View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Digital.CSIC

Journal of Agricultural Science (2009), **147**, 391–398. © *Cambridge University Press* 2009
doi:10.1017/S0021859609008673 *Printed in the United Kingdom*

MODELLING ANIMAL SYSTEMS PAPER

Rumen phosphorus metabolism in sheep

R. S. DIAS[1,2], S. LOPEZ[3]*, T. SILVA[2], R. M. P. PARDO[4], J. C. SILVA FILHO[5], D. M. S. S. VITTI[2], E. KEBREAB[6] AND J. FRANCE[1]

[1] *Centre for Nutrition Modelling, Department of Animal and Poultry Science, University of Guelph, Guelph, ON N1G 2W1, Canada*
[2] *Animal Nutrition Laboratory, Centro de Energia Nuclear na Agricultura, Caixa Postal 96, CEP 13400-970, Piracicaba, SP, Brazil*
[3] *Instituto de Ganadería de Montaña (IGM), Universidad de León – Consejo Superior de Investigaciones Científicas (CSIC), Departamento de Producción Animal, Universidad de León, E-24071, León, Spain*
[4] *Facultad de Ciencias Agropecuarias, University of Sucre, Carrera 28 5-267, Sincelejo, Sucre, Colombia*
[5] *Animal Research Laboratory, Federal University of Lavras, CEP 37200-000, Lavras, MG, Brazil*
[6] *Department of Animal Science, University of Manitoba, Winnipeg, MB R3T 2N2, Canada*

(*Revised MS received 12 December 2008; First published online 9 April 2009*)

SUMMARY

The objective of the present study was to examine the effect of the level of phosphorus (P) intake on ruminal P kinetics in sheep. Twelve Santa Inês male sheep (average body weight 36 kg) were fed a basal diet consisting of roughage (coast cross hay), concentrate mixture (cassava meal, soya bean meal and urea) and a mineral premix. The treatments consisted of the basal diet supplemented with 0, 1·5, 3 or 4·5 g/kg dry matter (DM) of mono-ammonium phosphate to provide increasing P levels representing treatments T0, T1, T2 and T3, respectively. The P content of experimental diets was 1·5; 2·0; 2·5 and 3·0 g P/kg DM, and considered highly deficient, deficient, adequate and in excess, respectively, compared with standard recommended allowances. Animals were injected with ^{32}P and thereafter samples of blood were collected over 7 days, while samples of rumen fluid and saliva were collected 4 and 6 days after injection. Phosphorus intake affected P concentration in ruminal fluid, whereas P concentration in saliva was not affected. The values for P turnover time in the rumen were 1·42, 1·23, 1·18 and 1·04 days, whereas values of endogenous P entry into the rumen were 1·05, 1·37, 1·53 and 1·91 g/day for T0, T1, T2 and T3, respectively, both affected by P intake. The specific activity (SA) of P in saliva, rumen and plasma were also all affected by P intake. The relationship between saliva and rumen SA emphasizes that most endogenous P in the rumen came from saliva. The possibility of an extra P source besides saliva contributing to endogenous P in the rumen is discussed. It is concluded from the results that, regardless of P intake, the flow of endogenous P into the rumen contributes to ensure a minimum supply of this essential element, which may be important in matching the requirements of the rumen microbes.

INTRODUCTION

Ruminal phosphorus (P) is provided mainly by endogenous P coming from saliva (Tomas 1973), which functions as a buffer to the volatile fatty acids produced when feed is fermented by microbes, thereby favouring the rumen environment (McDougall 1948). Microbial P requirements are relatively high, as this mineral is essential to rumen micro-organisms and necessary for the digestion of plant cell walls (Durand & Komisarczuk 1988; Bravo *et al*. 2003*a*). In the rumen, P may also affect the absorption of other minerals such as calcium and magnesium (Beardsworth *et al*. 1989).

Although endogenous P represents an important source contributing to the maintenance of rumen micro-organisms, its determination is not easily accomplished. Isotopic dilution methods may provide specific data on P kinetics, and hence can be

* To whom all correspondence should be addressed. Email: s.lopez@unileon.es

considered an interesting tool to quantify endogenous P flow to the rumen. For instance, although it has been commonly accepted that saliva is the main source of endogenous P entering the rumen, studies using ^{32}P have shown that endogenous P can also pass from blood to rumen (Lofgreen *et al.* 1952; Smith *et al.* 1955).

The current concern about P pollution necessitates research aimed at understanding the consequences of reducing P intake upon P metabolism in ruminants. For instance, it is well established that endogenous P is essential to the maintenance of a healthy rumen environment. Therefore it seems of interest to investigate the responses in endogenous P supply and P concentration in the rumen to different levels of P intake.

