ABSTRACT: A major objective of this study was to extend the Vitti-Dias model used to describe P metabolism in ruminants, by adding 2 new pools to the original model to represent the rumen and saliva. An experiment was carried out using 24 male sheep, initial BW of 34.5 kg, aged 8 mo, fed a basal diet supplied with increasing amounts of dicalcium phosphate to provide 0.14, 0.32, 0.49, and 0.65% P in the diet. Sheep were individually housed indoors in metabolic cages and injected with a single dose of 7.4 MBq of $^32$P into a jugular vein. Feed intake and total fecal and urinary outputs were recorded and sampled daily for 1 wk, and blood samples were obtained at 5 min, and 1, 2, 4, 6, 24, 48, 72, 96, 120, 144, and 168 h after $^32$P injection. Saliva and rumen fluid samples were taken on d 6, 7, and 8. Then, animals were slaughtered and samples from liver, kidney, testicle, muscle, and heart (soft tissue) and bone were collected. Specific radioactivity and inorganic P were then determined in bone, soft tissue, plasma, rumen, saliva, and feces, and used to calculate flows between pools. Increased P intake positively affected total P ($r = 0.97, P < 0.01$) and endogenous P excretion in feces ($r = 0.85, P < 0.01$), P flow from plasma to saliva ($r = 0.73, P < 0.01$), from saliva to rumen ($r = 0.73, P < 0.01$), and from lower gastrointestinal tract to plasma ($r = 0.72, P < 0.01$). Urinary P excretion was similar for all treatments ($P = 0.35$). It was, however, related to plasma P ($r = 0.63, P < 0.01$) and to net P flow to bone (accretion – resorption; $r = −0.64, P < 0.01$). Phosphorus intake affected net P flow to soft tissue ($P = 0.04$) but not net P flow to bone ($P = 0.46$). Phosphorus mobilized from bone was directed toward soft tissue, as suggested by the correlations between P flow from bone to plasma and net P flow to soft tissue ($r = 0.89, P < 0.01$), and P flow from plasma to soft tissue and net P flow to bone ($r = −0.76, P < 0.01$). The lack of effect of dietary P on net P accretion in bone suggests that P demand for bone formation was low and surplus P was partially used by soft tissue. In conclusion, the model resulted in appropriate biological description of P metabolism in sheep and added knowledge of the effects of surplus dietary P on P metabolism. Additionally, the model can be used as a tool to assess feeding strategies aiming to mitigate P excretion into the environment.

Key words: bone, modeling, phosphorus, rumen, saliva, soft tissue

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INTRODUCTION

A large number of ruminant producers provide dietary P supplementation in excess of requirements to guarantee animal performance, owing to the importance of this mineral in maintaining metabolic functions. Consequently, P excreted in feces represents a risk to the environment (Tamminga, 1996). Moreover, inorganic P supplemented to animal diets is a limited and expensive resource (Gilbert, 2009).
It is well established that, in ruminants, dietary P is mainly absorbed from the small intestine, with feces being the main route for disposing of unabsorbed P. Although the amount of urinary P excreted by ruminants is considered negligible, excretion of P in urine can represent a compensatory mechanism to eliminate surplus P when P secretion through saliva is not sufficient to maintain P homeostasis (Scott and Buchan, 1985, 1987).

When the ruminant is P deficient, P is mobilized from bone to attend to demands of various body tissues. Conversely, dietary P in excess of the requirements of an animal will affect P metabolism by increasing P in plasma, which in turn stimulates salivary P secretion into the rumen (Tomas et al., 1967) and hence the excretion of endogenous P in feces in addition to unabsorbed dietary P (Dias et al., 2009). There are still gaps in knowledge of the fates of dietary P, namely partition of retained P between bone and soft tissue and its involvement with P from other pools such as plasma, rumen, and saliva.

Mathematical models have been used for many years as tools to aid scientists gain information on P metabolism in ruminants (Lofgreen and Kleiber, 1953; Schneider et al., 1987; Vitti et al., 2000). In this context, a primary objective of this study was to extend the Vitti-Dias model (Dias et al., 2006) by including the rumen and saliva as additional, explicit pools to provide a more detailed and accurate description of the compartmentalization involved in P metabolism in growing sheep fed different amounts of dietary P.

**MATERIALS AND METHODS**

The experiment was conducted using a protocol approved by the Commission of Ethics in Experimentation with Animals and the Commission of Environmental Ethics of Centre for Nuclear Energy in Agriculture, University of Sao Paulo, Brazil.

**Animals and Diets**

Twenty-four Santa Inês male sheep [initial BW = 34.5 kg (SD = 1.38), aged 8 mo] were randomly allocated to 4 groups of 6 animals according to a completely random design. The sheep had an adequate nutritional status at the commencement of the trial, according to NRC (2007), whereas for diets with 0.32, 0.49, and 0.65% P [moderate (MP), high (HP), and surplus (SP) P diets, respectively], P intakes (3.5, 5.5, and 7.5 g of P/d, respectively) were greater than the requirements (2.9 g of P/d is the requirement for growing rams aged 8 mo with BW gain of 200 g/d).

