RELATIONSHIPS BETWEEN PHOSPHORUS INTAKE, PLASMA PHOSPHORUS AND FAECAL AND URINARY PHOSPHORUS EXCRETION IN YOUNG SHEEP

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SUMMARY
The effects of 6 levels of phosphorus (P) intake on plasma P concentrations and P excretion were studied in young sheep individually fed a basal diet of wheaten chaff, lucerne chaff, cane sugar and wheat gluten meal that provided 0.68 g P/day. Monosodium phosphate was used to supplement the remaining 5 diets with 1, 2, 3, 4 or 5 g P/day, to give intakes of 20-150 mg P/day per kg liveweight (LW). Faecal P excretion, apparent P absorption (P intake-faecal P) and total P excretion all showed marked linear responses to P intake ($P<0.001$). Urinary P excretion was low at intakes below 80 mg P/day per kg LW, but was higher and more variable above this intake. Urinary P excretion and apparent P absorption were closely related ($P<0.001$) in a manner well described by 2-phase linear regression with phases intercepting at an apparent absorption of 43.6 mg P/day per kg LW. Below this point, only about 13% of apparently absorbed P appeared in urine, but above it, urinary excretion of absorbed P appeared complete. The effect of P treatment on plasma P concentrations was not marked ($P=0.057$) and observed concentrations were low (<45 mg P/L) compared with published studies. Nevertheless, there was a significant ($P<0.001$) exponential relationship between urinary P excretion and plasma P, though this explained less than 60% of the variance in the former. Urinary P excretion was thus better predicted from P intake and faecal P, either by using them to calculate apparent P absorption, or by using them as separate terms in a multiple regression. It is suggested that, following field validation, these relationships could be useful in modelling P cycling in sheep grazing systems.

Keywords: phosphorus intake, urinary phosphorus excretion, nutrient cycling, plasma phosphorus

INTRODUCTION
The accurate modelling of phosphorus (P) cycling in grazing systems could assist livestock producers to make better decisions concerning P fertiliser use, but requires the establishment of relationships between P intake and its excretion in faeces and urine. The increasing use of plant wax alkanes for estimating diet composition and intake provides a vehicle for obtaining better estimates of P intake and faecal P excretion (Dove and Simpson 1997), but the field measurement of urinary P excretion remains very difficult.

At low daily P intakes (<30 mg/kg liveweight (LW); see Ternouth *et al*. 1996), urinary P excretion is very low and in general, P excretion in urine has received little attention in discussions of either P requirements (e.g. SCA 1990) or the modelling of P cycling in grazing systems (e.g. Nguyen and Goh 1992). Nevertheless, urinary P excretion can exceed 50-75 mg/day per kg LW in young sheep and cattle, and can account for more than 25% of total P excretion (Field *et al*. 1983; Challa and Braithwaite 1988a, b). It must, therefore, be accommodated in studies of P cycling in grazing systems. In the present study, we sought to examine the relationships between P intake, plasma P, and the excretion of P in faeces and urine in young sheep fed graded levels of P, while consuming a low-P diet. We aimed to define the level of P intake or apparent absorption (P intake-faecal P excretion) at which urinary P excretion became quantitatively important.

MATERIALS AND METHODS
Experimental animals, design and diets
Eighteen crossbred young castrate male sheep of mean LW 37.3 (s.d. 1.74) kg were allocated by stratified randomisation (based on LW) to 1 of 6 dietary treatments (3 sheep/treatment). All animals received a low-P (0.68 g P/kg DM) basal ration consisting (as fed) of 350 g/day chaffed wheat straw (0.21 g P/kg DM), 200 g/day lucerne chaff (3.39 g P/kg DM), 125 g/day cane sugar (nil P/kg DM) and 40 g/day wheat gluten meal (WGM; 1.19 g P/kg DM). Dietary treatments involved supplementation of the basal diet with 0, 1, 2, 3, 4 or 5 g P/day as monosodium phosphate, to achieve daily P intakes in the range, 20-150 mg P/kg LW, which encompasses the P intakes used in a number of previous studies with sheep and cattle (e.g. Grace 1981; Field *et al*. 1983; Challa and Braithwaite 1988a, b).

