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Application of a kinetic model to describe phosphorus metabolism in pigs fed a diet with a microbial phytase

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SUMMARY

The objective of the current study was to apply the Vitti–Dias model to investigate phosphorus (P) metabolism in growing pigs fed a diet supplemented with microbial phytase. The basal diet contained maize, defatted rice bran, vegetable oil, soybean meal, limestone, salt and a vitamin and mineral mix. There was no inorganic P in the diet and phytase was added at levels of 253, 759, 1265 and 1748 phytase units (PU)/kg of feed. The compartmental model included four pools of P: (1) gut lumen, (2) plasma, (3) bone and (4) soft tissue. A single dose of ³²P was administered, and specific radioactivity was measured in plasma, faeces, bone and soft tissue (muscle, heart, liver and kidney) at different times post-dosing for calculation of P flows between pools. Total P absorbed showed a negative relationship with total P excreted in faeces and was strongly correlated with bone P retention, suggesting that absorbed P was channelled to bone to address its physiological growth. Average efficiency of metabolic utilization of absorbed P was estimated to be 0.94, with 0.52 g/g of total net P balance being accreted in bone and the rest in soft tissue (including muscle and some vital organs). The Vitti–Dias model provided suitable representation of P interchange between compartments (in particular, flows between gut and plasma and partitioning of available P between bone and soft tissue), resulting in estimates of P flows comparable with values calculated from balance data.

INTRODUCTION

Phosphorus (P) is an essential mineral in pig nutrition and is one of the most expensive nutrients. However, most P in vegetable feedstuffs is in the phytate form, which is degraded only to a limited extent in the gastrointestinal tract of non-ruminant animals due to the lack of endogenous phytase (Cromwell 1979; Jongbloed 1987).

Addition of exogenous phytase aims to improve P availability, thus reducing environmental pollution as well as the cost of the diet (Cromwell *et al.* 1995*a, b*).

Phytase dose-related responses in pigs have been shown to vary among different categories of animal (i.e. gestating or lactating sows, growing–finishing pigs and piglets) (Kemme *et al.* 1997), type of phytase (Matsui *et al.* 2000), endogenous phytase present in the diet (Düngelhoef *et al.* 1994; Zimmermann *et al.* 2003), Ca:P ratio (Brady *et al.* 2002) and P level supplied by the diet (Zimmermann *et al.* 2002). Therefore, dose-response studies analysing effects of exogenous phytase upon P metabolism would help to elucidate how this enzyme affects P utilization.

Mathematical models can be used as a tool to investigate P metabolism, providing interesting information regarding the effects of different factors (such as phytase addition) upon P flows between body

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compartments and on P excretion. Vitti *et al.* (2000) and Dias *et al.* (2006) proposed a kinetic model for growing small ruminants with four P compartments in the body: (1) gut lumen, (2) plasma, (3) bone and (4) soft tissue, allowing flows between these four pools and out of the system to be determined. The objective of the current study was to investigate P exchanges between bone, soft tissue and other body compartments of growing pigs in response to ingestion of a diet containing microbial phytase by applying the Vitti–Dias model.

MATERIALS AND METHODS

Experimental procedures

Animals and diets

The study was carried out under the approval of animal experimental protocols by the Animal Care Committee of the Centro de Energia Nuclear na Agricultura (CENA), Piracicaba, Brazil. Twelve castrated crossbred pigs (cross-breeding of Agpic male (Hampshire with Landrace $\times$ Large White) and Camborough female (Duroc Pic with Landrace Pic $\times$ Large White Pic)) with mean body weight (BW) of 32 ± 1.6 kg were used.

All animals were fed a standard mash diet (Table 1) composed of maize, defatted rice bran, vegetable oil, soybean meal, limestone, salt and vitamin and mineral premixes. The feed mixture contained 7.4 g total P/kg and 1.7 g available P/kg. Experimental treatments consisted of four rates of exogenous phytase addition to the basal diet, namely 253, 759, 1265 and 1748 phytase units (PU)/kg feed, and are denoted by Ph1, Ph2, Ph3 and Ph4, respectively. The phytase supplement (Allzyme Phytase; Alltech Biotechnology, Inc, Nicholasville, KY, USA) used was a fungal enzyme obtained from *Aspergillus niger*, with a declared activity of 2300 PU/g, which also contained cellulase, protease, xylanase and acid phosphatase activities. The phytase activity, with 1 PU representing the amount of enzyme that under standard conditions releases 1 μ mol of inorganic P from sodium phytate in 1 min, was determined in a sodium-phytate substrate at 37 °C and pH 5.5.

Experimental diets were formulated according to Rostagno *et al.* (2000) and NRC (1998) (Table 1), and P availability in the basal diet was calculated according to Gomes *et al.* (1989). On the basis of the NRC (1998) approach, Rostagno *et al.* (2000) adjusted animal requirements and feed composition data to specific Brazilian conditions. The feed composition tables of Rostagno *et al.* (2000) include information on feedstuffs commonly used for pig feeding in Brazil, and pig nutrient requirements were calculated specifically taking into account particularities of pigs raised under Brazilian conditions, such as growth potential of Brazilian genotypes (considered as average genetic

Table 1. *Diet composition*

Ingredient	g/kg
Maize	619
Defatted rice bran	172
Vegetable oil	16
Soybean meal	170
Limestone	14
Salt	3.5
Vitamin premix*	4.0
Mineral premix†	1.0
Phytase enzyme	‡
Digestible energy (MJ/kg dry matter (DM))	13.7
Chemical composition (g/kg DM)	
Crude protein	173
Lysine	7.4
Tryptophan	1.9
Threonine	6.1
Methionine	2.7
Calcium	6.1
Total phosphorus	7.4
Available phosphorus	1.8

* Amount per kg of supplement: vitamin A, 1 500 000 UI; vitamin D3, 255 000 UI; vitamin E, 3000 mg; vitamin K3, 500 mg; thiamine, 2500 mg; riboflavin, 750 mg; pyridoxine, 250 mg; vitamin B12, 3 mg; calcium pantothenate, 3750 mg; niacin, 5500 mg; antioxidant, 6.25 g; growth promoter, 6.25 g; choline chloride, 75 g; selenium, 22.5 mg.