**Experimental Procedures**

Animals were placed initially in lot stalls where they received the corresponding experimental diet for 7 d, in 2 equal meals each day, at 0800 and 1700 h. Then, animals were transferred to individual cages specially designed for isotope studies. After a 7-d adaptation period for the sheep to become accustomed to the metabolic cages, animals received a single dose of 7.4 MBq of $^{32}$P injected into the right jugular vein, and subsequently blood samples (10 mL) were collected by venipuncture from the left jugular vein at 5 min, and 1, 2, 4, 6, 24, 48, 72, 96, 120, 144, and 168 h (first to seventh day of sampling period) after $^{32}$P dosing, and transferred to glass tubes containing heparin. Feed intake and total fecal and urinary outputs were recorded daily for 1 wk during the sampling period. Diets and feed refusals were weighed and sampled daily. All the feed refusal samples of each animal were combined, so that at the conclusion of the experimental period, feed and feed refusal composites were obtained for composition analyses. Total fecal output was collected and weighed daily, and representative samples of feces (10% of daily output) were collected and bulked at the end of the experimental period for further analysis of inorganic P and $^{32}$P. Urine was collected in buckets containing 10 mL of concentrated sulfuric acid, and after weighing total daily output, samples (10% of daily output) were frozen at −20°C. At the end of the collection period, the preserved urine samples were composited by animal for further P and $^{32}$P analyses. Mixed saliva samples and rumen fluid samples were taken only on d 6, 7, and 8 of the experiment to minimize stress to the animals. Saliva was collected using a pair of pincers and small pieces of plastic sponge, put directly into the mouth of the animal before feeding without impairing chewing movements. Rumen contents were collected in the morning before the first feeding using a stomach tube and a syringe to pump out ruminal fluid. To avoid excess saliva contamination of ruminal samples, the tube was inserted as quickly as possible by an experienced operator well into the rumen before fluid was collected. Then, the first approximately 200 mL withdrawn were discarded to minimize saliva contamination before a 50-mL sample was taken. On d 8, after samplings of feces, urine, saliva, and rumen fluid, animals were slaughtered by exsanguination via carotid arteries after intravenous injection of 0.05 mg of xylazine (Rompum; Bayer, Sao Paulo, Brazil)/kg of
BW. Samples from various soft tissues (liver, kidney, testicle, muscle, and heart) and bone (twelfth rib) were collected from each animal. Kidneys, heart, liver, and testicles were weighed to determine the percentage of these tissues in animal BW, whereas muscle was considered to be 26.9% of BW. For determination of the bone pool, total skeleton weight was assumed to be 8% of BW. Information regarding percentages of muscle and bone in BW was obtained from studies carried out using Santa Inês sheep at Centro de Energia Nuclear na Agricultura (D. M. S. S. Vitti, CENA, University of São Paulo, Brazil, unpublished data). These percentages are in close agreement with literature data (e.g., Butterfield, 1988).

### Analytical Methods

Samples of feed and feed refusals were dried at 60°C for 48 h, ground through a 1-mm screen in a Wiley mill and analyzed for DM, ash, CP, and ADF concentrations following recommendations of the Association of Official Analytical Chemists (AOAC, 1995). Crude protein was determined by the Kjeldahl method, and NDF according to Mertens (2002), without using amylase or sodium sulfite. Dried samples (1 g) were ashed at 500°C for 8 h, and then 5 mL of concentrated HCl (12 M) was added and heated to dissolve the ash completely. The mixture was filtered through P-free filter paper and P content in feed samples was determined by colorimetry using vanadate-molybdate reagent (Sarruge and Haag, 1974), whereas Ca was determined by atomic absorption spectrometry (Zagatto et al., 1979). The colorimetric determination of P was an adaptation of method 965.17 of AOAC (1995). A vanadate solution was prepared by dissolving 2.5 g of ammonium vanadate (NH₄VO₃) in 500 mL of boiling water. After cooling, 350 mL of concentrated HNO₃ was added and the solution was diluted to 1 L with distilled water. For the molybdate aqueous solution, 50 g of ammonium molybdate [(NH₄)₆Mo₇O₂₄] was dissolved in hot distilled water. Cooled vanadate and molybdate solutions were combined (1:1, vol/vol) to obtain the vanadate-molybdate reagent. Ash solution (2 mL) was mixed thoroughly with 2 mL of vanadate-molybdate reagent and 3 mL of distilled water. The mixture was allowed to stand for 5 min and absorbance was read at 420 nm against a standard P curve (using solutions of potassium dihydrogen phosphate) to estimate the P concentration in samples.

Inorganic P was determined in feces, urine, plasma, saliva, rumen, bone, and soft tissue samples. Fecal samples were dried at 60°C for 48 h. A sample (1 g) of dried feces was ashed at 500°C for 8 h, and then 5 mL of concentrated HCl (12 M) were added for wet ashing digestion. The mixture was filtered through P-free filter paper and P content was determined by the colorimetric method of Sarruge and Haag (1974). Thawed urine samples were processed as described by Morse et al. (1992), and inorganic P was analyzed by colorimetry according to the method of Fick et al. (1979). Blood samples were diluted in a solution of trichloroacetic acid (TCA; 100 g of TCA/L of distilled water), mix-
Radioactivity ($^{32}$P) was measured in feces, plasma, saliva, rumen fluid, and bone samples. Samples (1 mL) of centrifuged and filtered plasma, saliva, and rumen fluid were directly dispensed into borosilicate vials. Feces, bone, and soft tissue samples were ashed as for inorganic P determination. Ash samples obtained from fecal, bone, and soft tissue samples (1 g) were dissolved in 18 N H$_2$SO$_4$, and then 1 mL of each hydrolysate was transferred to counting vials for radioactivity measurement. All samples were diluted with a scintillation solution to 1:10 or 1:20 depending on the expected radioactivity values in each case. Finally, the radioactivity of $^{32}$P in each sample was measured in a liquid scintillation spectrometer (Beckman model LS 500-TA; Beckman-Coulter Inc., Fullerton, CA) using Cerenkov radiation. To improve the efficiency of radioactivity readings, an external standard calibration procedure of quench correction was used (Nascimento Filho, 1977).