Procedures

Feeding. Diets were introduced over a period of 18 days while animals were housed in individual pens. Initially the basal diet was based entirely on wheat straw, but the large feed refusals and resultant weight loss required that a proportion of lucerne chaff be included in the diet. When daily intakes had stabilised, animals were transferred to metabolism crates and were allowed a period of 2 weeks to acclimatise to the conditions, prior to the total collection of faeces and urine over a period of 6 days. Diets were fed once daily at 0900 h. The chaff components of the diet were mixed thoroughly in individual feed bins and the pre-weighed sugar, WGM and, where appropriate, P supplement were then sprinkled onto the chaff and mixed into it by hand. Animals had free access to drinking water throughout.

Measurements. Feed residues were collected and weighed daily. During excreta collection, feed residues were dried (70°C, 48 h), bulked within sheep and stored at -18°C pending later chemical analyses. Diet components were also sampled daily for DM determination, and a further sample bulked and frozen (-18°C). The daily total faecal output from each sheep was weighed and 10% by fresh weight was bulked and frozen. Two subsamples of 50 g fresh weight were also taken for DM determination. Urine was collected into 50 mL 10% hydrochloric acid. Daily urine output was weighed and a 10% subsample bulked and frozen pending later chemical analyses. At the end of the collection period, jugular blood samples were taken from all sheep into heparinised vacutainers, for the determination of plasma P content.

Chemical analyses

The P contents of feeds, feed refusals, urine and faeces were determined by the molybdenum-blue method, after digestion in sulphuric acid/hydrogen peroxide (Little *et al*. 1971). Plasma P content was similarly determined on the clear supernatant obtained by centrifugation, after 5 mL plasma from heparinised blood samples was deproteinised with 6 mL 10% trichloracetic acid (Grace 1981).

Statistical analyses

Responses to dietary treatment were examined initially using single classification analysis of variance, with the treatment term partitioned into linear and quadratic effects. In addition, relationships between plasma P, the components of P excretion and P intake, and apparent absorption, were further examined by simple, 2-phase or multiple regression analysis, using model terms as outlined in the results below.

RESULTS

Animals lost an average of 65 g LW/day over the course of the study, mostly during the initial adaptation period in pens, when they rejected the diet based entirely on wheat straw. There was no significant effect of P treatment on weight loss. Once intakes stabilised on the wheat straw/lucerne diet, feed refusals ranged from 4-49 g DM/day. Neither the amount nor the P content of refused feed was influenced by treatment. As a result, the intakes of P achieved were close to those intended (Table 1).

Regardless of whether results were expressed in g/day or mg/day per kg LW, dietary P content had marked linear effects ($P<0.001$) on P intake, faecal P excretion, apparent P absorption and total P excretion. Treatment effects on urinary P excretion and plasma P were less marked ($P<0.05$ and $P=0.057$, respectively). Although quadratic effects were not detected in the ANOVA model, some variates were clearly related in a curvilinear manner. There was also marked variation between animals within a treatment. The relationships between the measured variates were thus further examined by regression analysis; faecal P excretion was then related to P intake (both mg/day per kg LW) by the expression:

Faecal P excretion = 0.590 (s.e. 0.0570)*P intake + 8.76 (s.e. 5.51) ($r^2 = 0.862$, $P<0.001$).