The present work was conducted with the aim of improving understanding of the relationship between P intake and endogenous salivary and ruminal P, and to assess the effect of level of P intake upon ruminal P. The data elicited should contribute to current knowledge concerning P metabolism in ruminants.

MATERIAL AND METHODS

Animals and diets

The experiment was conducted using a protocol approved by the Animal Care Committee of the Centre of Nuclear Energy in Agriculture (CENA, University of São Paulo). Twelve male sheep of Santa Inês breed, initial live weight $36 \pm 0{\cdot}5$ kg and aged 8 months, were randomly allocated to one of the four experimental groups (three animals per treatment). Each lot was housed in a separate stall and received the assigned experimental diet for 10 days. Thereafter, the animals were placed in individual cages designed for isotopic studies.

The basal diet was a mixed ration with coast cross (*Cynodon dactylon* L. (Pers.) cv. 'coast cross') grass hay as roughage, and a concentrate mixture composed of (per kg concentrate): 820 g cassava meal, 130 g soya bean meal, 30 g urea and 20 g of a mineral premix (containing, per g of mixture, 0·4 g S, 0·337 g NaCl, 0·161 g MgO, 45·7 mg $FeSO_4$, 32 mg $ZnSO_4$, 14·8 mg $MnSO_4$, 9 mg $CuSO_4$, 0·9 mg KI, 0·1 mg Na_2SeO_3 and 0·08 mg $CoSO_4$). The treatments consisted of the basal diet supplemented with different amounts of mono-ammonium phosphate (0, 1·5, 3 and 4·5 g/day) and limestone to provide increasing P (1·5, 2·0, 2·5 and 3·0 g P/kg dry matter (DM); representing experimental treatments T0, T1, T2 and T3, respectively) and Ca levels respectively (Table 1). The chemical composition of hay, concentrate and experimental diets is presented in Table 2. Feed (1100 g/day equivalent to 30·6 g feed/kg live weight) was offered twice daily and feed refusals were collected daily and weighed to determine DM intake (DMI).

Table 1. *Feed ingredients and composition of experimental diets*

	Treatments*			
Ingredient (g/day)	T0	T1	T2	T3
Hay	800	800	800	800
Concentrate	300	300	300	300
Limestone	–	2·5	5	7
Mono-ammonium phosphate	–	1·5	3	4·5

* T0, T1, T2 and T3 correspond to treatments providing 1·5; 2·0; 2·5 and 3·0 g P/kg DM.

Table 2. *Chemical composition of hay, concentrate and experimental diets (g/kg DM unless otherwise stated).*

			Experimental diets*			
	Hay	Concentrate	T0	T1	T2	T3
DM (g/kg)	893	906	896	897	897	896
Organic matter	947	975	955	960	957	967
CP	58	150	81	80	81	84
Ether extract	19	21	19	18	19	20
NDF	784	90	574	549	571	606
ADF	460	52	337	322	335	355
Phosphorus	1·5	1·43	1·48	2·04	2·57	3·04

* T0, T1, T2 and T3 correspond to treatments providing 1·5; 2·0; 2·5 and 3·0 g P/kg DM.

Following an adaptation period of 1 week, a single dose of 7·4 MBq of ^{32}P in 1 ml of saline solution (8·5 g NaCl/l distilled water) was injected into the jugular vein, and subsequently samples of plasma were collected every morning before feeding. Blood samples (10 ml) were taken (collected in Vacutainer® tubes) from the left jugular vein every 24 h for the 6 days after injection of ^{32}P dose. Saliva and rumen fluid samples were taken during the morning before feeding on days 4 and 6 after injection. No more samples of saliva or rumen fluid were collected so as to minimize possible stress caused to the animals. Before feeding the animals, saliva was collected on small pieces of plastic sponge placed directly into the animal's mouth and held by a pair of pincers, without impairing chewing movements. After a short time, soaked sponges were squeezed and a sample of 10 ml of saliva was gathered and thereafter frozen and

stored until further analysis. Rumen contents were withdrawn using an oesophageal tube (10 mm diameter) and a syringe to pump out 50 ml of sample at each time of collection. Rumen fluid was obtained after filtering the rumen sample using gauze, and centrifuging the filtrate at 3000 rpm for 15 min.

Animals were slaughtered on the last day of collection, following which rumen contents were weighed and ruminal volume was estimated ('physiological volume'). Thereafter the carcasses were disposed of in specially designed concrete holes.

