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Effects of Dietary Supplementation on Phosphorus Metabolism in Sheep

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Abstract: Phosphorus (P) metabolism in sheep was studied using 98 Suffolk sheep, which were given various levels of P supplementation in their diet and administered a single dose of isotopic ^{32}P in their blood. About 62% of P intake was excreted in faeces. Endogenous loss in faeces ranged from 8.6 to 84.0 mg P/kg body weight (BW) daily and was linearly related to P intake. Minimum endogenous faecal P loss was estimated at 8.63 mg/kg BW/day. Dietary P absorption was positively and linearly related to P intake at all intake levels, but the efficiency of absorption decreased at higher intakes. This suggests that P absorption involves not only a passive mechanism but also an active process, possibly due to homeostatic control. Taking an average 63% absorption efficiency, requirement of dietary P for maintenance was calculated to be 28.3 mg/kg BW/day. For a 35-kg sheep, this is equivalent to 0.99 g P/day to replace endogenous P losses fed at maintenance. Non linear relationships of salivary P with absorbed, endogenous feacal P and dry matter intake were established, suggesting that regulation of P secretion in saliva could be by active and passive mechanisms. Urinary loss of P was low, even at high levels of P intake. The paper demonstrates the relevance of understanding P metabolism and the need to estimate P requirements for ruminants accurately, in order to optimise production but avoid excessive P outputs to the environment.

Key words: Phosphorus, Sheep, Endogenous Phosphorus loss

Introduction

Phosphorus (P) is an essential nutrient and is involved as phosphate in most of the metabolic activities of the body, as well as in bone formation (Kebreab and Vitti, 2005). Animals, therefore, need an adequate supply of P for optimum growth and production.

December 9, 2004ietary P deficiency is a problem in animal production in many countries. In most tropical countries, dietary P deficiency is one of the predominant problems of mineral imbalance in ruminant production from extensive agricultural systems. Most pastures in Brazil, for example, have low P and as a result productivity from ruminants is low. Therefore, P needs to be supplemented in many forage-based diets but due to the cost of P and potential environmental pollution (Tamminga, 1996), the amount of supplemental P needs to be estimated accurately. The absence of appropriate P data, especially from kinetic studies of P metabolism (e.g., Vitti, 2000, for goats), has led to a variety of estimates of P requirements in ruminants (ARC, 1980; AFRC, 1991, NRC, 2001; Silva, 1995).

A substantial amount of P recycling takes place through saliva. Kebreab *et al.* (2005) reported that about 40% of P in the duodenum was salivary P. Faeces is the main route of P excretion in ruminants and accounts for about 70% of total P output. Phosphorus in faeces is exogenous, mainly residual from the ration (Bromfield and Jones, 1970), or endogenous, comprising P of bodily origin (Wadsworth and Cohen, 1976). Reports in the literature show that endogenous faecal loss varies with P intake and this fraction constitutes over two-thirds of total faecal P in cattle and sheep (e.g., Scott *et al.*, 1995). Although it is accepted that P homeostasis in ruminants is achieved largely through faecal loss, the exact mechanisms involved are uncertain (Challa *et al.*, 1989). It is not clear whether P homeostasis is brought about by control of P absorption, control of salivary P, or both.

The objectives of this study are to (i) improve understanding of the control of P homeostasis in sheep fed various levels of P, (ii) obtain more information on P utilisation and the relationships between various pools of P in the body, and (iii) make estimates of P excretion in order to provide recommendations for minimum P requirements.

Material and Methods

Experimental procedure: Ninety eight castrated Suffolk sheep aged 18-24 months and weighing 38-40 kg, were housed indoors in metabolism crates designed for isotope studies and handling of faeces and urine at the Centre for Nuclear Energy in Agriculture (CENA), University of São Paulo. All sheep received a diet consisting of a concentrate mixture (cassava meal, urea, mineral mixture) and Brachiaria decumbens hay (Table 1). The hay was offered ad libitum and P supplementation was offered in different sources and amounts (Table 2) to obtain required P intake for each treatment. Feed was given twice a day for a 28-d period.