The mean values of $^{32}$P counting determined in saliva and rumen samples collected on d 6, 7, and 8 were used for the model calculations. Radioactivity of each soft tissue was measured and a mean value (considering the relative weight of each organ) was determined. For the calculation of the pool size of soft tissue, P concentration was determined in each organ and the relative weight of each tissue in the animal BW was considered for the calculation.

**Model Description**

The model of whole-body P metabolism is an extension of the one proposed by Vitti et al. (2000) and revised by Dias et al. (2006) for use with ruminants (see France et al., 2010 for review). The extended model is illustrated in Figure 1. The 6 pools of P are 1) rumen, 2) lower gastrointestinal tract (GIT), 3) saliva, 4) plasma, 5) bone, and 6) soft tissue. The entry of P to the system is via intake ($F_{01}$), and exit via feces ($F_{02}$) and urine ($F_{04}$). These 3 flows are experimental measurements. A single dose of $^{32}$P (D, MBq) is administered into systemic blood at time zero, and specific $^{32}$P radioactivity (SRA, MBq/g of P in sample) of all pools monitored [$s_1$, $s_2$, $s_3$, $s_4$, $s_5$, and $s_6$ for SRA in rumen, GIT (measured in feces), saliva, plasma, bone, and soft tissue, respectively]. The $Q_i$ to $Q_6$ are the quantities of P in the respective pools, and $q_1$ to $q_5$ are the quantities of labeled P in the respective pools. The $F_{ij}$ is the flow of P to pool $i$ from $j$. $F_{sij}$ denotes an external flow into pool $i$, and $F_{0ij}$ denotes a flow from pool $j$ out of the system. The scheme assumes there is no re-entry of labeled P from external sources. Basic mathematical notation is summarized in Table 2.

Conservation of mass principles can be applied to each pool in Figure 1 to generate differential equations. For labelled P,

$$\frac{dQ_1}{dt} = F_{10} + F_{13} - F_{21}, \quad [1a]$$

$$\frac{dQ_2}{dt} = F_{21} + F_{24} - F_{02} - F_{42}, \quad [1b]$$

$$\frac{dQ_3}{dt} = F_{34} - F_{13}, \quad [1c]$$

$$\frac{dQ_4}{dt} = F_{42} + F_{45} + F_{46} - F_{24} - F_{21} - F_{54} - F_{64}, \quad [1d]$$

$$\frac{dQ_5}{dt} = F_{54} - F_{45}, \quad [1e]$$

$$\frac{dQ_6}{dt} = F_{64} - F_{46}, \quad [1f]$$

and for unlabelled P,

$$\frac{dq_1}{dt} = s_3F_{13} - s_1F_{21}, \quad [2a]$$

$$\frac{dq_2}{dt} = s_1F_{21} + s_1F_{24} - s_2(F_{02} + F_{42}), \quad [2b]$$

$$\frac{dq_3}{dt} = s_4F_{34} - s_3F_{13}, \quad [2c]$$

$$\frac{dq_4}{dt} = s_2F_{42} + s_2F_{45} + s_6F_{46} - s_4(F_{04} + F_{24} + F_{34} + F_{54} + F_{64}), \quad [2d]$$
Table 2. Principal symbols used in the model

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
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<tbody>
<tr>
<td>$F_{ij}$</td>
<td>Flow of P to pool $i$ from $j$; $F_0$ denotes an external flow into pool $i$ and $F_0$ denotes a flow from pool $j$ out of the system; g/d</td>
</tr>
<tr>
<td>$D$</td>
<td>Dose of labeled P administered to plasma at time zero, MBq</td>
</tr>
<tr>
<td>$Q_i$</td>
<td>Quantity of P in pool $i$, g</td>
</tr>
<tr>
<td>$q_i$</td>
<td>Quantity of labeled P in pool $i$, MBq</td>
</tr>
<tr>
<td>$s_i$</td>
<td>Specific $^{32}$P radioactivity of pool $i$ ($=q_i/Q_i$), MBq/g</td>
</tr>
<tr>
<td>$k$</td>
<td>Relative rate of change of plasma specific activity, per day</td>
</tr>
<tr>
<td>$N$</td>
<td>Number of days, d</td>
</tr>
</tbody>
</table>

\[
\frac{d q_5}{dt} = s_1 F_{34} - s_4 F_{45}, \quad \text{[2e]}
\]

\[
\frac{d q_6}{dt} = s_1 F_{64} - s_6 F_{66}, \quad \text{[2f]}
\]

Let

\[
s_4(t) = s_4(0)e^{-kt},
\]

where $s_4(0)$ is SRA at time of dosing, $k$ (d$^{-1}$) is a relative rate constant, and $t$ is time (d) after dosing. Then,

\[
\frac{d s_4}{dt} = -ks_4(0)e^{-kt}
\]

and

\[
\frac{1}{s_4} \frac{ds_4}{dt} = -k.
\]

Consider

\[
\frac{ds_5}{dt} = \frac{d(q_5Q_5^{-1})}{dt} = Q_5^{-1} \frac{dq_5}{dt} - q_5Q_5^{-2} \frac{dQ_5}{dt}. \quad \text{[3]}
\]