Analysis of samples

Samples of feed and feed refusals were analysed for DM, crude protein (CP), P and acid detergent fibre (ADF) following recommendations of the Association of Official Analytical Chemists (1995). Neutral detergent fibre (NDF) was determined according to Mertens (2002), without using amylase or sodium sulphite (Table 2).

Blood samples were diluted in a solution of trichloroacetic acid (TCA; 100 g TCA/l distilled water) mixing 1 ml with 9 ml TCA, and then centrifuged at 1100 ***g*** for 10 min for plasma separation and protein precipitation. Concentration of inorganic P in plasma was determined by colorimetry (Fiske & Subbarow 1925). Samples of saliva and rumen fluid were diluted in a solution of TCA (100 g TCA/l distilled water) for protein precipitation (0·5 ml saliva + 9·5 ml TCA and 0·5 ml rumen fluid + 4·5 ml TCA), and then inorganic P was measured by colorimetric analysis (Fiske & Subbarow 1925).

Radioactivity of ^{32}P was measured in a Packard Liquid Scintillation Spectrometer (model 2450B) using Cerenkov radiation. For estimation of specific radioisotope activity, a standard ^{32}P solution (7·4 MBq ^{32}P diluted in 1 litre distilled water) was prepared, and then 1 ml of standard solution was transferred to a counting vial for radioactivity measurement. The value recorded for this solution (standard radioactivity = 278 245 dpm) was used as the reference for the specific activity (SA) calculations. For ^{32}P measurements, 1 ml of liquid samples (plasma, saliva or rumen fluid) was diluted with 19 ml of distilled water into counting vials and radioactivity (^{32}P) measured in the scintillation counter.

Calculations

Specific activities of ^{32}P in plasma, rumen and saliva (SA_p, SA_r and SA_s) were calculated as proportion of standard radioactivity/g of P, where proportion activity is calculated from the relation between activity measured in samples of plasma, rumen fluid or saliva and that determined in the standard solution, and the value was expressed per g of P in the corresponding sample (Roque *et al.* 2007).

The calculations proposed by Smith *et al.* (1955) were used to estimate flow of endogenous P into the rumen and P turnover time in the rumen. Secretion rate of endogenous P into the rumen (S_r; g/day) was calculated as

$$S_r = \frac{R \times SA_r(t) + D \int_0^t SA_r dt}{\int_0^t SA_p dt - \int_0^t SA_r dt}$$

where R is the pool size of P in the rumen (grams of P), t is the time after ^{32}P injection, $SA_r(t)$ is the value of SA_r recorded at 6 days after ^{32}P injection and D is the dietary P intake (g/day). Measured values of rumen volume (litres) and of P concentration in rumen fluid (C_r; g/l) were used to estimate R. The SA of the ruminal and plasma P over time following tracer administration ($\int_0^t SA_r dt$ and $\int_0^t SA_p dt$, respectively) was calculated from the corresponding SA *v.* time curves. Turnover time of rumen P (T; day) was

$$T = R/(S_r + D).$$

In order to calculate S_r, SA_r and SA_p, data at different times post-injection were used. Data reported by Smith *et al.* (1955) were adjusted for dose and combined with SA_r and SA_p data measured in the present study for the purposes of curve fitting. Thereafter the areas under the curve were calculated for each animal. Based on the plots of SA_r against time, a linearly increasing trend was assumed from 0 to 8 h, and then a negative exponential curve was fitted to the assembled SA_r data to describe the period 8 to 144 h after ^{32}P injection. As for SA_p *v.* time curves, the initial segment between 0 and 4 h was assumed to follow a decreasing linear trend, and thereafter the same negative exponential curve was fitted to the assembled SA_p data for the period beyond 4 h post-injection (4–144 h).

Statistical analysis

Experimentally measured data (P intake, saliva, rumen and saliva P concentrations and isotopic SA in saliva, rumen and plasma) and estimated values of endogenous P entering the rumen and P turnover time were analysed by one-way analysis of variance (ANOVA) according to a completely random design. Data were obtained from 12 animals, three for each treatment (P level). A completely random design with treatments assigned to subjects (parallel design) was appropriate and sufficient for the objectives of the present study, assuming that there were no systematic differences between the groups of subjects, and any differences can be attributed to treatment effects. A repeated measurements design (crossover, changeover or Latin-square) could, in theory, be an alternative to reduce the error variance and the individual subject differences from the overall treatment effect, because in these crossover designs each subject