After 21 d each animal was administered, as a single dose via the right jugular vein, 7.4 MBq ^{32}P in 1 ml sterile isotonic saline (9 g/l sodium chloride). Blood samples (10 ml) were taken by vacutainer from the left jugular vein at 24-h intervals, after isotope administration, for 7 d. Blood was centrifuged and plasma removed for analysis, as most of mobile P in blood is found in plasma. Nine ml trichloroacetic acid (100 g/l) was added to 1 ml plasma for protein precipitation. After centrifugation (1100 g) inorganic P in plasma was determined by colorimetric analysis (Fiske and Subbarow, 1925).

Dry matter intake, faeces and urine were recorded daily and representative samples were retained for further analysis. Faecal samples (1 g) were dried overnight (105°) and ashed (500° for 8h). Concentrated hydrochloric acid (HCl) was added to the ash and P content was determined by a calorimetric method (Sarruge and Haag, 1974). A similar procedure was followed to determine P content of dry matter intake. Urine samples (30 ml) were added to HCl (12N), dried (55°) and ashed (500°). Ashed samples were diluted (3N HCl) and volume made up to 10 ml (Morse *et al.*, 1992). Inorganic P was determined using vanadate-molibdate reagents (Sarruge and Haag, 1974). In

most cases, P in urine was too low to be determined and thus not included in calculations of P metabolism.
For radioactivity measurements, 1 ml plasma and urine samples were added to 19 ml distilled water and placed in counting vials, and ashed faecal samples (1 g) were dissolved in 18N sulphuric acid (H_2SO_4) and placed in counting vials. Radioactivity of ^{32}P was measured in a Packard Liquid Scintillation Spectrometer (model 2450B) using Cerenkov radiation. Specific activities in plasma and faeces were determined according to Lofgreen and Kleiber (1953).

The model and mathematical calculations: A whole body P metabolism model of Vitti *et al.* (2000) is adopted to represent P flows in sheep (Figure 1a). The model contains four pools of P: 1) gut lumen, 2) blood, 3) bone, and 4) soft tissue. Flows of P between pools and into and out of the system are shown as arrowed lines (mg/kg body weight (BW)/day). The gut lumen, bone, and soft tissue pools interchange bi-directionally with the blood pool, with flows F_{21} and F_{12}, F_{23} and F_{32}, and F_{24} and F_{42}, respectively. Phosphorus enters the system via intake, F_{10}, and exits via faeces, F_{01}, and urine, F_{02}. The scheme adopted for the movement of label is shown in Figure 1b (Vitti *et al.*, 2000).
P metabolism was described quantitatively using equations taken from previously published work. Total endogenous P in faeces (F_{e01}, mg/kg BW/day) was calculated as follows (Vitti *et al.*, 2000):

$$FF_{e01} = \quad (1)$$

where F_{01} is P in faeces, F_{10} is P intake, F_{12} is flow of P from blood to the gut pool, and s_1 and s_2 are specific activities (dpm/g) of the faeces and blood pools, respectively. Truly absorbed P, which is the dietary P absorbed by the sheep, (F_{abs}, mg/kg BW/day), its efficiency of absorption ($å_{abs}$) and non absorbed P, which is dietary P directly excreted in faeces (F_{non}, mg/kg BW/day) were calculated as follows (Grace, 1981; Braithwaite, 1984; Fernandez, 1995):

$F_{abs} = F_{10} - (F_{01} - F_{e01})$ (2)

$å_{abs} = F_{abs} / F_{10}$ (3)

$F_{non} = F_{01} - F_{e01}$ (4)

Due to low P content in urine, P retention (F_{ret}, mg/kg BW/day) was calculated as:

$F_{ret} = F_{10} - F_{01}$ (5)

Salivary P (F_{sal}, mg/kg BW/day) was calculated as follows (Young *et al.*, 1966):

$F_{sal} = F_{e01} /(1 - F_{abs})$ (6)

Statistical analysis: Data were analyzed statistically using ANOVA (SAS, 1999) (n = 98). Normality test was carried out and significant variability of the data was determined at $P < 0.05$ level. Where significant differences were established, models that fit the data were assessed using the PROC REG procedure (SAS, 1999). The simplest model, which did not increase the residual sum of squares significantly when compared to a more complex model, was selected. SE of coefficients and r^2 are reported.