† Amount per kg of feed: Mn, 45 mg; Cu, 15 mg; Zn, 80 mg; Fe, 88 mg; I, 1 mg.

‡ Added at 110, 330, 550 and 760 mg/kg of feed in each of the experimental diets to reach levels of phytase activity of 253, 759, 1265 and 1748 PU/kg.

potential) and production in a hot tropical climate (mean annual temperatures between 20 and 25 °C, with maximum temperatures of 30–39 °C). These adjusted values were used in the present study. Similarly, P availability values are relative to Brazilian feed sources, in an attempt to obtain results more reflective of local conditions. In the case of P allowances, a basal diet with a low available P content was formulated to target maximum response to phytase addition (Qian *et al.* 1996; Brady *et al.* 2002). As no inorganic P was incorporated into the mixed diet and the main source of P was rice bran (a feedstuff with a low P availability of 0.20 according to Rostagno *et al.* 2000), the amount of total P had to be higher than the recommended allowance in order to match animal requirements for P.

Balance trials and sample collection

A preliminary period of adaptation to diet in metabolism cages lasted for 10 days. After adaptation, a dose of 7.4 MBq of 32 P was injected into the jugular vein at 07.00 h, prior to feeding the animals. Radioactive P ($\text{Na}_2\text{H}^{32}\text{PO}_4$) was acquired from Instituto de

Table 2. Chemical composition of ingredients (g/kg DM)

Nutrient	Ingredients			
	Maize	Soybean meal	Defatted rice bran	Lime
Crude protein	95.3	466.0	200.2	—
Ether extract	69.7	33.2	38.7	—
Crude fibre	47.8	81.8	107.3	—
Total P	3.3	7.6	23.3	—
Available P	1.0	2.3	4.6	—
Ca*	0.2	3.6	1.1	373.2
DE (MJ/kg DM)	14.6	14.1	10.1	—
Lysine*	2.3	28.7	6.2	—
Methionine*	1.7	6.5	2.9	—
Threonine*	3.4	17.8	5.6	—
Tryptophan*	0.8	6.7	1.6	—

* Based on tables by Rostagno *et al.* (2000).

Pesquisas Energéticas e Nucleares (IPEN), São Paulo, Brazil. During the sampling period, urine and faeces were quantitatively collected from each animal every day for 7 days before morning feed (at 07.00 h) to calculate P balance. A representative sample (100 g/kg total faecal output) of faeces from each animal was taken. Daily samples were pooled for each pig at the end of the 7-day collection period, and the composite sample was air-dried in a forced-air oven at 105 °C and ground to pass through a 1 mm screen. Both ground faeces and urine samples were kept frozen (−4 °C) until used for subsequent analyses.

Blood samples were withdrawn from the jugular vein at 5 min, 1, 2, 4 and 6 h after ³²P dosing and, thereafter, every 24 h until the last sample was taken at 144 h. Specific radioactivity (SRA) was measured in plasma samples and the plasma SRA decay curve subsequently constructed. Animals were slaughtered on day 7 after ³²P dosing, and samples from liver, kidney, muscle, heart and the third and fourth metatarsal bones were collected for further analysis.

Analysis of samples

Feed samples were analysed following the methods of the Association of Official Analytical Chemists (AOAC 1995) (Table 2). Crude protein was determined by the Kjeldahl method and P content by colorimetry (Sarruge & Haag 1974).

For inorganic P determination, dry faecal samples were ashed at 500 °C, and the residue dissolved in 5 ml of HCl. Afterwards P content was determined by a colorimetric method using vanadate–molybdate reagents (Sarruge & Haag 1974). For radioactivity detection, ashes obtained from faeces were digested using 10 ml of H₂SO₄ (18 N) for 1 h; the remaining hydrolysate added to a scintillation vessel and topped

up to 20 ml with distilled water prior to recording radioactivity.

Blood samples were centrifuged for plasma separation. Determination of inorganic P in plasma and urine (diluted 1:10) samples was undertaken by the colorimetric method of Fiske & Subbarow (1925). For radioactivity detection, plasma and urine samples (0.5 ml) were diluted with 19.5 ml of deionized water in scintillation vessels.

Bones were cleaned and degreased; thereafter samples were acid-digested on a hot plate with the addition of an aqueous solution of nitric acid (500 ml HNO₃/l) and then ashed in a muffle at 550 °C. The ash residues were further digested using 10 ml of an aqueous solution of HCl (100 ml HCl/l) and filtered into a 25 ml volumetric flask through phosphorus-free filter paper, and inorganic P content was determined (Sarruge & Haag 1974). Samples of the third and fourth metatarsal bones were analysed separately and average P concentration values were used subsequently for calculations. Inorganic P in each soft tissue sample (liver, kidney, muscle and heart) was determined by the method of Fiske & Subbarow (1925). Samples of bone and soft tissue were digested in 18 N H₂SO₄ and the extract was transferred to counting vials for determination of associated radioactivity. Radioactivity in soft tissue (*s₄*) was calculated from the measured SRA and the relative proportion of each tissue.

The radioactivity counting was carried out by liquid scintillation spectrometry using Cerenkov radiation (Nascimento Filho & Lobão 1977; IAEA 1979). Radioactivity counting was expressed in terms of SRA relative to the counting in a standard solution of ³²P and calculated as

$$\text{SRA}(\text{g total P}) = \frac{\text{cpm}_{\text{sample}}}{\text{cpm}_{\text{std}}} \div P_{\text{sample}}$$

where $\text{cpm}_{\text{sample}}$ and cpm_{std} are scintillation counts per minute in the sample (per g or per ml of sample) and in the standard solution, respectively, and P_{sample} is the total inorganic P content of the sample. The standard solution was prepared diluting 7.4 MBq of ³²P in 1 litre of distilled water. Thereafter, 1 ml of this standard solution was transferred to a counting vial for radioactivity measurement.