Table 3. *Observed and predicted SA of P in plasma and rumen*

	Plasma SA_p*	Rumen SA_r*
Observed mean	0·0808	0·0667
Predicted mean	0·0770	0·0689
Pearson correlation coefficient	0·949	0·936
Lin concordance coefficient	0·944	0·933
MSPE†	0·000149	0·000102
Root MSPE	0·0122	0·0101
MSPE		
Bias‡	0·102	0·046
Slope§	0·020	0·038
Random‖	0·878	0·917

* SA expressed as proportion of standard radioactivity/g of *P*; values corresponding to mean from 4, 5 and 6 days after ^{32}P injection for SA_p and 4 and 6 days after ^{32}P injection for SA_r.
† Mean square prediction error.
‡ Error due to central bias expressed as a proportion of MSPE.
§ Error due to slope bias expressed as a proportion of MSPE.
‖ Error due to random variation expressed as a proportion of MSPE.

(experimental unit) is sequentially exposed to all treatments. However, such designs could not be used in a study of this nature because the animals had to be slaughtered at the end of a treatment and residual radioactivity in animal organs or tissues would affect measurements in following experimental periods when another treatment was applied to the same subject. The statistical analysis was performed using the GLM procedure (SAS 1999*a*), with level of P intake as the only source of variation (degrees of freedom of the error = 8). Standard error of means was obtained using the SAS LSMEANS procedure.

For the integrals in the S_r formula, the area under the curve (AUC) was calculated using a SAS macro (SAS 1999*b*; Yeh 2002). To build up the SA *v.* time curves to an acceptable number of data points, data measured in our experiment were merged with those reported by Smith *et al.* (1955). To validate this approach, a negative exponential function was fitted to the pooled dataset, and SA_r and SA_p values predicted from the fitted curves were compared with those measured experimentally (SA_r at 4 and 6 days, and SA_p at 4, 5 and 6 days after ^{32}P injection) using the concordance or reproducibility coefficient (Lin 1989) and the analysis of mean square prediction error (MSPE) (Table 3), calculated as

$$\mathrm{MSPE} = \frac{1}{n}\sum_{i=1}^{n}(O_i - P_i)^2$$

where n is the number of observations, O_i is the observed value and P_i is the predicted value. Root MSPE expressed as a proportion of the observed mean gives an estimate of the overall prediction error. The MSPE was decomposed into error due to random variation, error due to deviation of the regression slope from unity, and error due to central (mean) bias (Bibby & Toutenburg 1977). High correlation and concordance coefficients between observed and predicted values (Table 3) indicate a high degree of reproducibility between both values. The MSPE analysis also showed that predicted values of SA_p and SA_r were close to observed values (Table 3) and, in both cases, random variation was the major source of error (Table 3).

RESULTS

DMI was not significantly different among treatments ($P > 0{\cdot}05$) with average daily intakes of 972, 943, 971 and 1017 g DM/day (S.E.M. = 47·5) for T0, T1, T2 and T3, respectively. Average daily intake was 27·1 g DM/kg live weight (S.E. = 0·60).

There were significant differences between treatments in daily P intake (Table 4), which increased, as expected, with increased amounts of P provided as mono-ammonium phosphate. The concentration of P in feed DM was below National Research Council (NRC) (1985) recommended allowances in treatments T0 and T1 (1·5 and 2·0 g/kg DM), while T2 was close (2·5 g/kg DM) to the recommended value (2·4 g/kg DM) and T3 was above (3·0 g/kg DM) requirements established by NRC (1985).

Salivary P concentration (C_s; g/l) values are for mixed saliva, due to the method used for saliva collection. In studying ruminant saliva, McDougall (1948) observed that mixed saliva usually consists largely of parotid saliva. Average C_s values were similar for all treatments ($P > 0{\cdot}05$) (Table 4), with a significant linear correlation between C_s and DMI (g/day):

$$C_s = 0{\cdot}0007(\pm 0{\cdot}00016)\mathrm{DMI} - 0{\cdot}036(\pm 0{\cdot}15)$$
$$(n = 12;\ R^2 = 0{\cdot}70)$$

Mean rumen volume was 5·9, 5·7, 5·8 and 5·5 litres (S.E.M. = 0·37) representing 0·16, 0·15, 0·16 and 0·15 kg/kg live weight for T0, T1, T2 and T3, respectively.