Results and Discussions

The results were corrected for variations in BW and are expressed in mg P/kg BW/day. The regression equations were obtained using individual animal data. The amount of P consumed ranged from 38.3 to 199 mg/kg BW/day, and total P excreted in faeces ranged from 21 to 146 mg P/ kg BW/day.
There was a highly significant linear ($P < 0.01$) relationship between total P in faeces and P intake (Figure 2):

F_{01} = 7.95 (SE 6.68) + 0.62 (SE 0.05) F_{10} (7)

r^2 = 0.60; n 98

Endogenous P losses in faeces varied from 8.6 to 84.0 mg P/kg BW/day. A linear correlation between endogenous faecal P and P intake was not found over this range in this study, although a number of reports in the literature indicate that endogenous P is linearly related to intake (Challa *et al.*, 1989; Louvandini, 1995; Salviano, 1996). This might be due to the high levels of dietary P used in the present experiment. If only P intakes of up to 120 mg P/kg BW/day are considered, a linear relationship was established ($P < 0.01$) (Figure 3):

F_{e01} = 8.63 (SE 5.05) + 0.27 (SE 0.05)F_{10} (8)

r^2 = 0.40; n 40

There was also a linear relationship between P intake and absorbed P ($P < 0.01$) (Figure 4a). P absorption varied from 88% of dietary P at the lowest intake used in this study (38 mg P/kg BW/day) to 53% at the highest (199 mg P/kg BW/day). Efficiency of absorption varied from 0.20 to 0.89 and decreased linearly with P intake (Figure 4b):

F_{abs} = 15.99 (SE 7.18) + 0.46 (SE 0.06)F10 (9)

r^2 = 0.42; n 98

$å_{abs}$ = 0.82 (SE 0.058) - 0.0017 (SE 0.0005)F10 (10)

r^2 = 0.12; n 98

Non-absorbed dietary P increased exponentially ($P < 0.01$) as P intake increased (Figure 5a). Non-absorbed P had a stronger and linear relationship ($P < 0.01$) with P in faeces (Figure 5b):

F_{non} = 5.95 (SE 1.41) exp[0.015 (SE 0.0012)F_{10}] (11)

r^2 = 0.57; n 98

F_{non} = -18.04 (SE 4.59) + 0.82 (SE 0.05)F_{01} (12)

r^2 = 0.73; n 98

There was a strong and positive linear ($P < 0.01$) relationship between amount of P retained in the body and truly absorbed P (Figure 6):

F_{ret} = -12.87 (SE 3.18) + 0.72 (SE 0.054)F_{d21} (13)

r^2 = 0.65; n 98

Salivary secretion of P increased exponentially as P absorption increased ($P < 0.05$) (Figure 7a). Salivary P was also related to

endogenous P in faeces ($P < 0.01$) (Figure 7b), to efficiency of P absorption ($P < 0.01$) (Figure 7c) and to dry matter intake (DMI) ($P < 0.05$) (Figure 7d), as follows:

$F_{sal} = 26.7$ (SE 6.12) exp[0.016 (SE 0.002)F_{abs}] (14)

$r^2 = 0.22$; n 98

$F_{sal} = 54.9$ (SE 8.80) exp[0.02 (SE 0.0033)F_{e01}] (15)

$r^2 = 0.51$; n 98

$F_{sal} = 9.03$ (SE 2.19) exp[3.36 (SE 0.311)$\hat{a}_{abs}$] (16)

$r^2 = 0.70$; n 98

$F_{sal} = 33.05$ (SE 9.93) exp[0.06 (SE 0.0127)DMI] (17)

$r^2 = 0.26$; n 98

P in plasma was not correlated with other measured variables.

Phosphorus intakes between 20-30 mg/kg BW/day are considered deficient for sheep and values between 80-100 mg/kg BW/day are considered adequate (Challa and Braithwaite, 1988). Accordingly, the experiment was designed to achieve P intakes that were moderately deficient (30-80 mg P/kg BW/day), adequate (80-100 mg P/kg BW/day) and excessive (>100 mg P/kg BW/day) in order to determine their effects on various pools of P in the body.

Faeces was the major route of P excretion and level of P intake had direct influence ($P < 0.01$) on total faecal P loss. Faecal P, including endogenous P loss, represented about 60% of P intake similar to reports by Braithwaite (1986) for lactating ewes. However, values of faecal loss as high 95% of total P intake have been reported (Salviano, 1996).