Pool size of P in bone (Q_3) and soft tissue (Q_4) were calculated from P concentrations determined in each tissue and the relative proportion of each tissue in the body mass, assumed to be on average 60, 410, 20, 1.2 and 3 g/kg animal BW for bone, muscle, liver, kidney and heart, respectively. These values are based on measurements in pigs obtained from several experiments carried out at CENA over the last 15 years (data not published), and are well in agreement with values published in the literature (Anugwa *et al.* 1989; Kerr *et al.* 1995; Dutra *et al.* 2001; Vasupen *et al.* 2008).

Fig. 1. Schematic representation of the model of phosphorus metabolism in growing animals. F_{ij} represents total P flow to pool i from j , F_{i0} denotes ingestion of P, F_{01} excretion of P in faeces, F_{02} excretion of P in urine.

Table 3. Principal symbols used in the model

F_{ij}	Flow of P to pool i from pool j . F_{i0} denotes an external flow into pool i , and F_{0j} a flow from pool j out of the system. A tilde indicates a flow that can be measured experimentally. Units are g/day as calculated, mg/(kg BW $\times$ day) as presented in tables.
Q_i	Quantity of P in pool i : g
s_i	SRA in pool i : /g P
k	Fractional rate of change of SRA in pool i : per day
t	Time: day

Mathematical procedures

The Vitti–Dias model

The Vitti–Dias model (Vitti *et al.* 2000), as revised by Dias *et al.* (2006), was used to examine P metabolism in the current study. A detailed description of the model is given by Dias *et al.* (2006) and the relevant calculations are summarized below. The model is shown in Fig. 1. It contains four pools: (1) gut lumen, (2) plasma, (3) bone and (4) soft tissue, and the flows of P between pools and into and out of the system are shown as arrowed lines. Labelled P (^{32}P) was administered intravenously as a single dose D at time zero, and SRA in faeces (s_1), bone (s_3) and soft tissue (s_4) were measured after 7 days. The value of s_2 at 7 days was obtained after fitting the negative exponential equation $s_2 = Ae^{-kt}$ to the plasma SRA decay curve, where t (day) is the time after dosing ^{32}P , A is the extrapolated value of s_2 at $t = 0$ and k (/day) is the fractional rate of change of s_2 (i.e. $(ds_2/dt)/s_2 = -k$). The scheme assumes no re-entry of isotopic P from external sources.

After 7 days, pool 1 (gut lumen) is assumed to be in complete steady state (i.e. isotopic and non-isotopic

steady state) and pool 2 (plasma) in non-isotopic steady state. Algebraic solution to the model (Dias *et al.* 2006; see Table 3 and Fig. 1 for principal mathematical notation) then gives:

$$F_{12} = \frac{s_1}{s_2 - s_1} \tilde{F}_{10} \tag{1}$$

$$F_{21} = \tilde{F}_{10} + F_{12} - \tilde{F}_{01} \tag{2}$$

$$F_{32} = \frac{k s_3 Q_3}{s_2 - s_3} \tag{3}$$

$$F_{42} = \frac{k s_4 Q_4}{s_2 - s_4} \tag{4}$$

$$\overline{F_{23}} + \overline{F_{24}} = \tilde{F}_{02} + F_{12} + F_{32} + F_{42} - F_{21} \tag{5}$$

$$F_{23} = \frac{s_4 - s^*}{s_4 - s_3} \overline{F_{23}} + \overline{F_{24}} \tag{6}$$

$$F_{24} = \overline{F_{23}} + \overline{F_{24}} - F_{23} \tag{7}$$

Symbols with a tilde represent flows determined experimentally ($\tilde{F}_{10}$, $\tilde{F}_{01}$, $\tilde{F}_{02}$ for P intake and total P

Table 4. Phosphorus intake and excretion, retained P and P absorbed from the diet and SRAs measured or calculated

Phytase (PU/kg of feed)	Symbol	Dietary treatments				S.E.M.
		Ph1 253	Ph2 759	Ph3 1265	Ph4 1748	
Flows [mg P/(kg BW × day)]						
Intake	$\tilde{F}_{10}$	314	308	288	299	8.6
Faeces	$\tilde{F}_{01}$	174	191	200	191	22.4
Urine	$\tilde{F}_{02}$	0.62	0.38	0.32	0.58	0.179
P apparent absorption	P_{aa}	140	117	88	108	21.2
P balance	$P_{balance}$	139	116	88	107	21.2
SRA ^a (per g P)						
Faeces	s_1	0.96	0.94	1.02	1.10	0.061
Plasma	s_2	22	22	25	25	1.4
Bone	s_3	9.2	8.1	9.0	9.5	0.48
Soft tissue	s_4	9.7	8.7	9.2	9.8	0.45
Bone and soft tissue	s^*	9.4	8.3	9.1	9.6	0.46

The values shown are treatment means (three animals per treatment).

^a s_2 and s^* were calculated as described in the text.

excreted in faeces and urine, respectively). Regarding the formula for F_{23} , a typographical error appears in Vitti *et al.* (2000) that has been corrected in Eqn (6).

Q_3 and Q_4 are the pool size of P in bone and soft tissue, respectively, and both were determined at the end of the experimental period (7 days after dosing ^{32}P).