Phosphorus concentration in rumen fluid (C_r; g/l) was different among treatments ($P < 0{\cdot}05$) and was significantly correlated with P intake (D; g/day):

$$C_r = 0{\cdot}27(\pm 0{\cdot}018)D + 0{\cdot}20(\pm 0{\cdot}037)$$
$$(n = 12;\ R^2 = 0{\cdot}96)$$

Specific activities of isotope ^{32}P in saliva (SA_s), rumen (SA_r) and plasma (SA_p) decreased with increasing P levels in diet ($P < 0{\cdot}05$; Table 4). SA values in the three compartments were significantly correlated with

Table 4. *Phosphorus measurements, specific activities and estimated values of endogenous secretion and turnover time of P in the rumen*

		Treatment*				
	Symbol	T0	T1	T2	T3	S.E.M.
DMI (g/day)		973	943	971	1017	47·5
P measurement						
Intake (g/day)	D	1·44	1·92	2·48	3·07	0·071
Saliva (g/l)	C_s	0·67	0·67	0·72	0·74	0·040
Rumen (g/l)	C_r	0·59	0·70	0·82	0·93	0·030
Plasma (g/l)	C_p	0·081	0·075	0·091	0·083	0·004
SA†						
Saliva	SA_s	0·0910	0·0707	0·0380	0·0346	0·0082
Rumen	SA_r	0·0825	0·0709	0·0436	0·0345	0·0134
Plasma	SA_p	0·0990	0·0726	0·0365	0·0331	0·0058
P in the rumen						
Endogenous P flow to the rumen (g/day)	S_r	1·05	1·37	1·53	1·91	0·110
P turnover time (day)	T	1·42	1·23	1·18	1·04	0·095

* T0, T1, T2 and T3 correspond to treatments providing 1·5; 2·0; 2·5 and 3·0 g P/kg DM.
† SA expressed as proportion of standard radioactivity/g of P; values corresponding to 6 days after ^{32}P injection.

each other (Pearson correlation coefficients of 0·89 ($SA_s - SA_r$); 0·81 ($SA_s - SA_p$) and 0·82 ($SA_r - SA_p$)).

Mean values for secretion rate of endogenous P into the rumen (S_r; g/day) were different among treatments ($P < 0·05$; Table 4), and this secretion rate was related to P intake (D; g/day), P in rumen fluid (C_r; g/l) and P turnover time (T; day):

$$S_r = 0·67(\pm 0·091)D - 0·10(\pm 0·190) \quad (n=12;\ R^2 = 0·84)$$
$$S_r = 2·3(\pm 0·38)C_r - 0·26(\pm 0·297) \quad (n=12;\ R^2 = 0·77)$$
$$S_r = -1·3(\pm 0·39)T + 3·1(\pm 0·48) \quad (n=12;\ R^2 = 0·53)$$

The mean values of P turnover time (T; day) are shown in Table 4, and were significantly different among treatments. S_r increased and T decreased as P intake became higher.

DISCUSSION

Phosphorus intake increased with addition of mono-ammonium phosphate for treatments T1, T2 and T3, whereas in treatment T0 animals did not receive this mineral source of P. Increased levels of Ca corresponding to increased levels of P were established with the aim of maintaining the Ca:P ratio equal to 2:1 for all treatments. Several researchers have shown Ca may affect P metabolism (Challa *et al*. 1989; Dias *et al*. 2006), therefore keeping the Ca:P ratio similar among treatments precludes possible influence of Ca upon P.

Mean values of salivary P concentration (C_s) ranged between 0·67 and 0·74 g/l, only slightly higher than values reported by McDougall (1948) but noticeably higher than those reported by Bailey (1961). Differences in the procedures for saliva collection may explain these discrepancies (Kincaid & Rodehutscord 2005). The current method was similar to that of McDougall (1948).

A relationship between C_s and DMI was observed. This was in agreement with Bravo *et al*. (2003*b*) who showed that DMI affected salivary P concentration. Karn (2001) suggested that whereas saliva secretion is related to DMI, salivary P concentration is related to plasma P concentration. According to Durand & Kawashima (1979), there is a relationship between salivary P concentration and rate of secretion in saliva. Therefore, DMI may be also related to salivary P concentration. The relationship between P intake and salivary P concentration remains uncertain, although it has been suggested that at higher P intakes salivary P flow decreases whereas at lower P intakes salivary P flow remains constant (Coats & Wright 1957; Bravo *et al*. 2003*b*).

The relationship between salivary and plasma P concentrations is well known, as demonstrated by several researchers (Scott & Beastall 1978; Mañas-Almendros *et al*. 1982; Challa *et al*. 1989). The lack of differences in concentration of salivary P between treatments may be related to the similar values of plasma P concentration between treatments. The ratio of salivary to plasma P varied from 6·2:1 to 10·5:1, with most values ranging between 8 and 9:1. These ratios are close to those reported by Tomas *et al*. (1967) but lower than the ratio 15:1 reported by McDougall (1948) in sheep.