Endogenous faecal P loss at zero P intake was calculated to be 8.63 mg P/kg BW/day (Equation 8), close to the value for sheep obtained by Braithwaite (1983) (11 mg P/kg BW/day). This minimum value represents the inevitable loss that would occur if sheep were not consuming any P. It is also the minimum amount that the animals need to absorb from dietary P sources to meet their maintenance demand. This minimum endogenous P value is in accordance with calculations of ARC (1994), who give 9-12 mg P/kg BW/day for P requirement at maintenance.

The linear relationship between P absorption and P intake (Equation 9) obtained in this study is in agreement with Braithwaite (1984). Average efficiency of conversion of dietary P to truly absorbed P was about 63%. Efficiency of absorption of dietary P decreased, although P absorption increased with P intake. There was an exponential increase in non-absorbed P as P intake increased, particularly above 100 mg/kg BW/day (Figure 5a). The decrease in efficiency of P absorption may indicate that P absorption involves not only a passive mechanism but also an active process, which is subject to regulation. Saturation of the active mechanism of P absorption may be involved in animals given P intakes in excess of their P requirement. The major route of excreting non-absorbed P was through faeces (Fig. 5b).

No net gain or loss of P in the body is expected at maintenance so P retained in body should be equal to zero. Thus, the animals need to absorb 17.88 mg P/kg BW/day just to remain in balance. There was large variation in efficiency of conversion of dietary P to truly absorbed P so assuming a mean efficiency of absorption of 63%, the sheep need to consume 28.4 mg P/kg BW/day. This value represents 0.99 g P/d, for a 35-kg sheep. Vitti *et al.* (2000) calculated maintenance intake of approximately 24.4 mg P/kg BW/day in goats, which is slightly lower than the present study suggests possibly because of the limited range of P intakes considered in the goat study. P requirement for a 35-kg sheep is between 0.6 and 0.8 g P/d according to ARC (1994). The apparent discrepancy is because P requirement calculated by ARC (1994) assumed a 73% efficiency of P absorption, which is higher than the mean value determined in the present study. If however a 73% efficiency of P absorption is assumed in the present study, P requirement would be 0.86, which is in agreement with ARC (1994).

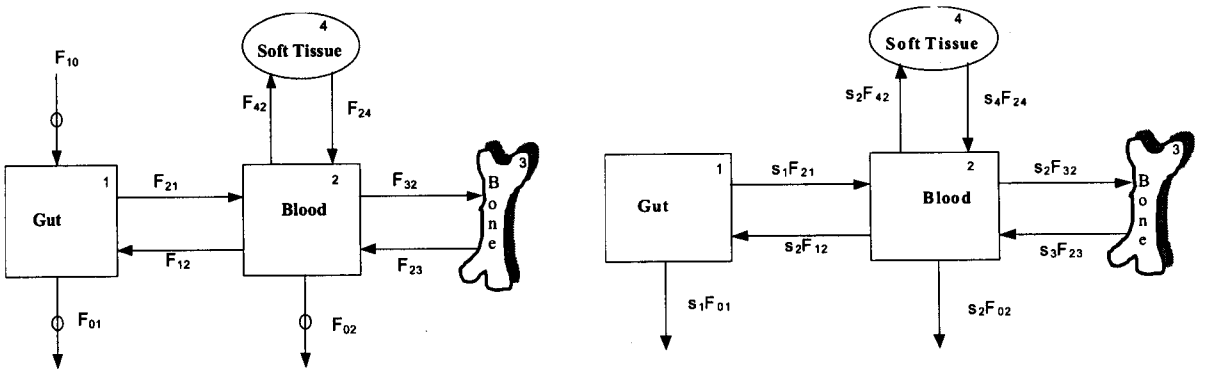

Fig. 1: Schematic representation of the model of phosphorus metabolism: (a) unlabeled P, (b) labeled P. F_{ij} is the total flow of pool i from j, F_{i0} is an external flow into pool i and F_{0j} flow from pool j out of the system. Specific activity of pool i is represented by s_i, and circles denote flows measured experimentally (Vitti *et al.*, 2000)

Relationships between salivary secretion of P and P absorption and endogenous faecal P show that salivary P secretion plays an important key role in P homeostasis. Correlation between salivary P and efficiency of P absorption (r^2 0.70) and endogenous faecal P (r^2 0.51) are in agreement with the literature (FSAL, 1990; Scott *et al.*, 1995). Although variations in salivary P secretion can be attributed to dry matter intake, a linear relationship with P intake could not be established. The curvilinear relationship found may indicate that there is saturation of the mechanism of salivary P secretion. Challa *et al.* (1989), however, suggested that increase in salivary P is probably uncontrolled and is only related to plasma P concentrations, but it was not established in this study.