SRA (SRA/g P) was measured in each pool i and represented by s_i . The average SRA in a joint pool (s^*) formed by combining pools 3 and 4 was calculated as

$$s^* = \frac{s_3 Q_3 + s_4 Q_4}{Q_3 + Q_4} \quad (8)$$

It is assumed that fractional rate of change of SRA has the same magnitude in plasma, bone and soft tissue pools after 7 days, giving:

$$-\frac{1}{s_2} \frac{ds_2}{dt} = \frac{1}{s_3} \frac{ds_3}{dt} = \frac{1}{s_4} \frac{ds_4}{dt} = k \quad (9)$$

Finally, the model is solved by computing Eqns (1)–(9).

Endogenous P in faeces (P_e) was calculated according to Lofgreen & Kleiber (1953) as $P_e = \tilde{F}_{01} \times s_1/s_2$, where s_1 and s_2 are SRA in faeces and plasma, respectively, and $\tilde{F}_{01}$ is the total faecal P. Estimates of P retention in bone (P_b) and soft tissue (P_t) were obtained by measuring the difference between the flow from plasma to tissue (total accretion) and that from tissue to plasma (mobilization). Therefore, $P_b = F_{32} - F_{23}$ and $P_t = F_{42} - F_{24}$.

Supplementary calculations were performed. The amount of P apparently absorbed from the gut

(P absorption, P_{aa}) was calculated as the difference between total P absorption from (flow gut to plasma, F_{21}) and P secretion to (flow plasma to gut, F_{12}) the gut (i.e. $P_{aa} = F_{21} - F_{12} = \tilde{F}_{10} - \tilde{F}_{01}$). The amount of P truly absorbed (P_{ta}) was estimated as $P_{ta} = \tilde{F}_{10} - (\tilde{F}_{01} - P_e)$. P balance was calculated as the difference between all inputs and outputs in the system: $P_{balance} = \tilde{F}_{10} - \tilde{F}_{01} - \tilde{F}_{02}$.

All model flows were weight-scaled and expressed in units of mg P/kg BW/day to take into account differences among animals in BW.

Statistical analyses

Experimental measurements of P intake and excretion, total P in bone and tissue, and P in plasma, as well as the flows calculated from the model, were analysed by analysis of variance (ANOVA) as a completely randomized design. The General Linear Model Procedure (SAS 1999) was used to perform one-way ANOVA with phytase level as the treatment effect in the statistical model. Correlation and regression analyses between variables (calculated flows) were carried out using PROC CORR and PROC REG procedures (SAS 1999), respectively.

RESULTS

Total P intake ($\tilde{F}_{10}$, mg/(kg BW × day)) was similar for all treatments (Table 4). Phosphorus excreted in urine ($\tilde{F}_{02}$), was negligible for all treatments (Table 4). There were no significant differences among dietary treatments in total amount of P excreted in faeces ($\tilde{F}_{01}$), in apparent absorption of P from the gut to

Table 5. Average value, standard error and range of values for the model outputs (flows of P calculated using the model) and for calculated P balance measures (absorption and retention) considering all the animals in the pooled dataset

	Symbol	Average	S.E.M.	Range	
				Minimum	Maximum
Intake	$\tilde{F}_{10}$	302	4.6	274	332
Faeces	$\tilde{F}_{01}$	189	10.0	121	244
Urine	$\tilde{F}_{02}$	0.48	0.086	0.12	0.99
Flows (mg P/(kg BW $\times$ day))					
Gut to plasma	F_{21}	127	10.8	75	203
Plasma to gut	F_{12}	14	0.4	12	16
Plasma to bone	F_{32}	456	35.6	237	639
Bone to plasma	F_{23}	396	35.7	202	581
Plasma to soft tissue	F_{42}	289	30.0	164	457
Soft tissue to plasma	F_{24}	237	30.5	110	412
P apparent absorption	P_{aa}	113	10.6	61	188
Endogenous faecal P	P_c	8.1	0.44	5.5	10.6
P retention in bone	P_b	60	5.8	33	99
P retention in soft tissue	P_t	52	7.2	12	96
P balance	$P_{balance}$	113	10.6	61	187
Pool size (g P)					
Plasma	Q_2	0.10	0.003	0.08	0.12
Bone	Q_3	143	4.8	115	171
Soft tissue	Q_4	88	9.4	46	140

the plasma (P_{aa}) or in P balance (Table 4). SRAs measured in faeces, plasma, bone and soft tissue were unaffected by the level of addition of phytase (Table 4).

Owing to the lack of significant effects of phytase on P excretion, absorption and balance, data from each treatment were assembled in a single dataset, which was analysed to assess pair-wise relationships between flows estimated by the model. Average value, standard error and range of values for model outputs (flows of P calculated using the model) considering all the animals in the pooled dataset are presented in Table 5. Large ranges in the variables allow for robust correlation and regression analysis.

DISCUSSION

Phosphorus intake ($\tilde{F}_{10}$; Table 4) was adequate (on average 10.5 g/day) for growing pigs of this weight (32 kg on average) according to NRC (1998) recommendations.

Effects of phytase addition on P utilization

Based on theoretical content of available P in the basal diet (1.7 mg/g feed), expected supply of available P from the basal diet without phytase would be 70–75 mg/(kg BW $\times$ day), noticeably lower than that observed in all the experimental treatments

(with values for F_{21} ranging between 101 and 154 mg/(kg BW $\times$ day)). This observation is in agreement with Cromwell *et al.* (1995a), who reported an increase of 15% in P availability from the basal level when 2000 units of the same phytase used in the present trial (Allzyme) were added/kg diet. However, in the present study, there were no differences between experimental treatments (levels of addition of microbial enzyme ranging from 253 to 1748 PU/kg of feed) in P digestibility or P utilization by growing pigs.