By contrast, urinary P excretion was not related to P intake in a simple manner. It remained low until P intake exceeded 2.67 g/d (72.5 mg/day per kg LW). Urinary P excretion in some animals then increased dramatically, but there was marked between-animal variability (Figure 1a). Urinary P excretion was more closely related to the amount of P apparently absorbed (Figure 1b). Below an apparent absorption of about 30 mg P/day per kg LW (a daily P intake of about 90 mg/day per kg LW; Table 1), urinary P excretion was negligible. Above about 40mg P/day per mg LW, it increased

rapidly and ultimately approached 40% of total P excretion. A 2-phase linear regression model was fitted to these data. The slopes of the 2 phases provide an estimate of the proportion of apparently absorbed P excreted in urine, whilst the intersection of the phases indicates the P absorption above which urinary P excretion increases markedly. The equation for phase 1 was constrained through the origin, since the unconstrained regression had a negative y-intercept, which implied negative urinary excretion. In our data, the phases intersected at an apparent P absorption of 43.6 (s.e. 2.58) mg P/day per kg LW (P intake of about 125 mg/day per kg LW). Below this point, only about 13% of apparently absorbed P was excreted in urine (slope = 0.132), but above it, urinary excretion of absorbed P appeared to be complete (slope = 1.032).

Table 1. Treatment differences in the components of daily phosphorus (P) balance and in plasma P.

Variate	Treatment (g/day supplemental P)						s.e. for a
	0	1	2	3	4	5	linear contrast
g P/day							
P eaten	0.68	1.67	2.67	3.66	4.66	5.66	0.002***
Faecal P	0.68	1.33	2.06	2.60	2.62	3.84	0.054***
P apparently absorbed	-0.01	0.34	0.62	1.06	2.03	1.81	0.054***
Urinary P	0.02	0.02	0.06	0.21	0.65	0.43	0.048*
Total P excretion	0.70	1.35	2.11	2.81	3.28	4.27	0.023***
mg P/kg LW/day							
P eaten	18.2	45.7	72.5	93.3	124.1	155.2	0.53***
Faecal P	18.3	36.4	55.7	66.8	69.8	105.7	1.63***
P apparently absorbed	-0.2	9.3	16.9	26.5	54.3	49.5	1.40***
Urinary P	0.4	0.6	1.6	5.1	17.6	11.5	1.27*
Total P excretion	18.8	37.0	57.2	71.9	87.3	117.2	0.85***
Plasma P (mg P/L)	32.3	28.0	36.2	36.0	37.6	43.2	1.12 (P=0.057)

***;* Significant linear effect of treatment (P<0.001; P<0.05). Quadratic effects were not significant (P>0.05).

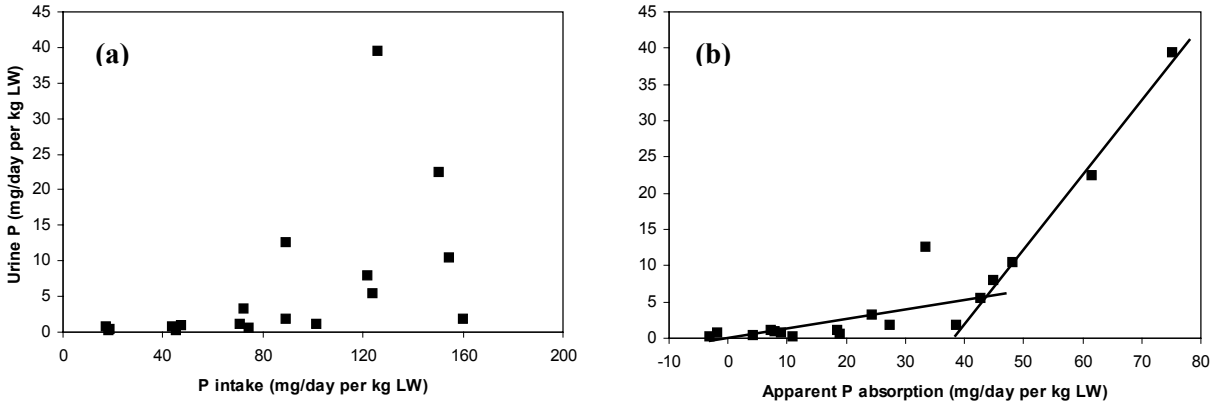

Figure 1. Relationships between urinary P excretion and either (a) daily P intake, or (b) daily apparent absorption of P. The solid lines in Figure 1b are the result of a fitted 2-phase regression (r^2 = 0.939, P<0.001):

Phase 1: Urinary P excretion = 0.132 (s.e. 0.0302)*P absorption

Phase 2: Urinary P excretion = 1.032 (s.e. 0.1080)*P absorption – 39.289.