Table 1: Feed ingredients and diet composition

Ingredient (g/100g DM)	
hay	78.78
cassava meal	18.20
salt*	1.21
urea	1.81
Composition (g/kg DM)	
crude protein	87.6
crude fiber	301.5
Ca	2.0
P 1.3	

*As a sodium chloride and mineral mixture containing: $FeSO_4$ (0.457g), $CuSO_4$ (0.03g), $MnSO_4$ (0.148g), $ZnSO_4$ (0.32g) $CoSO_4$ (0.0008g), KI (0.009g), NaCl (3.4g) and S (4.0g).

Table 2: Composition of phosphorus sources (g/kg)

Source	DM	Ash	Phosphorus	Calcium	Ca:P*	Fluorine
dicalcium phosphate	982.0	821.9	160.0	270.0	1.69:1	0.8
Patos phosphate	993.5	978.8	100.0	340.0	3.40:1	12.0
Tapira phosphate	874.5	985.0	162.0	347.0	2.14:1	16.0
Finos de tapira	883.3	969.5	150.0	480.0	3.20:1	10.6
bone meal	987.4	780.0	118.8	320.0	2.70:1	0.6
superphosphate	932.0	911.5	189.8	151.1	0.80:1	10.0
monoamonium	942.0	405.1	220.2	6.3	0.03:1	3.9
acidulated phosphate	986.0	909.9	104.8	237.0	2.26:1	16.7*

For treatments with a ratio of Ca:P less than 2:1, calcium carbonate was added to increase the proportion to 2.5:1.

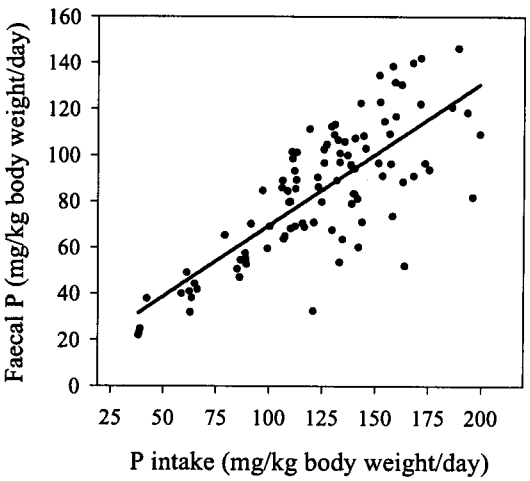

Fig. 2: Relationship between P intake and faecal P for sheep given various treatments. Fitted line is given by Equation (7). Standard error of intercept and slope were 6.68 and 0.05, respectively

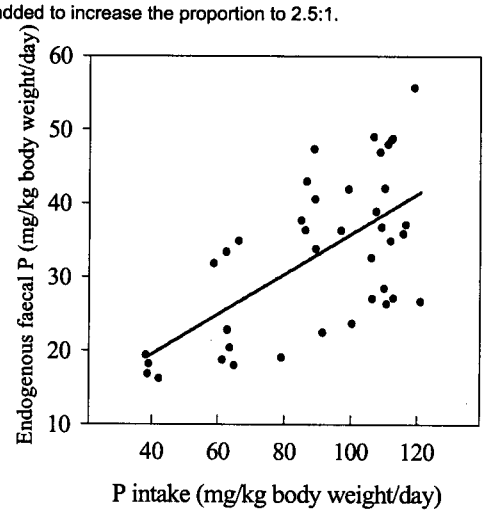

Fig. 3: Relationship between P intake and endogenous faecal P for sheep with intakes not exceeding 120 mg/kg BW/day. Fitted line is given by Equation (8). Standard error of intercept and slope were 5.06 and 0.05, respectively

Urinary P excretion is affected by type of diet offered, such that animals fed a finely ground diet show an increase in urinary excretion of P and reduction in faecal and endogenous faecal P excretion (Scott and Buchan, 1988). However, P balance was not altered. In sheep, P concentration in urine was high at low salivary secretion rates and low at high rates (Bailey and Balch, 1961). Thus, reduced saliva flow may not necessarily be accompanied by a reduction in P excretion. It can be inferred that the salivary glands play a more important role in regulation of P metabolism than the kidneys when fibrous diets are fed. In the present study, animals were fed a roughage diet, which probably was reflected in the low level of P in urine. Plasma P concentration can be another factor influencing urinary P excretion. According to Scott and McLean (1981), an increase in P in urine was observed only when P plasma concentration exceeded a threshold value, between 2 and 3 mmol/l. In this study, P in plasma was within the normal range, which might account for the low excretion of P in urine.