The capacity of microbial phytases to enhance P absorption and growth performance and to reduce P excretion in pigs offered diets with inadequate P levels is well documented (Cromwell *et al.* 1995b; Zimmermann *et al.* 2002). However, the reported effects of phytase addition on the improvement of P digestibility have shown great variation (Johansen & Poulsen 2003), and inconsistent results from phytase studies in pigs can be due to source of phytase, and a number of feed and animal factors (Bedford & Schulze 1998). The response to microbial phytases may be affected interactively by several factors, such as the particular phytase used, inclusion rate of phytase, dietary substrate levels, diet composition (ingredients with intrinsic phytases, Ca:P ratio, balance with other nutrients) and the physiological status of the animal (Johansen & Poulsen 2003; Selle & Ravindran 2008).

Regarding the studies that report favourable response of pigs to phytase in terms of P digestibility, it is important to point out that these responses are rather variable. For instance, Cromwell *et al.* (1995a) observed that pigs fed maize and soybean meal supplemented with increasing levels of microbial phytase did not consistently decrease their P excretion in faeces. The current results support studies which have reported decreasing improvements in P digestibility with increasing levels of phytase (Young *et al.* 1993; Schindler *et al.* 1997; Traylor *et al.* 2001). Moreover, decreased P utilization related to an increase in available P has been observed by several researchers. Although not working with phytase, Ketaren *et al.* (1993) reported a decrease in P utilization with higher available P intakes in grower-finisher pigs, suggesting a decrease in efficiency of P absorption. Rodehutsord *et al.* (1999) suggested a possible adaptation of the pig to increased P supply that could explain the inhibition in net absorption of P.

In relation to the effects of diet composition on response to phytase, it is well known that Ca and P levels may affect the efficiency of phytase addition, but there is still a need to explore other aspects of the complex interaction between these minerals (Selle *et al.* 2009) and phytase. The effect of phytase on P availability in maize and soybean meal diets may also be affected by the level of lysine in the diet (Brumm 2001).

Several studies have shown a significant effect of phytase level on P accumulation in bone and soft tissue (Young *et al.* 1993; Han *et al.* 1997; Kies *et al.* 2005). The animals used in the present study were growing pigs so it is expected that absorbed P is accreted in bone to facilitate animal growth. As P provision was not affected by level of phytase (there were no differences among treatments in absorbed P), no response in P accretion in bone could be expected with the different doses of phytase used in the present experiment.

Phosphorus absorption and excretion

Flows between gut and plasma and out of the system were calculated from P intake, P in faeces and urine and SRA determined in faeces and plasma.

Endogenous P in faeces (P_e) was within the range of values reported by other authors (Jongbloed 1987; Ajakaiye *et al.* 2003). Endogenous faecal P was closely correlated with total faecal P, accounting for 0.04 g P_e /g of total P in faeces ($\tilde{F}_{01}$), based on the relationship:

$$P_e = 0.040(\pm 0.0056)\tilde{F}_{01} + 0.42(\pm 1.083) \\ (R^2 = 0.84, n = 12, P < 0.0001)$$

Ajakaiye *et al.* (2003) pointed out that endogenous P is important in partitioning whole-body P utilization and maintaining P homeostasis in the growing pig. Rodehutsord *et al.* (1999) suggested that

endogenous faecal P losses do not influence regulatory P excretion in growing pigs fed increasing P concentrations in the diet.

The mean value of P excreted in urine (0.42 mg/(kg BW $\times$ day)) was slightly higher than the value 0.35 mg/(kg BW $\times$ day) reported by Rodehutsord *et al.* (1998) as the daily inevitable urinary loss for growing pigs fed suboptimal P. Urinary P ($\tilde{F}_{02}$) accounted on average for 1.6 mg/g of P ingested, comparable to the value reported by Figueredo *et al.* (2000) studying pigs fed rolled rice meal with phytase added to the ration. Rodehutsord *et al.* (1999) studied P excretion in pigs in response to increasing concentrations of P in the diet, and concluded that an adaptation to surplus P supply occurred initially in the gut, whereas a similar adaptation to an excess in P provision would be observed in the kidneys when P body stores were replete. Therefore the negligible P urinary excretion reported herein could indicate that body stores were not fully replete.

Apparent absorption of P (P_{aa}) and faecal excretion of P ($\tilde{F}_{01}$) showed a significant and negative correlation as given by the equation (with P_{aa} and $\tilde{F}_{01}$ in mg/(kg BW $\times$ day)):

$$P_{aa} = -0.958(\pm 0.146)\tilde{F}_{01} + 294(\pm 28.1) \\ (R^2 = 0.81, n = 12, P < 0.0001)$$

A similar relationship was found between F_{21} (flow of P from gut lumen to plasma) estimated by the model and faecal excretion of P (both in mg/(kg BW $\times$ day)):

$$F_{21} = -0.962(\pm 0.156)\tilde{F}_{01} + 308(\pm 29.9) \\ (R^2 = 0.79, n = 12, P < 0.0001)$$

In contrast, apparent absorption of P (P_{aa}) was not correlated with P intake ($r = 0.35$, $P = 0.262$), owing to lack of a significant relationship between faecal excretion of P and P intake ($r = 0.091$, $P = 0.779$).

Model performance

The Vitti-Dias model was built on the assumption that all P was accounted for in the calculations, considering a single input through dietary intake and that no P disappeared from the system, except from defined exits (faeces and urine). The Vitti-Dias model is formulated using rate:state principles, is dependent on SRA measurements and has been demonstrated as applicable to both goats (Vitti *et al.* 2000) and pigs (Schulin-Zeuthen *et al.* 2005). There is no reported information on the actual values of exchanges of P between plasma and bone or soft tissue that could be compared with the present values obtained using isotopic tracer methodology and applying the Vitti-Dias model. Therefore, the model was refined in the current study and applied to study P metabolism of growing pigs, examining the relationships between

some of the flows to appraise the feasibility and suitability of the values determined by the model. Flows derived from the model follow a similar trend to those reported by Dias *et al.* (2007) for sheep, although values were dissimilar as expected considering that animal species, diets and P supplies were different in both experiments.