Some researchers have proposed that salivary P is not under homeostatic regulation, and that P intake rather than DMI or salivary flow rate is the major

factor influencing changes in endogenous P secretion (Scott *et al.* 1995). Although in the current study P intake and salivary P concentration were not correlated, there was, however, a significant effect of P intake on the rate of endogenous P secreted into the rumen (S_r).

Phosphorus concentration in rumen fluid (C_r) was different between treatments ($P<0{\cdot}05$) and was correlated with dietary P intake, in agreement with Tomas *et al.* (1967) and Nel & Moir (1974) who observed a similar relationship in their studies. When C_r (mg/l) was plotted against dietary P (g P/kg DM), the relationship was linear ($C_r = 282{\cdot}3$ dietary P $+ 175{\cdot}4$; $n=12$; $R^2=0{\cdot}87$) and closely matched the relationship obtained by Witt & Owens (1983). Those authors found that with 1 g P/kg DM in the diet the concentration of P in ruminal fluid was 417 mg/l, whereas according to the equation in the present study for 1 g P/kg DM in diet the ruminal P concentration would be 457 mg/l. The present results also indicate that at higher P intakes, ruminal P concentrations would be expected to increase linearly. Mean C_r found for animals fed 1·44 g P/kg DM in the diet was 595 mg/l, a value close to 619 mg/l reported by Witt & Owens (1983) for sheep fed a diet containing 1·66 g P/kg DM. Conversely, Evans & Davis (1966) reported a positive relationship between dietary and rumen P concentrations, up to a plateau immediately above 1·6 g dietary P/kg DM.

Although Tomas (1973) suggested that most of the inorganic P in rumen fluid originates from saliva secretion, in the present study salivary and ruminal P concentrations were not correlated, in agreement with Tomas *et al.* (1967). Concentration of P in the rumen seemed to be determined mainly by P intake. Kincaid & Rodehutscord (2005) suggested that rumen P is directly affected by dietary P concentration and indirectly by a subsequent salivary effect. The concentration of P in the rumen can also be affected by the rate of phosphate absorption from the rumen and by phosphate solubility (Bailey 1961). Both processes are influenced, in turn, by the chemical form of dietary P, what could explain some of the differences in C_r between T0 (all dietary P is organic) and the other treatments (a significant amount of dietary P is from a mineral salt).

In order to calculate S_r (endogenous P secreted into the rumen), SA_p and SA_r data measured in the current study were combined with those reported by Smith *et al.* (1955). The predicted values of SA_p and SA_r were in close agreement with current observed values, given the low MSPE and high correlation and concordance coefficients between both values (Table 3). This agreement may be explained by the fact that data reported by Smith and co-workers were also measured in growing sheep (4 and 10 months) and, although in the present work different levels of P were supplied to animals, the level of dietary P does not affect the decay rate of ^{32}P in plasma and rumen (unpublished data) validating the combination of data from both studies. Random variation was the main source of error indicating that there was no major bias in the predictive ability of the equations used in the current study and the predicted SA_p and SA_r can be used with confidence to calculate S_r.

Measurements of SA_s, SA_r and SA_p were statistically different between treatments as a result of increasing P ingestion. When compared within each treatment, SA_r was in most cases higher than SA_s and SA_p, suggesting that a possible endogenous source other than saliva may have contributed to total endogenous P flowing to the rumen. The significant relationship between SA_s and SA_r would confirm that saliva is the main source of endogenous P flowing into rumen, although the contribution of endogenous P coming from other sources may not be ruled out. In agreement, Smith *et al.* (1955) reported that salivary secretion could not account for the quantity of endogenous P that entered the rumen and suggested that part of endogenous P is not of salivary origin, probably entering directly through the rumen wall. Scarisbrick & Ewer (1951), studying the absorption of inorganic phosphate from the rumen in sheep, observed that ^{32}P injected into the rumen appeared in the carotid artery to a lesser extent than in the ruminal vein, indicating that absorption of inorganic phosphate from the rumen is plausible. In addition, Scarisbrick & Ewer (1951) reported that inorganic phosphate could also flow from blood to the rumen. Supporting these observations, Parthasarathy *et al.* (1952) demonstrated that movement of inorganic P may occur in both directions across the rumen epithelium. Breves *et al.* (1988) concluded that the reticulo-rumen wall is permeable to inorganic phosphate, which can pass though in either direction, with a significant relationship between the concentration of inorganic phosphate in the fluid and its net flux across the wall.

