mixture or entirely legume. Estimation of Diet P concentration was improved (R^2 = 0.67) when F.NIRS measurement of diet N concentration was included in the prediction model.

6.3 The acid-insoluble P concentration of forages provides a measure of the availability of P to the ruminant. However, inclusion of the measured acid-insoluble P concentration in faeces was of little additional value to estimate diet P concentration from measurements of faeces.

6.4 Diet P concentration is more reliably estimated from a number of measurements in faeces and use of multiple regression models.

6.5 A consequence of the poor correlation between faecal P concentration and diet P concentration is that indicators of diet P adequacy which include the faecal P concentration as a ratio (e.g F-TP/Diet ME) may not be reliable. Therefore the evaluation of the adequacy of P in the diet should depend on use of the diet P concentration in conjunction with other information about the diet (e.g. the ME and N contents of the diet from F.NIRS) and the physiological state and productivity of the animal, to indicate whether the diet is likely to adequate in P.

6.6 Adjustment of the estimated diet P concentration will be necessary for animals in physiological states other than dry animals in slow LW loss through to slow LW gain. The excretion of P in faeces will be affected by a number of animal factors including rapid LW loss or gain, conceptus growth in late pregnancy, milk synthesis, and net deposition or mobilization of bone P. These were not accommodated within the data set and the multiple regression models. The adjustments for LW change, conceptus growth and milk can be estimated from established nutritional information. However there is no currently available on-farm procedure to measure net bone P deposition of mobilization.

6.7 The equations to estimate diet P concentration were only applicable when cattle were ingesting tropical forage diets and without P supplements and without concentrate supplements.

6.8 Despite the limitations described above the present study indicated that measurements of faeces should provide a useful guide of the diet P intake of grazing cattle in many circumstances and indicate when cattle are likely to respond to P supplements. Most reliable estimates should be obtained for growing cattle which have been grazing the paddock(s) of interest through a wet season, and with faeces measured late in the wet season when any bone P reserves should be depleted and the animals are in moderate growth rate. This is the approach used for the P-Screen test. Faecal measurements to estimate diet P concentration are likely to be most useful as a guide where it is not possible to obtain blood samples to conduct the P-Screen diagnostic test.

6.9 The diet P concentrations estimated from faecal measurements in cattle grazing various pastures through a number of annual cycles at Swans Lagoon Research Station in the northern speargrass seasonally dry coastal tropics were consistent with expectations of diet P status from past research on the site and knowledge of the land systems and the pastures.

6.10 The estimation of diet P concentration from measurements in faeces should be reconsidered when information on markers indicative of net bone P deposition and mobilization is available from Project B.NBP.0689 ("Improved management of cattle phosphorus status through applied physiology"). Inclusion of such markers with faecal sampling should improve estimation of diet P concentration from measurements of faeces.

6.11 The 2012 Producer P Manual requires revision to incorporate the new information derived from the present study, and information from other projects addressing the phosphorus requirements of beef cattle in northern Australia. It is recommended that a revised manual should emphasise that the P-Screen test with both blood and faecal sampling is expected to provide the most reliable diagnosis of diet P deficiency in grazing cattle. Faecal testing should continue to be described in a revised P Manual but with caution with respect to the class of cattle and the time of year, and a recommendation that it is most reliable when sampling is from a sentinel herd which has grazied the paddock(s) of interest during the wet season, and that sampling should be in the late wet season. A revised approach to evaluation of diet P concentration from faeces should: (i) continue to measure the concentration of total P in faeces, but also include F.NIRS analyses to estimate diet DM digestibility, diet total N concentration and faecal N concentration, (ii) be clear that faecal sampling without blood sampling is a less reliable diagnostic which may be useful as a guide but will not be correct in some circumstances.

6.13 A measurement system suitable for on-farm use is needed to routinely evaluate the P reserves (i.e. the P status, as opposed to the current diet intake of P) of grazing cattle. The need is to measure the extent of mineralization of the skeleton of cattle and most likely as bone mineral density or bone mineral mass.

7 Key messages

7.1 Measurements in faeces of cattle grazing tropical pastures provide a guide to the concentration of P in the diet in some circumstances. Consideration must be given to the class of cattle, the season of year and the recent nutritional history of the cattle. The concentration of P in the diet selected by cattle grazing tropical pastures and without P or concentrate supplements can be estimated from measurements in faeces as a guide to diet P concentration with reasonable confidence from faecal measurements in dry cattle which are not losing or gaining LW rapidly.

7.2 Multiple regression equations have been developed to estimate diet P concentration from measurements of faeces. However caution is needed to apply these regression models correctly and only in appropriate circumstances. Adjustments will need to be applied for cattle in rapid liveweight loss or liveweight gain, and in other specified physiological circumstances.

7.3 Consideration of the estimated faecal P concentration with knowledge obtained from F.NIRS evaluation of diet will allow improved evaluation of the likely adequacy of diet P for cattle grazing tropical pastures in many situations.

7.4 The P-Screen test requiring blood and faecal samples from a defined class of cattle in the late wet season is expected to provide the most reliable diagnosis of the P status of cattle and is the preferred option for implementation of P supplementation program. Inclusion of F.NIRS measurements will improve the reliability of the P-screen test. Evaluation of the P status of cattle from measurements only of faeces should be adopted only when it is not feasible to use the P-screen diagnostic test.

7.5 The estimation of the P status and of the responses to supplementary P by grazing cattle will be markedly improved if field tests to measure skeletal P and the net mobilisation or deposition of bone P can be developed.

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9 Appendix

Publications from the project:

Dixon RM, Reid DJ and Coates DB (2015) Estimating phosphorus intake from faeces in cattle grazing tropical pastures. In: TropAg2015. pp. 148, #P139. 16-18 November 2015, Brisbane, Australia.

Dixon RM, Quigley SP, Mayer RJ, Isherwood P (2016) Effects of time of sampling during the day on the concentration of phosphorus in faeces of cattle. *Animal Production Science. Proceedings of the 2016 ASAP conference*.

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