Using Eq. 1e and 2e in Eq. 3 gives

\[
\frac{1}{s_5} \frac{ds_5}{dt} = \frac{s_4 - s_5}{s_5Q_5} F_{54}. \quad \text{[4]}
\]

**Figure 1.** Whole-body P metabolism model for growing ruminants. The circles identify the measured flows: $F_{10} = $ P intake, $F_{02} = $ P excreted in feces, and $F_{34} = $ P in urine. The arrows represent the flows between pools: $F_{21} = $ from rumen to lower gastrointestinal tract (GIT), $F_{24} = $ from GIT to plasma, $F_{34} = $ from plasma to GIT, $F_{44} = $ from plasma to soft tissue, $F_{46} = $ from soft tissue to plasma, $F_{54} = $ from plasma to bone, $F_{45} = $ from bone to plasma, $F_{45} = $ from plasma to saliva, $F_{13} = $ from saliva to rumen.
Similarly,\[ \frac{1}{s_6} \frac{ds_6}{dt} = \frac{s_4 - s_6}{s_6} Q_{66}. \] [5]

At time \( N \) (d) after dosing (\( N \) is assumed to be about 1 wk), assume both gut pools reach complete steady state:

\[ \frac{dQ_1}{dt} = F_{10} + F_{13} - F_{21} = 0, \] [6a]

\[ \frac{dQ_2}{dt} = F_{21} + F_{24} - F_{02} - F_{42} = 0, \] [6b]

\[ \frac{dq_1}{dt} = s_4 F_{13} - s_1 F_{21} = 0, \] [6c]

\[ \frac{dq_2}{dt} = s_1 F_{21} + s_4 F_{24} - s_3 (F_{02} + F_{42}) = 0, \] [6d]

and that the saliva and plasma pools reach non-isotopic steady state:

\[ \frac{dQ_3}{dt} = F_{34} - F_{13} = 0, \] [7a]

\[ \frac{dQ_4}{dt} = F_{42} + F_{45} + F_{46} - F_{04} - F_{24} - F_{34} - F_{54} - F_{64} = 0. \] [7b]

Solving Eq. 6a and 6c,

\[ F_{13} = \frac{s_1 F_{10}}{s_4 - s_1}. \] [8]

Equation 6a then gives

\[ F_{21} = F_{10} + F_{13}. \] [9]

Solving Eq. 6b and 6d,

\[ F_{24} = \frac{s_1 - s_2}{s_2 - s_4} F_{21}. \] [10]

Equations 6b and 7a then yield, respectively,

\[ F_{42} = F_{21} + F_{24} - F_{02}, \] [11a]

\[ F_{34} = F_{13}. \] [11b]

Assume further that at time \( N \), the relative rates of change of specific activity in plasma, bone, and soft tissue have the same magnitude:

\[ \frac{1}{s_5} \frac{ds_5}{dt} = \frac{1}{s_6} \frac{ds_6}{dt} = k. \]

Equations 4 and 5 then yield, respectively,

\[ F_{54} = \frac{k s_5}{s_4 - s_5}, \] [12a]

\[ F_{64} = \frac{k s_6}{s_4 - s_6}. \] [12b]

Alternatively, at time \( N \), approximate \( ds_5/dt \) in Eq. 4 by \( s_5/N \) and \( ds_6/dt \) in Eq. 5 by \( s_6/N \). Equations 4 and 5 then give, respectively,

\[ F_{54} = \frac{s_5 Q_5}{(s_4 - s_5) N}, \] [13a]

\[ F_{64} = \frac{s_6 Q_6}{(s_4 - s_6) N}. \] [13b]

Equation 7b now yields

\[ |F_{45} + F_{46}| = F_{04} + F_{24} + F_{34} + F_{54} + F_{64} - F_{42}. \] [14]

The combined flow \( |F_{45} + F_{46}| \), which denotes the sum of outflow from pool 5 and pool 6,

\[ |F_{45} + F_{46}| = F_{45} + F_{46}, \] [15]

can be uncoupled by combining pools 5 and 6. Let \( s^* \) denote the SRA of the combined pool. This is calculated as

\[ s^* = \frac{s_5 Q_5 + s_6 Q_6}{Q_5 + Q_6}. \] [16]

The outflow of label from the combined pool is the sum of the outflow of label from pool 5 and the outflow of label from pool 6:

\[ s^* |F_{45} + F_{46}| = s_5 F_{45} + s_6 F_{46}. \] [17]

Algebraic manipulation of Eq. 15 and 17 gives

\[ F_{46} = \frac{s^* - s_5}{s_6 - s_5} \times |F_{45} + F_{46}|, \] [18a]

\[ F_{45} = |F_{45} + F_{46}| - F_{46}. \] [18b]

The model can be applied by computing the flows using Eq. 8 to 12, 14, 16, and 18. A second solution to the model can be found using Eq. 13 instead of Eq. 12.
to compute the flows $F_{54}$ and $F_{64}$. Radioactive measurements of $^{32}$P and inorganic P determination used to calculate SRA were determined accurately to minimize errors in output flow calculations. Endogenous P in feces may be calculated as $F_{02end}$, g of P/d = $(s_f/s_i)F_{02}$.