Phosphorus retention in bone was positively correlated with flow of P from gut to plasma:

$$P_b = 0.415(\pm 0.107)F_{21} + 7.8(\pm 14.1) \\ (R^2 = 0.60, n = 12, P = 0.003)$$

indicating that P retention in bone was highly determined by P provision from the gut (0.415 mg of P retained in bone per g of P transferred to plasma from the gut). There was also a significant relationship between P retention in bone and urinary P excretion:

$$P_b = 53(\pm 13.1)\tilde{F}_{02} + 35(\pm 7.3) \\ (R^2 = 0.62, n = 12, P = 0.002)$$

Phosphorus retention in soft tissue was also significantly related to the flow of P from gut to plasma:

$$P_t = 0.563(\pm 0.113)F_{21} - 19.1(\pm 14.8) \\ (R^2 = 0.71, n = 12, P = 0.0005)$$

The efficiency of utilization of truly absorbed P (P_{ta}) was estimated as $P_{balance}/P_{ta}$, showing that, on average, 0.937 (± 0.0081) g of P truly absorbed were effectively accreted in the body of the pig ($R^2 = 0.99$, $P < 0.001$), equivalent to 0.374 g of P accreted per g of P ingested. The partitioning of net total P retention between bone and soft tissue was estimated regressing P retained in bone against P balance (regression constrained to pass through the origin), giving:

$$P_b = 0.525(\pm 0.0323)P_{balance} \\ (R^2 = 0.541, n = 12, P = 0.004)$$

indicating that in the current study approximately half of the P was retained in bone and half in soft tissue. Vitti *et al.* (2000), studying P metabolism in growing goats, reported greater amounts of P transferred from plasma to bone (F_{32}) for goats receiving a lower P level than for goats receiving higher levels, suggesting that metabolism in growing animals works essentially to provide P for bone formation even if the animal ingests a low level of this mineral element. The animals used in the present study were growing pigs, so it is expected that absorbed P be accreted in bone to facilitate animal growth.

There was a close relationship between the flow of P from plasma to bone (F_{32}) and P returned from bone to plasma (F_{23}):

$$F_{23} = 0.99(\pm 0.051)F_{32} - 56(\pm 24.1) \\ (R^2 = 0.97, n = 12, P < 0.0001)$$

As a result, only 0.14 g of total P flowing from plasma to bone is actually retained in osteoid tissue. Similarly, the uptake of P from the plasma to soft tissue was balanced to some extent by the mobilization of P from these tissues, so that net P retention in soft tissue was only 0.20 g/g total P flowing from plasma to these tissues. The relationship between flow of P from plasma to soft tissue (F_{42}) and P mobilized from soft tissue to plasma (F_{24}) was

$$F_{24} = 0.99(\pm 0.076)F_{42} - 49(\pm 23.2) \\ (R^2 = 0.94, n = 12, P < 0.0001)$$

Kemme *et al.* (2006) suggested that different phosphates (penta, tetra, tri, di and mono) could be formed in the upper gastrointestinal tract of pigs, and that inositol phosphates of different molecular weight may have different fates after being absorbed.

Based on P flows to and from bone and soft tissue, and pool size of these compartments (Table 5), turnover rates (accretion and mobilization rates) can be calculated, resulting in values of 0.102 and 0.104 per day for P accretion in bone and soft tissue, respectively, and of 0.088 and 0.086 per day for P resorption from bone and P mobilization from soft tissue, respectively. These values suggest that there is an important turnover of P in bone and soft tissue, and that both P resorption from bone and P mobilization from soft tissue contribute to maintain P levels in the central pool (plasma) for normal functions of the animal body (Fernández 1995). The current values are within the ranges (0.09–0.17/day in bone and 0.07–0.14/day in soft tissue) calculated from flows and pool sizes reported by Schulin-Zeuthen *et al.* (2005) for growing pigs fed increasing levels of P. However, values reported by Dias *et al.* (2007) for sheep fed increasing levels of P were slightly higher (0.13–0.21 per day), probably because ruminants excrete greater amounts of faecal endogenous P than non-ruminants. Unfortunately, the scientific literature provides scarce quantitative data of P turnover rates in bone and soft tissue, and thus a detailed comparison of P turnover in tissues from the current study with those obtained by others is not possible. This emphasizes the importance of the present work.

In conclusion, the model provides suitable representation of P flows between the central pool (plasma) and the gut (absorption of dietary P and resorption of endogenous P) and other peripheral compartments, resulting in appropriate estimates of P flows between compartments (comparable with values calculated from balance data) and allowing for quantification of the partition of available P between bone and soft tissue. The Vitti–Dias model may be considered a useful tool for studying the effect of different factors on P metabolism in pigs.