Despite the between-animal variability obvious in the excretion of P in faeces, and particularly in urine (Figure 1a), when these were related to P intake, the relationship between total P excretion (faecal P plus urinary P) and P intake (both mg/day per kg LW) was very close and described by the expression:

Total P excretion = 0.703 (s.e. 0.0241)*P intake + 5.286 (s.e. 2.3280) (r^2 = 0.982, P<0.001).

At a given P intake, animals thus excreted very similar amounts of P, but the route of excretion differed between animals. The close relationships between urinary P excretion and apparent P absorption, and between total P excretion and P intake, implied the existence of an inter-relationship between faecal and urinary P excretion. Urinary P excretion in the present study could thus be predicted from a multiple regression including P intake and faecal P excretion, such as the following (values mg P/day per kg LW):

Urine P = 0.543 (s.e. 0.0528)*P intake - 0.728 (s.e. 0.0836)*Faecal P + 2.9 (s.e. 1.98) (r^2 = 0.865, P<0.001).

When added to this model, the effect of plasma P was not quite significant (P=0.054) and only explained a further 3% of the variance. This is probably because there was no marked response of plasma P to P intake (Table 1). However, the relationship between urinary P excretion (mg/day per kg LW) and plasma P (mg/L) could be described by the following exponential expression, though it should be noted that this relationship still accounted for less than 60% of the variance in urine P excretion:

$$\text{Urine P excretion} = 0.011 * e^{0.1448 * \text{Plasma P}} \quad (r^2 = 0.560, P<0.001).$$

DISCUSSION

The response of P excretion to changes in P intake in the present study accords with previous indoor studies, and with estimates of P intake and faecal P excretion in grazing sheep. For example, the present relationship between faecal P excretion and P intake (both mg P/day per kg LW) is similar to that reported by Dove and Simpson (1997), and does not differ from it in slope:

Present study	Faecal P excretion = 0.590*P intake + 8.76
Dove and Simpson (1997)	Faecal P excretion = 0.616*P intake + 16.10.

The faecal endogenous P losses implied by these equations (8.8-16.1 mg/day per kg LW) are similar to published values of 9-14 mg P/day per kg LW for sheep (Grace 1980; Field *et al*. 1983). This gives some confidence in the wider applicability of the present results, though cautious interpretation is required because of the possibility of seasonal differences in the extent of P absorption (Dove and Simpson 1997).

The nature of the response of urinary P excretion to P intake or apparent P absorption is similar to previous observations in sheep (Field *et al*. 1983) and cattle (Challa and Braithwaite 1988a, b; Ternouth *et al*. 1996). In our study, urinary P excretion was not important until P intake exceeded 80-90 mg/day per kg LW (Figure 1). This is considerably higher than the threshold intakes of 30-50 mg/day per kg LW in the above reports. These studies also demonstrated marked curvilinear responses of urinary P excretion to plasma P, in which urinary P excretion increased rapidly once plasma P concentrations exceeded 60 mg/L. In the present study, there was not a marked response of plasma P to P intake or apparent absorption. Moreover, the exponential relationship between urinary P excretion and plasma P only explained 56% of the variance in urinary P excretion. These observations may relate to the fact that the plasma P concentrations observed in the present study were low and never approached the values of 60-120 mg/L observed in some studies relating urinary P excretion to plasma P (e.g. Challa and Braithwaite 1988a, b).

Although plasma P concentrations were not useful for predicting urinary P excretion in the present study, the close relationship between urinary P excretion and apparent P absorption (Figure 1b), or the multiple regression between urinary P excretion, P intake and faecal P excretion should allow estimation of urinary P excretion in the absence of its measurement. Alkane-based estimates of P intake (Dove and Simpson 1997) can also provide the estimate of faecal P excretion required to use these relationships. However, before using such relationships in models of P cycling in sheep grazing systems, they should be validated under field conditions and with different classes of animals.

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