**Sensitivity Analysis**

A sensitivity analysis was conducted by ascribing the mean values across all animals to each measurement. The model was solved by perturbing each SRA and the k value in turn by 0, ±10, and ±20%. Each calculated flow ($y$, g/d) was then plotted against perturbation ($x$, %), and a 5-point linear regression of $y$ on $x$ performed to determine the average slope of the line produced. Each average slope was subsequently scaled by its corresponding unperturbed average flow value, to give the scaled slopes dimensions of percentage change in $y$ per percentage change in $x$.

**Statistical Analysis**

Experimental measurements of P intake, and P concentrations in rumen, saliva, bone, and soft tissue, SRA in rumen, feces, saliva, plasma, bone, and soft tissue, and P flows given by the model were analyzed as a one-way completely random design. Animals (6 per experimental group) were the experimental units. Analysis of variance was performed using the GLM procedure (SAS Inst. Inc., Cary, NC) with P content in the diet as the only source of variation. Polynomial orthogonal contrasts were performed and probability values for linear and quadratic components are reported. The pairwise correlations between variables were determined using PROC CORR of SAS.

**RESULTS AND DISCUSSION**

**P Intake, Excretion, and Secretion**

The basal diet was supplemented with dicalcium phosphate to provide increasing P intake. Dry matter intakes were 1.041, 1.118, 1.130, and 1.156 kg/d for LP, MP, HP, and SP, respectively. Except for LP, DMI were statistically similar for all the other 3 treatments ($P = 0.07$). Feed intake in animals fed the LP diet was decreased by 8% ($P < 0.01$), probably due to an induced P deficiency (Ternouth et al., 1993; NRC, 2007). As the mineral supplement used was dicalcium phosphate, not only P but also Ca provision was increased across the LP to SP diets. The experiment was designed so that the Ca:P ratio was similar for supplemented diets (2.27, 2.21, and 2.19 for MP, HP, and SP diets, respectively) and greater for LP so that this diet would be below P requirements ($2.9$ g of P/d) but sufficient to match Ca requirements ($3.9$ g of Ca/d) for lambs of this age and BW (NRC, 2007). When P is adequate, the Ca:P ratio is not influential, but if P is limiting, a Ca:P ratio greater than 3.6 can be detrimental for sheep (Wan Zahari et al., 1990).

Greater P intakes ($F_{00}$) were associated with increased total ($F_{02}$; $r = 0.97$) and endogenous ($F_{02end}$; $r = 0.85$) P excretion in feces. Urinary P excretion ($F_{04}$) was similar for all treatments (Table 3) and was related to P concentration in plasma ($r = 0.63$, $P < 0.01$). Although it is considered negligible, P excretion in urine of ruminants contributes to P homeostasis. According to various studies (Field et al., 1983; Scott et al., 1984; Scott and Buchanan, 1985), when plasma P increases above a concentration threshold (about 2 mmol/L), the reabsorptive capacity of kidney tubules is exceeded and extra P is excreted in urine. In this study, plasma P concentrations were above this concentration (2.15, 2.55, 2.83, and 2.81 mmol/L for LP, MP, HP, and SP, respectively). Therefore, it would seem reasonable to suggest that P excretion via urine was regulated to adjust plasma P even though urinary P excretion was modest. Calcium intake varied among diets, but the Ca:P ratio was similar for diets providing P above requirements. Vitti et al. (2010) pointed out that adverse effects of impaired Ca:P ratios on Ca or P absorption could be critical only when P is inadequate. Even with the LP diet, the Ca:P ratio of the diets used in this experiment was within a range causing no adverse effects on P absorption or fecal and urinary P excretion in sheep (Wan Zahari et al., 1990; Vitti et al., 2010).

Plasma P concentration was affected linearly by P intake ($r = 0.52$, $P = 0.01$). According to Bravo et al. (2003), only 17% of the variation in plasma P concentration was explained by differences in P intake in ruminants. Vitti et al. (2010) concluded that plasma is not a reliable indicator of P status because of homeostatic responses, as P is mobilized from bone to maintain normal concentrations in blood when animals suffer severe P deficiency. Thus, factors that can affect P concentration in plasma such as P mobilization from bone and soft tissue may also interfere in the relationship between plasma P and dietary P intake. For example, in our study P concentration in plasma was inversely related to net P flow to bone ($r = -0.63$, $P < 0.01$) suggesting that plasma P was affected by other factors besides P intake. Furthermore, P concentration in plasma was related to endogenous P excreted in feces ($F_{02end}$; $r = 0.71$, $P < 0.01$), indicating greater P in plasma might have induced a greater secretion of endogenous P (Mañas-Almendros et al., 1982; Scott et al., 1984) and, consequently, a greater excretion in feces. In agreement with Preston and Pfander (1964) and Vitti et al. (2005), endogenous (also called metabolic) fecal P increased with increasing dietary P. Endogenous fecal P can be derived either from inevitable fecal losses or from fecal excretion of surplus P (Pfeffer et al., 2005). Although it is impossible to differentiate both sources, the increase in endogenous fecal P between LP and the other diets can be attributed in part to inevitable fecal losses significantly related to DMI (Spiekers et al.,
likely resulted in the greater P flows from rumen to GIT was greater than P intake. The intake by sheep affected salivary P secretion. In agreement, Scott et al. (1984) reported that variation in P intake by sheep affected salivary P secretion and P absorption in the gut. Saliva is known to be the main contributor to P entering the gut, explaining how increasing P flows from plasma to saliva (F$_{34}$; r = 0.73) and from saliva to rumen (F$_{13}$; r = 0.73), and from rumen to GIT (F$_{21}$; r = 0.96). Intake of P was also related to P flow from GIT to plasma (F$_{12}$; r = 0.72), which represents P absorption. In agreement, Scott et al. (1984) reported that variation in P intake by sheep affected salivary P secretion and P absorption in the gut. Saliva is known to be the main contributor to P entering the gut, explaining how increasing P flows from plasma to saliva (F$_{34}$) and then from saliva to rumen (F$_{13}$; Tomas, 1973; Dias et al., 2009) are followed by concomitant greater P flows from rumen to GIT (F$_{12}$; Clark, 1953). The flow of P from rumen to GIT was greater than P intake. The increasing contribution of salivary P related to P intake likely resulted in the greater P flows from rumen to GIT, and subsequently in a greater fecal excretion of endogenous P. Other studies have shown that P interchanges through the rumen wall (both absorption and secretion) can be considered negligible (Scarisbrick and Ewer, 1951; Parthasarathy et al., 1952; Care, 1994), leaving salivary P as the major non-dietary source of P entering the rumen. Based on these studies, the model assumed there was no absorption of P from rumen to plasma or flow in the opposite direction.