The present study showed that there is an inevitable endogenous loss of P in faeces, even at low P intakes. An increase in P intake is accompanied by an increase in P absorption, faecal dietary P and faecal endogenous P. However, at high levels of P intake, which exceed P requirements, P regulation is achieved by a decreased efficiency of absorption, which leads to an increase in faecal P loss.

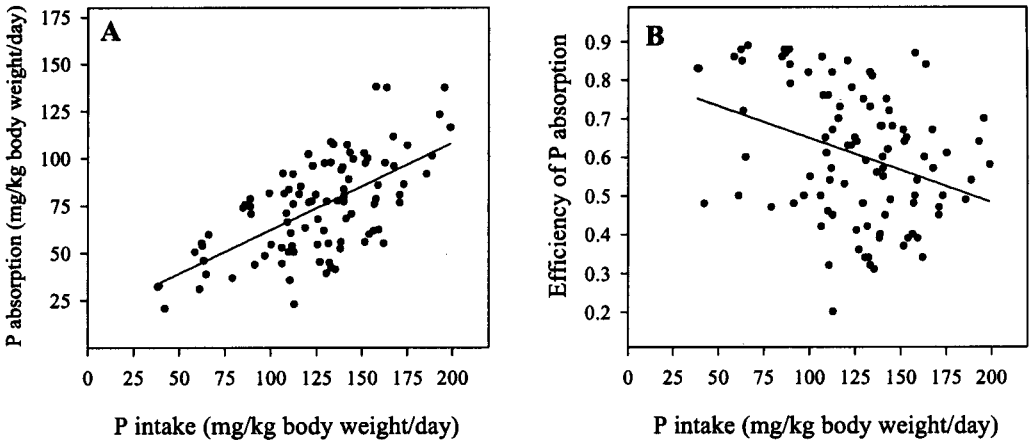

Fig. 4: Relationship between P intake and (a) P absorption, and (b) efficiency of P absorption for sheep given various treatments. Fitted lines are given by Equations 9 and 10 respectively

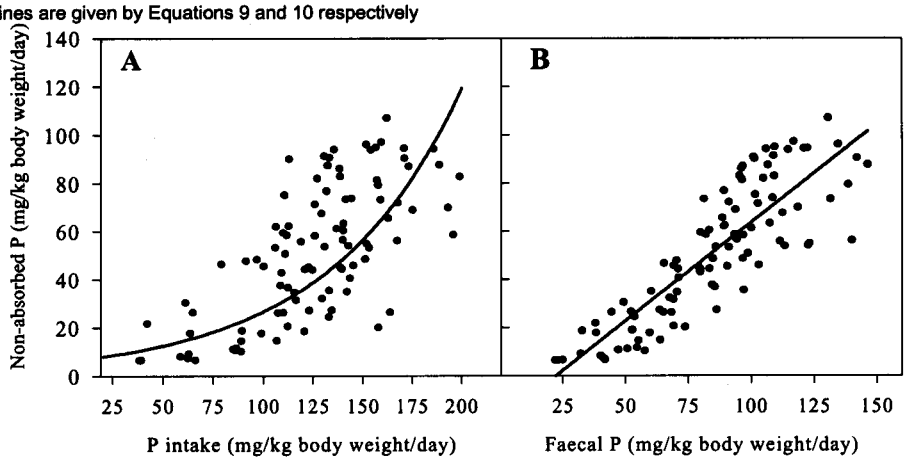

Fig. 5: Relationship between non-absorbed P and (a) P intake, and (b) faecal P for sheep given various treatments. Fitted lines are given by Equations 11 and 12 respectively