REFERENCES

- AJAKIYE, A., FAN, M. Z., ARCHBOLD, T., HACKER, R. R., FORSBERG, C. W. & PHILLIPS, J. P. (2003). Determination of true digestive utilization of phosphorus and the endogenous phosphorus outputs associated with soybean meal for growing pigs. *Journal of Animal Science* **81**, 2766–2775.
- ANUGWA, F. O. I., VAREL, V. H., DICKSON, J. S., POND, W. G. & KROOK, L. P. (1989). Effects of dietary fiber and protein concentration on growth, feed efficiency, visceral organ weights and large intestine microbial populations of swine. *Journal of Nutrition* **119**, 879–886.
- ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. (1995). *Official Methods of Analysis of AOAC*, 16th edn. Arlington, VA: Association of Official Analytical Chemists International.
- BEDFORD, M. R. & SCHULZE, H. (1998). Exogenous enzymes for pigs and poultry. *Nutrition Research Reviews* **11**, 91–114.
- BRADY, S. M., CALLAN, J. J., COWAN, D., MCGRANE, M. & O'DOHERTY, J. V. (2002). Effect of phytase inclusion and calcium/phosphorus ratio on the performance and nutrient retention of grower–finisher pigs fed barley/wheat/soya bean meal-based diets. *Journal of Science and Food Agriculture* **82**, 1780–1790.
- BRUMM, M. C. (2001). Phytase in swine diets. *2001 Nebraska Swine Report*, pp. 4–7. Lincoln, NE: University of Nebraska. Available online at <http://digitalcommons.unl.edu/coopextswine/93>
- CROMWELL, G. L. (1979). Availability of phosphorus in feedstuffs for swine. *Proceedings of the Distillers Feed Conference* **34**, 40–52.
- CROMWELL, G. L., COFFEY, R. D., MONEGUE, H. J. & RANDOLPH, J. H. (1995a). Phytase of low-activity, microbial phytase in improving the bioavailability of phosphorus in corn–soybean meal diets for pigs. *Journal of Animal Science* **73**, 449–456.
- CROMWELL, G. L., COFFEY, R. D., PARKER, G. R., MONEGUE, H. J. & RANDOLPH, J. H. (1995b). Efficacy of a recombinant-derived phytase in improving the bioavailability of phosphorus in corn–soybean meal diets for pigs. *Journal of Animal Science* **73**, 2000–2008.
- 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.
- DIAS, R. S., KEBREAB, E., VITTI, D. M. S. S., PORTILHO, F. P., LOUVANDINI, H. & FRANCE, J. (2007). Phosphorus kinetics in lambs fed different levels of dicalcium phosphate. *Journal of Agricultural Science, Cambridge* **145**, 509–516.
- DÜNGELHOEF, M., RODEHUTSCORD, M., SPIEKERS, H. & PFEFFER, E. (1994). Effects of supplemental microbial phytase on availability of phosphorus contained in maize, wheat and triticale to pigs. *Animal Feed Science and Technology* **49**, 1–10.
- DUTRA, W. M. JR., FERREIRA, A. S., DONZELE, J. L., EUCLYDES, R. F., TAROUÇO, J. U. & CARDOSO, L. L. (2001). Predição de curvas de crescimento de tecidos de fêmeas suínas por intermédio da função alométrica estendida. *Revista Brasileira de Zootecnia – Brazilian Journal of Animal Science* **30**, 1007–1014.
- FERNÁNDEZ, J. A. (1995). Calcium and phosphorus metabolism in growing pigs. I. Absorption and balances studies. *Livestock Production Science* **41**, 233–241.
- FIGUEREDO, A. V., FIALHO, E. T., VITTI, D. M. S. S., LOPES, J. B., SILVA FILHO, J. C., TEIXEIRA, A. S. & LIMA, J. A. F. (2000). Ação da fitase sobre a disponibilidade biológica do fósforo, por intermédio da técnica de diluição isotópica, em dietas com farelo de arroz integral para suínos. *Revista Brasileira de Zootecnia – Brazilian Journal of Animal Science* **29**, 177–182.
- FISKE, C. H. & SUBBAROW, Y. (1925). The colorimetric determination of phosphorus. *Journal of Biological Chemistry* **66**, 375–400.
- GOMES, P. C., ROSTAGNO, H. S., PEREIRA, J. A. A., COSTA, P. M. A. & LIMA, J. A. F. (1989). Exigência de fósforo total e disponível para suínos na fase de crescimento. *Revista Brasileira de Zootecnia – Brazilian Journal of Animal Science* **18**, 232–239.
- HAN, Y. M., YANG, F., ZHOU, A. G., MILLER, E. R., KU, P. K., HOGBERG, M. G. & LEI, X. G. (1997). Supplemental phytase of microbial and cereal sources improve dietary phytate phosphorus utilization by pigs from weaning through finishing. *Journal of Animal Science* **75**, 1017–1025.
- INTERNATIONAL ATOMIC ENERGY AGENCY (IAEA). (1979). *Laboratory Training Manual on the Use of Nuclear Techniques in Animal Research*. Technical Report Series No. 193. Vienna, Austria: IAEA.
- JOHANSEN, K. & POULSEN, H. D. (2003). Substitution of inorganic phosphorus in pig diets by microbial phytase supplementation – a review. *Pig News and Information* **24**, 77N–82N.
- JONGBLOED, A. W. (1987). Phosphorus in the feeding of pigs: effect of diet on the absorption and retention of phosphorus by growing pigs. Ph.D. dissertation, Wageningen Agricultural University, The Netherlands.
- KEMME, P. A., JONGBLOED, A. W., MROZ, Z. & BEYENEN, A. C. (1997). The efficacy of *Aspergillus niger* phytase in rendering phytate phosphorus available for absorption in pigs is influenced by pig physiological status. *Journal of Animal Science* **75**, 2129–2138.
- KEMME, P. A., SCHLEMMER, U., MROZ, Z. & JONGBLOED, A. W. (2006). Monitoring the stepwise phytate degradation in the upper gastrointestinal tract of pigs. *Journal of Science of Food and Agriculture* **86**, 612–622.
- KERR, B. J., McKEITH, F. K. & EASTER, R. A. (1995). Effect on performance and carcass characteristics of nursery to finisher pigs fed reduced crude protein, amino acid-supplemented diets. *Journal of Animal Science* **73**, 433–440.
- KETAREN, P. P., BATTERHAM, E. S., WHITE, E., FARRELL, D. J. & MILTHORPE, B. K. (1993). Phosphorus studies in pigs. 1. Available phosphorus requirements of grower/finisher pigs. *British Journal of Nutrition* **70**, 249–268.
- KIES, A. K., GERRITS, W. J. J., SCHRAMA, J. W., HEETKAMP, M. J. W., VAN DER LINDEN, K. L., ZANDSTRA, T. & VERSTEGEN, M. W. A. (2005). Mineral absorption and excretion as affected by microbial phytase, and their effect on energy metabolism in young piglets. *Journal of Nutrition* **135**, 1131–1138.
- LOFGREEN, G. P. & KLEIBER, M. (1953). The availability of the phosphorus in alfalfa hay. *Journal of Animal Science* **12**, 366–371.