The mean values of the P flows from rumen to GIT (F$_{21}$) and from GIT to plasma (F$_{42}$) are comparable with the values of 7.88 and 5.05 g/d obtained by Schneider et al. (1987) for chaff-fed sheep. Endogenous P excreted in feces was correlated with P flows from plasma to saliva (r = 0.54, P = 0.01), from rumen to GIT (F$_{21}$; r = 0.78, P < 0.01), from GIT to plasma (F$_{12}$; r = 0.85, P < 0.01), and with P recycled from plasma to GIT (F$_{24}$; r = 0.75, P < 0.01), demonstrating the pathway of endogenous P through the body of the animal (Scott et al., 1995).

The flow of P recycled to GIT (F$_{24}$) and P absorption (F$_{42}$) as a proportion of P ingestion decreased at the greatest P intake (SP). Accordingly, researchers have observed a decrease in efficiency of P absorption with increasing P intake in ruminants (Scott et al., 1984; Vitti et al., 2005) and nonruminants (Ketaren et al., 1993), suggesting that P absorption involves not only a passive diffusion mechanism.

The flows of P from plasma to bone (F$_{34}$) and from plasma to soft tissue (F$_{35}$) were calculated using 2 approaches: applying the relative rate parameter k estimated from the exponential decay curve obtained by plotting SRA (specific activity) in plasma vs. time from injection (Eq. 12a and 12b), and by using Eq. 13a and 13b. The flows resulting from using Eq. 12a and 12b (Table 4) can be considered more accurate, for these calculations are theoretically more realistic, as they consider the exponential decay of plasma SRA for each animal, which reflects the P kinetics for each individ-

<table>
<thead>
<tr>
<th>Item</th>
<th>Symbol</th>
<th>P content in diet, %</th>
<th>SEM</th>
<th>P-value</th>
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<tr>
<td></td>
<td></td>
<td>0.14</td>
<td>0.32</td>
<td>0.49</td>
</tr>
<tr>
<td>Flow, g of P/d</td>
<td>$F_{10}$</td>
<td>1.46</td>
<td>3.53</td>
<td>5.53</td>
</tr>
<tr>
<td>Intake</td>
<td>$F_{20}$</td>
<td>1.09</td>
<td>3.18</td>
<td>4.39</td>
</tr>
<tr>
<td>Feces</td>
<td>$F_{42}$</td>
<td>0.60</td>
<td>1.54</td>
<td>2.39</td>
</tr>
<tr>
<td>Endogenous</td>
<td>$F_{34}$</td>
<td>0.012</td>
<td>0.010</td>
<td>0.071</td>
</tr>
<tr>
<td>Urine</td>
<td>$F_{44}$</td>
<td>23.0</td>
<td>13.1</td>
<td>8.9</td>
</tr>
<tr>
<td>Rumen</td>
<td>$s_1$</td>
<td>41.8</td>
<td>22.8</td>
<td>18.1</td>
</tr>
<tr>
<td>Feces</td>
<td>$s_2$</td>
<td>60.4</td>
<td>37.6</td>
<td>29.4</td>
</tr>
<tr>
<td>Saliva</td>
<td>$s_3$</td>
<td>76.3</td>
<td>50.2</td>
<td>33.3</td>
</tr>
<tr>
<td>Plasma</td>
<td>$s_4$</td>
<td>5.1</td>
<td>3.1</td>
<td>3.6</td>
</tr>
<tr>
<td>Bone</td>
<td>$s_5$</td>
<td>17.9</td>
<td>14.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Bone and soft tissue</td>
<td>$s^*$</td>
<td>6.4</td>
<td>4.3</td>
<td>4.3</td>
</tr>
<tr>
<td>Pool size, g</td>
<td></td>
<td>147.4</td>
<td>148.9</td>
<td>149.2</td>
</tr>
<tr>
<td>Bone</td>
<td>$Q_{b1}$</td>
<td>18.9</td>
<td>18.6</td>
<td>21.6</td>
</tr>
<tr>
<td>Soft tissue</td>
<td>$Q_{b2}$</td>
<td>147.4</td>
<td>148.9</td>
<td>149.2</td>
</tr>
<tr>
<td>Bone</td>
<td>$Q_{b1}$</td>
<td>18.9</td>
<td>18.6</td>
<td>21.6</td>
</tr>
</tbody>
</table>

1SRA values are expressed per gram in model equations.
Phosphorus metabolism model