The proportion of S_r relative to P intake tended to decrease from T0 to T3 (0·73, 0·71, 0·62 and 0·62 for T0, T1, T2 and T3, respectively), suggesting a compensation for the lower P contribution to the rumen when P intake is lower. As P intake is increased, S_r will also increase, but its relative contribution to total P reaching the rumen will gradually decrease.

Turnover time of P in the rumen (T; day) was calculated according to Smith *et al.* (1955). The turnover times obtained in the current study (Table 4) are comparable with the value (27·7 h or 1·15 day) obtained by Smith *et al.* (1955) in sheep aged 10 months, who also suggested that turnover time could be shorter in younger animals. Guyton *et al.* (2003) reported shorter ruminal P turnover times as P intake was increased in lactating cows fed phytic acid, in agreement with current results. The longest turnover time was observed in sheep fed the diet with the

lowest P level, and decreased in response to supplementation with inorganic P. Increasing levels of dietary P and, in particular, of inorganic P may have favoured P fluxes into and out of the rumen, shortening the turnover time. Rumen P turnover could be important in providing P to ruminal micro-organisms to match their requirements, as the element is essential for the maintenance and growth of ruminal microbes (Durand & Komisarczuk 1988), and this is one of the key targets of P metabolism in ruminants. Nel & Moir (1974) concluded that hardly any P deficiency can possibly be so severe as to cause a P deficiency in the rumen as far as the requirements of the rumen microbes are concerned.

The present results indicate that both dietary and endogenous provisions of P to the rumen are increased with higher P intakes. However, the relative contribution of endogenous P secretion to the total flow of P entering the rumen decreases gradually as P intake becomes higher, indicating that this endogenous secretion is relatively more important at lower P intakes, as a means of ensuring a minimum supply of this mineral to the rumen. It would be of interest to ascertain at what metabolic expense P is supplied to rumen micro-organisms without impairing overall P metabolism in the animal.

The authors thank Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP Proc. 2004/14532-5) for financial support. Funding was provided, in part, by the Canada Research Chairs Program.

REFERENCES

Association of Official Analytical Chemists (1995). *Official Methods of Analysis of AOAC*, 16th edn. Arlington, VA: Association of Official Analytical Chemists International.

Bailey, C. B. (1961). Saliva secretion and its relation to feeding in cattle. *British Journal of Nutrition* **15**, 489–498.

Beardsworth, L. J., Beardsworth, P. M. & Care, A. D. (1989). The effect of ruminal phosphate concentration on the absorption of calcium, phosphorus and magnesium from the reticulo-rumen of the sheep. *British Journal of Nutrition* **61**, 715–723.

Bibby, J. & Toutenburg, H. (1977). *Prediction and Improved Estimation in Linear Models*. London, UK: Wiley.

Bravo, D., Sauvant, D., Bogaert, C. & Meschy, F. (2003*a*). II. Quantitative aspects of phosphorus absorption in ruminants. *Reproduction Nutrition Development* **43**, 271–284.

Bravo, D., Sauvant, D., Bogaert, C. & Meschy, F. (2003*b*). III. Quantitative aspects of phosphorus excretion in ruminants. *Reproduction Nutrition Development* **43**, 285–300.

Breves, G., Holler, H., Packheiser, P., Gabel, G. & Martens, H. (1988). Flux of inorganic phosphate across the sheep rumen wall *in vivo* and *in vitro*. *Quarterly Journal of Experimental Physiology* **73**, 343–351.

Challa, J., Braithwaite, G. D. & Dhanoa, M. S. (1989). Phosphorus homeostasis in growing calves. *Journal of Agricultural Science, Cambridge* **112**, 217–226.

Coats, D. A. & Wright, R. D. (1957). Secretion by the parotid gland of the sheep: the relationship between salivary flow and composition. *The Journal of Physiology* **135**, 611–622.

Dias, R. S., Kebreab, E., Vitti, D. M. S. S., Roque, A. P., Bueno, I. C. S. & France, J. (2006). A revised model for studying phosphorus and calcium kinetics in growing sheep. *Journal of Animal Science* **84**, 2787–2794.

Durand, M. & Kawashima, R. (1979). Influence of minerals in rumen microbial digestion. In *Digestive Physiology and Metabolism in Ruminants* (Eds Y. Ruckebush & P. Thivend), pp. 375–408. Lancaster, UK: MTP Press Ltd.

Durand, M. & Komisarczuk, S. (1988). Influence of major minerals on rumen microbiota. *Journal of Nutrition* **118**, 249–260.