- MATSUI, T., NAKAGAWA, Y., TAMURA, A., WATANABE, C., FUJITA, K., NAKAJIMA, T. & YANO, H. (2000). Efficacy of yeast phytase in improving phosphorus bioavailability in a corn-soybean meal-based diet for growing pigs. *Journal of Animal Science* **78**, 94–99.
- NASCIMENTO FILHO, V. F. & LOBÃO, A. O. (1977). Detecção de P-32 em amostras de origem animal e vegetal por efeito Cerenkov, cintilação líquida e detector GM. Boletim Científico, 48. Piracicaba, Brazil: CENA.
- NATIONAL RESEARCH COUNCIL. (1998). *Nutrient Requirement of Swine*. 10th revised edn, Washington, DC: National Academy Press.
- QIAN, H., KORNEGAY, E. T. & CONNER, D. E. JR. (1996). Adverse effects of wide calcium:phosphorus ratios on supplemental phytase efficacy for weanling pigs fed two dietary phosphorus levels. *Journal of Animal Science* **74**, 1288–1297.
- RODEHUTSCORD, M., HAVERKAMP, R. & PFEFFER, E. (1998). Inevitable losses of phosphorus in pigs, estimated from balance data using diets deficient in phosphorus. *Archives of Animal Nutrition* **51**, 27–38.
- RODEHUTSCORD, M., FAUST, M. & PFEFFER, E. (1999). The course of phosphorus excretion in growing pigs fed continuously increasing phosphorus concentrations after a phosphorus depletion. *Archives of Animal Nutrition* **52**, 323–334.
- ROSTAGNO, H. S., ALBINO, L. F. T., DONZELE, J. L., GOMES, P. C., FERREIRA, A. S., OLIVEIRA, R. F. & LOPES, D. C. (2000). *Tabelas Brasileiras para Aves e Suínos: Composição de Alimentos e Exigências Nutricionais (Brazilian Tables for Poultry and Swine: Feed Composition and Nutrient Requirements)*, 1st edn. Viçosa, Brazil: Imprensa Universitária.
- SARRUGE, J. R. & HAAG, H. P. (1974). *Análises Químicas em Plantas [Chemical Analysis of Plants]*. Piracicaba, Brazil: Departamento de Química, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo.
- SAS (1999). *SAS/STAT User's Guide*, Release 8.00. Cary, NC: SAS Institute.
- SCHINDLER, B., LANTZSCH, H.-J., MOSENTHIN, R., BIESALSKI, H. K. & DROCHNER, W. (1997). Dose-response effects of microbial phytase on P absorption in growing pigs, fed P reduced diets. In *Digestive Physiology in Pigs, Proceedings of the VIIth International Symposium on Digestive Physiology in Pigs* (Eds J. P. LAPLACE, C. FÉVRIER & A. BARBEAU), pp. 441–445. EAAP Publication No. 88. Saint Malo, France: European Association for Animal Production, Institut National de la Recherche Agronomique.
- SCHULIN-ZEUTHEN, M., LOPES, J. B., KEBREAB, E., VITTI, D. M. S. S., ABDALLA, A. L., HADDAD, M. D., CROMPTON, L. A. & FRANCE, J. (2005). Effects of phosphorus intake on phosphorus flow in growing pigs: application and comparison of two models. *Journal of Theoretical Biology* **236**, 115–125.
- SELLE, P. H. & RAVINDRAN, V. (2008). Phytate-degrading enzymes in pig nutrition. *Livestock Science* **113**, 99–122.
- SELLE, P. H., COWIESON, A. J. & RAVINDRAN, V. (2009). Consequences of calcium interactions with phytate and phytase for poultry and pigs. *Livestock Science* **124**, 126–141.
- TRAYLOR, S. L., CROMWELL, G. L., LINDEMANN, M. D. & KNABE, D. A. (2001). Effects of level of supplemental phytase on ileal digestibility of amino acids, calcium, and phosphorus in dehulled soybean meal for growing pigs. *Journal of Animal Science* **79**, 2634–2642.
- VASUPEN, K., YUANGKLANG, C., WONGSUTHAVAS, S., PANYAKAEW, P., MITCHAOTHAI, J. & BEYNEN, A. C. (2008). Growth performance, carcass and meat characteristics of female and male Kadon pigs. *Journal of Biological Sciences* **8**, 671–674.
- VITTI, D. M. S. S., KEBREAB, E., LOPES, J. B., ABDALLA, A. L., DE CARVALHOS, F. F. R., DE RESENDE, K. T., CROMPTON, L. A. & FRANCE, J. (2000). A kinetic model of phosphorus metabolism in growing goats. *Journal of Animal Science* **78**, 2706–2712.
- YOUNG, L. G., LEUNISSEN, M. & ATKINSON, J. L. (1993). Addition of microbial phytase to diets of young pigs. *Journal of Animal Science* **71**, 2147–2150.
- ZIMMERMANN, B., LANTZSCH, H.-J., MOSENTHIN, R., SHONER, F.-J., BIESALSKI, H. K. & DROCHNER, W. (2002). Comparative evaluation of the efficacy of cereal and microbial phytases in growing pigs fed diets with marginal phosphorus supply. *Journal of Science of Food and Agriculture* **82**, 1298–1304.
- ZIMMERMANN, B., LANTZSCH, H.-J., MOSENTHIN, R., BIESALSKI, H. K. & DROCHNER, W. (2003). Additivity of the effect of cereal and microbial phytases on apparent phosphorus absorption in growing pigs fed diets with marginal P supply. *Animal Feed Science Technology* **104**, 143–152.