Table 4. Phosphorus flows calculated using the model

<table>
<thead>
<tr>
<th>Flow to/d</th>
<th>Symbol</th>
<th>0.14</th>
<th>0.31</th>
<th>0.49</th>
<th>0.65</th>
<th>SEM (n = 6)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saliva to rumen</td>
<td>$F_{13}$</td>
<td>0.94</td>
<td>2.08</td>
<td>2.52</td>
<td>3.59</td>
<td>0.389</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>GIT to plasma</td>
<td>$F_{21}$</td>
<td>2.40</td>
<td>5.61</td>
<td>8.05</td>
<td>11.1</td>
<td>0.343</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Plasma to GIT</td>
<td>$F_{22}$</td>
<td>2.64</td>
<td>4.99</td>
<td>8.30</td>
<td>8.18</td>
<td>0.653</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Plasma to saliva</td>
<td>$F_{54}$</td>
<td>0.94</td>
<td>2.08</td>
<td>2.52</td>
<td>3.59</td>
<td>0.389</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Plasma to bone</td>
<td>$F_{64}$</td>
<td>2.17</td>
<td>2.28</td>
<td>5.24</td>
<td>6.00</td>
<td>1.15</td>
<td>0.01</td>
</tr>
<tr>
<td>Bone to plasma</td>
<td>$F_{65}$</td>
<td>2.57</td>
<td>3.27</td>
<td>5.97</td>
<td>7.25</td>
<td>4.11</td>
<td>0.01</td>
</tr>
<tr>
<td>Plasma to soft tissue</td>
<td>$F_{56}$</td>
<td>1.08</td>
<td>1.73</td>
<td>2.68</td>
<td>2.93</td>
<td>0.494</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Soft tissue to plasma</td>
<td>$F_{66}$</td>
<td>0.33</td>
<td>0.41</td>
<td>0.88</td>
<td>1.12</td>
<td>0.240</td>
<td>0.02</td>
</tr>
<tr>
<td>Net flow bone</td>
<td>$F_{64} - F_{65}$</td>
<td>-0.40</td>
<td>-0.98</td>
<td>-0.73</td>
<td>-1.24</td>
<td>0.378</td>
<td>0.19</td>
</tr>
<tr>
<td>Net flow soft tissue</td>
<td>$F_{64} - F_{66}$</td>
<td>0.75</td>
<td>1.32</td>
<td>1.79</td>
<td>1.81</td>
<td>0.274</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total P retention</td>
<td>$F_{65}$</td>
<td>0.36</td>
<td>0.34</td>
<td>1.07</td>
<td>0.57</td>
<td>0.220</td>
<td>0.17</td>
</tr>
</tbody>
</table>

$^1$GIT = gastrointestinal tract.
$^2$Flows calculated according to Eq. 12a and 12b.

Dias et al. (2007) found a linear effect of dietary P on net P flow to bone in growing sheep fed different amounts of dicalcium phosphate. Preston and Pfander (1964) reported a greater P balance with increasing dietary P supply, although these authors did not differentiate between retention in bone and in soft tissue. Effects of P intake on bone are more apparent with decreased P intakes. Scott et al. (1994) observed less bone formation with P-deficient diets, whereas bone resorption was not affected. When P deficiency becomes severe, bone tissues can be affected by increased degradation (Breves and Prokop, 1990). Benzie et al. (1959) observed significant resorption by the skeleton of ewes fed both moderate and low-P rations between mid-gestation and mid-lactation. On the other hand, P accretion by the skeleton seems to show only a small response to increased P intake in excess of adequate supply, so that increasing the provision of dietary P mainly affects fecal and urinary P excretion (Pfeffer et al., 2005). Challa and Braithwaite (1988) reported P resorption from the skeleton in calves remained almost unaffected by P intake, but our results show that P mobilized from bone to plasma increased linearly with P intake. Differences between results could be due to the different experimental methods, type of animals or dietary P intakes used in each experiment. Several studies have shown that excess dietary P is associated with decreased P retention in bone in swine (Reinhart and Mahan, 1986), mice (Yuen and Draper, 1983), and dogs (Laflamme and Jowsey, 1972), and greater and prolonged bone mobilization in transition cows (Moreira et al., 2009). Excess P in relation to Ca can lead to skeletal softening, and a maximum tolerable dietary P of 0.6% in the ration has been recommended (NRC, 2007). Calcium intake varied among diets (increasing from 4.1 to 16.4 g/d), but had no significant effect on net P accretion in bone. Calcium provision was always above requirements (3.9 g/d), and the Ca:P ratio in the diets should not have affected P absorption, so minor effects would be expected of Ca on bone accretion. Pfeffer et al. (1995) observed that although P intake has a
significant effect on P retained in goat kids, increasing Ca in the diet (from 0.39 to 1.09%) had no effect, and Benzie et al. (1959) concluded that bone resorption was affected to a greater extent by low-P than by low-Ca diets.