Evans, J. L. & Davis, G. K. (1966). Dietary phosphorus, sulfur and molybdenum and mineral composition of rumen fluid. *Journal of Animal Science* **25**, 1010–1013.

Fiske, C. H. & Subbarow, Y. (1925). The colorimetric determination of phosphorus. *Journal of Biological Chemistry* **66**, 375–400.

Guyton, A. D., McKinney, J. M. & Knowlton, K. F. (2003). The effect of steam-flaked or dry ground corn and supplemental phytic acid on phosphorus partitioning and ruminal phytase activity in lactating cows. *Journal of Dairy Science* **86**, 3972–3982.

Karn, J. F. (2001). Phosphorus nutrition of grazing cattle: a review. *Animal Feed Science and Technology* **89**, 133–153.

Kincaid, R. L. & Rodehutscord, M. (2005). Phosphorus metabolism in rumen. In *Nitrogen and Phosphorus Nutrition of Cattle – Reducing the Environmental Impact of Cattle Operations* (Eds E. Pfeffer and A. Hristov), pp. 187–193. Cambridge, MA: CABI Publishing.

Lin, L. I. K. (1989). A concordance correlation coefficient to evaluate reproducibility. *Biometrics* **45**, 255–268.

Lofgreen, G. P., Kleiber, M. & Smith, A. H. (1952). The excretion of injected P^{32} into the gastrointestinal tract of the young calf. *The Journal of Nutrition* **47**, 561–569.

Mañas-Almendros, M., Ross, R. & Care, A. D. (1982). Factors affecting the secretion of phosphate in parotid saliva in the sheep and goat. *Quarterly Journal of Experimental Physiology* **67**, 269–280.

Mcdougall, E. I. (1948). Studies on ruminant saliva. 1. The composition and output of sheep's saliva. *Biochemical Journal* **43**, 99–109.

Mertens, D. R. (2002). Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. *Journal of AOAC International* **82**, 1217–1240.

National Research Council (NRC) (1985). *Nutrient Requirements of Sheep*, Sixth Revised edition. Washington, DC: National Academy Press.

Nel, J. W. & Moir, R. J. (1974). The effect of ruminal and duodenal application of different levels of calcium and

phosphorus to sheep on semi-purified diets. *South African Journal of Animal Science* **4**, 1–20.

Parthasarathy, D., Garton, G. A. & Phillipson, A. T. (1952). The passage of phosphorus across the rumen epithelium of sheep. *Biochemical Journal* **52**, xvi–xvii.

Roque, A. P., Dias, R. S., Vitti, D. M. S. S., Silva Bueno, I. C., Cunha, E. A., Santos, L. E. & Bueno, M. S. (2007). True digestibility of calcium from sources used in finishing lamb diets. *Small Ruminant Research* **71**, 243–249.

SAS Institute Inc. (1999*a*). *SAS/STAT User's Guide Version 8*. Cary, NC: SAS Institute Inc.

SAS Institute Inc. (1999*b*). *SAS Macro Language: Reference Version 8*. Cary, NC: SAS Institute Inc.

Scarisbrick, R. & Ewer, T. K. (1951). The absorption of inorganic phosphate from the rumen of the sheep. *Biochemical Journal* **49**, lxxix.

Scott, D. & Beastall, G. (1978). The effects of intravenous phosphate loading on salivary phosphate secretion and plasma parathyroid hormone levels in the sheep. *Quarterly Journal of Experimental Physiology* **63**, 147–156.

Scott, D., Rajaratne, A. A. J. & Buchan, W. (1995). Factors affecting faecal endogenous phosphorus loss in the sheep. *Journal of Agricultural Science, Cambridge* **124**, 145–151.

Smith, A. H., Kleiber, M., Black, A. L. & Baxter, C. F. (1955). Transfer of phosphate in the digestive tract. II. Sheep. *Journal of Nutrition* **57**, 507–527.

Tomas, F. M. (1973). Parotid salivary secretion in sheep: its measurement and influence on phosphorus in rumen fluid. *Quarterly Journal of Experimental Physiology* **58**, 131–138.

Tomas, F. M., Moir, R. J. & Somers, M. (1967). Phosphorus turnover in sheep. *Australian Journal of Agricultural Research* **18**, 635–645.

Witt, K. E. & Owens, F. N. (1983). Phosphorus: ruminal availability and effects on digestion. *Journal of Animal Science* **56**, 930–937.

Yeh, S. T. (2002). Using trapezoidal rule for the area under a curve calculation. In *Proceedings of the 27th Annual SAS® User Group International* (*SUGI*) *Conference*, Paper 229. Orlando, FL.