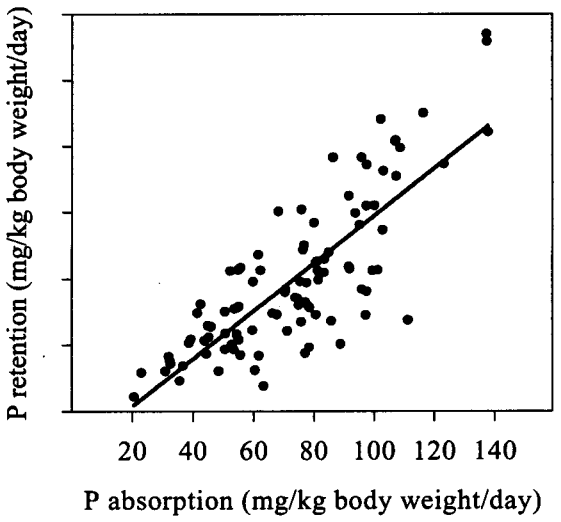

Fig. 6: Relationship between P retention and P absorption in sheep. Fitted line is given by Equation (13)

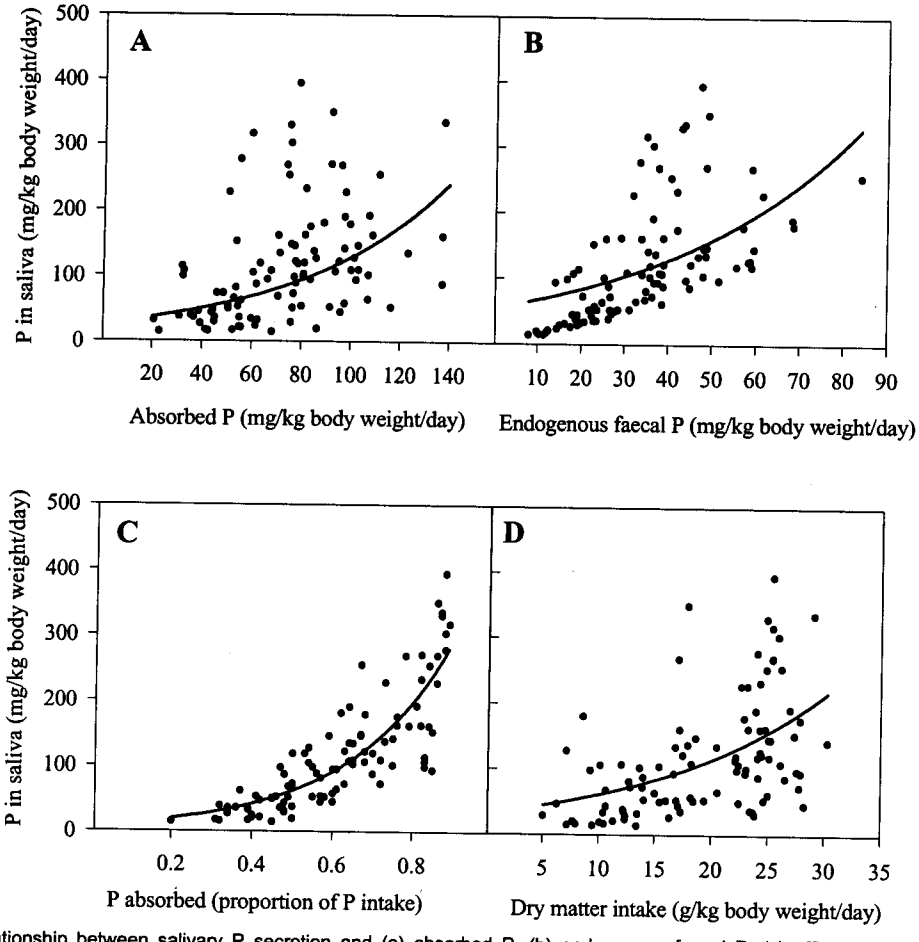

Fig. 7: Relationship between salivary P secretion and (a) absorbed P, (b) endogenous faecal P, (c) efficiency of absorption, and (d) dry matter intake in sheep. Fitted lines are given by Equations 14 to 17 respectively

The results indicate that there is saturation of the mechanism of P absorption at high intakes. A 40-kg sheep, in the conditions of the experiment, fed 3g P/d would excrete 62% of this in faeces. It can be inferred that excess of P in the diet is reflected directly in P voided in faeces, which might contribute to environmental phosphorus pollution. Isotope dilution techniques permits us to determine more precisely P requirement of the animal, and thus obtain more adequate values of dietary recommendations without an excessive safety margin.

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