The negligible effect of dietary P on net P flow to bone indicates a low demand for P by bone, likely due to excess dietary P in most treatments. Consequently, surplus P not deposited in bone was distributed in the plasma and channeled to soft tissue, as indicated by the negative relationships between net P flow to bone (P retention in bone) and P in plasma ($r = −0.63$, $P < 0.01$) and P accretion in soft tissue ($F_{64}$; $r = −0.76$, $P < 0.01$). The relationship between P mobilized from bone and net P flow to soft tissue is highlighted by the strong correlation between P flow from bone ($F_{45}$) and net P flow to soft tissue ($r = 0.89$, $P < 0.01$). In this particular study, P intake had an effect on net P flow to soft tissue, indicating demand for P by soft tissue during animal growth. The results of this study indicate that excess dietary P increased plasma P, consequently affecting the fates of P in the body of the animal. Soft tissue would take precedence over bone for the supply of available plasma P. Furthermore, there were inverse relationships between net P retention in bone and urinary P ($r = −0.64$, $P < 0.01$) and endogenous fecal P ($r = −0.55$, $P = 0.01$), indicating that P mobilized and not utilized by soft tissue was excreted in feces and urine. Schneider et al. (1982), examining the effects on P metabolism in sheep receiving an infusion of 2 g of P/d, suggested that bone P was mobilized, impairing elimination of this mineral. The excess of phosphates released from bone resorption is excreted from the body via the kidneys in young animals (Todd et al., 1962; Iqbal et al., 2005), indicating that P intakes with the HP and SP diets were in excess of animal requirements, increasing urinary P to maintain homeostasis, as observed by Taylor et al. (2009). The demand for P retention in the skeleton also has an effect (inverse relationship) on the rates of endogenous fecal loss and efficiency of P absorption in the gut (Braithwaite, 1984).

The results of the sensitivity analysis are summarized in Table 5. These scaled values indicate an error of 1% in an input causes, on average, an error of less than 1.5% in a flow sensitive to that input. They demonstrate measurement of SRA in plasma ($s_4$) is the most crucial due to its involvement in the calculation of several flows. However, although influencing a lesser number of flows, SRA in soft tissue ($s_5$) affects flows ($F_{54}$, $F_{45}$, and $F_{46}$) to a greater extent than the other SRA. As ruminal fluid was collected using a stomach tube, samples could have been potentially contaminated with saliva, although the fact that SRA values in ruminal and salivary samples were so different (Table 3) would suggest that this contamination was minimal. Nevertheless, sensitivity analysis (Table 5) indicates that errors (e.g., due to saliva contamination) in the SRA measured in the rumen ($s_4$) would affect estimates of P flows between saliva, GIT, rumen, and plasma pools, but in all cases to a small extent.

The extended model of P metabolism in sheep proposed herein provides meaningful biological understanding of the metabolic fate of ingested P. The correlations between flows and measured parameters offer a sensible description of P metabolism at the P intakes and with the animals considered in this study. Additionally, the model shows that excess P intake is not efficiently used. As a result, P supplementation often represents an unnecessary feeding cost and potential damage to the environment, not only due to increased excretion of unabsorbed dietary P in feces but also due to increased endogenous P excretion.

### Table 5. Average slope for the flows calculated using the model after perturbing specific $^{32}$P radioactivity (SRA) and relative rate of change of SRA in plasma ($k$ value)

<table>
<thead>
<tr>
<th>Flow, g/d</th>
<th>Symbol</th>
<th>$s_1$</th>
<th>$s_2$</th>
<th>$s_3$</th>
<th>$s_4$</th>
<th>$s_5$</th>
<th>$s_6$</th>
<th>$k$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endogenous</td>
<td>$F_{64}$</td>
<td>0.00</td>
<td>1.00</td>
<td>0.00</td>
<td>−1.02</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Saliva to rumen</td>
<td>$F_{14}$</td>
<td>1.55</td>
<td>0.00</td>
<td>−1.67</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Rumen to GIT$^3$</td>
<td>$F_{23}$</td>
<td>0.54</td>
<td>0.00</td>
<td>−0.58</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>GIT to plasma</td>
<td>$F_{34}$</td>
<td>0.27</td>
<td>1.63</td>
<td>−0.94</td>
<td>−1.12</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Plasma to GIT</td>
<td>$F_{43}$</td>
<td>−0.68</td>
<td>3.29</td>
<td>−0.58</td>
<td>−2.28</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Plasma to saliva</td>
<td>$F_{54}$</td>
<td>1.55</td>
<td>0.00</td>
<td>−1.67</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Plasma to bone</td>
<td>$F_{64}$</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>−1.13</td>
<td>1.08</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Bone to plasma</td>
<td>$F_{65}$</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>−1.43</td>
<td>0.78</td>
<td>0.58</td>
<td>1.14</td>
</tr>
<tr>
<td>Plasma to soft tissue</td>
<td>$F_{64}$</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>−1.45</td>
<td>0.00</td>
<td>1.36</td>
<td>1.00</td>
</tr>
<tr>
<td>Soft tissue to plasma</td>
<td>$F_{66}$</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>−1.43</td>
<td>0.78</td>
<td>0.58</td>
<td>1.14</td>
</tr>
<tr>
<td>Net flow bone</td>
<td>$F_{54} - F_{65}$</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>−2.76</td>
<td>−0.57</td>
<td>3.16</td>
<td>1.79</td>
</tr>
<tr>
<td>Net flow soft tissue</td>
<td>$F_{64} - F_{66}$</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>−1.46</td>
<td>−0.30</td>
<td>1.67</td>
<td>0.94</td>
</tr>
</tbody>
</table>

$^1$Results are presented as the average slope, percentage change in flow per percentage change in SRA or $k$ value.

$^2$s$_1$ = SRA rumen, s$_2$ = SRA feces, s$_3$ = SRA saliva, s$_4$ = SRA plasma, s$_5$ = SRA bone, s$_6$ = SRA soft tissue.

$^3$GIT = gastrointestinal tract.

### LITERATURE CITED


