In Vitro Procedure for Predicting the Enzymatic Dephosphorylation of Phytate in Corn−Soybean Meal Diets for Growing Swine

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An *in vitro* procedure was developed to simulate the digestive system of growing swine for the purpose of predicting phosphorus release from corn−soybean meal diets. The procedure consisted of separate peptic and pancreatic digestion periods. Peptic digestion was carried out by incubating 1.0 g of feed sample with 3000 units of pepsin at pH 2.5 for 75 min. Pancreatic digestion was conducted in a pH 6.0 dialyzing medium for 1−5 h after the pepsin digesta were mixed with 2.4 mg of pancreatin. The i*n vitro* P release responded linearly and quadratically ($p < 0.0001$) to increasing concentrations of added P and microbial phytase, respectively. The recovery rate of added inorganic P averaged 98%. The *in vitro* P release correlated ($r = 0.999$) with *in vivo* P digestibility and growth performance. In conclusion, this *in vitro* procedure is a valid, simple, and economical method for predicting the enzymatic dephosphorylation of phytate in corn−soybean meal diets fed to growing swine.

Keywords: *In vitro procedure; phosphorus; phytase; swine*

INTRODUCTION

Phytate-bound P, the major form of P in plant origin feed ingredients, must be hydrolyzed from the inositol ring by phytase before it can be absorbed by animals (Pointillart et al., 1984). Monogastric animals such as pigs cannot utilize phytate-bound P, due to a lack of intestinal phytase (Pointillart et al., 1984). Only 15 and 25% of the P in corn and dehulled soybean meal, two major feed ingredients for pigs in the United States, are nutritionally available to pigs, respectively (National Research Council, 1988). Thus, inorganic P is added to swine diets to meet the available P requirement, which increases feed cost. Furthermore, all of the nondigested phytate P is excreted in the manure, which is an environmental concern.

Supplementation of microbial phytase significantly improves P availability in corn−soybean meal diets for pigs (Näsi, 1990; Simons et al., 1990; Cromwell et al., 1995). The digestibility or availability of dietary P, the most widely used index of the efficacy of microbial phytase in dephosphorylation of phytate in the diet, can be determined by conducting an *in vivo* digestion or balance study. However, this is an expensive and time-consuming procedure. Therefore, development of a simple, economical, and effective *in vitro* procedure for predicting dephosphorylation of phytate by phytase in swine diets would be useful.

Successful *in vitro* approaches have been used to predict the digestibilities of protein (Babinszky et al., 1990; Drake et al., 1991; Van der Meer and Perez, 1992) and minerals such as Fe (Miller et al., 1981), Zn (Lease, 1967; Wolters et al., 1993), and P (Zyla et al., 1995). Zyla et al. (1995) developed an *in vitro* procedure for studying enzymatic dephosphorylation of phytate by phytase in corn−soybean meal diets for turkey poults by simulating the digestive conditions of the crop, gizzard, and duodenum of poultry. The amount of P hydrolyzed from feed samples by *in vitro* digestions was significantly correlated with growth performance and toe ash of turkey poults.

There is no *in vitro* procedure available for predicting P availability in diets for pigs. The purpose of this research was to develop an *in vitro* procedure that simulates the digestive system of growing swine and accurately predicts the availability of P in corn−soybean meal diets fed to swine.

MATERIALS AND METHODS

Enzymes and Enzyme Activity Measurements. The microbial phytase (EC 3.1.3.8) used in the *in vitro* study (Natuphos, BASF, Mount Olive, NJ) has a declared phytase activity of ≥5000 phytase units (PU)/mL. The activity of the phytase preparation was confirmed to be 6800 PU/mL by the method of Engelen et al. (1994). One PU is defined as the amount of enzyme that liberates 1 μmol of inorganic P per minute from 5.1 mM of sodium phytate from rice (P-3168, Sigma Chemical Co., St. Louis, MO) at 37 °C and pH 5.5. A dry powdered phytase preparation containing 47 000 PU/g (declared by BASF) was used as a standard reference. Porcine pepsin (P-6887; EC 3.4.23.1) and pancreatin (P-7545; activity = 8 × USP) were purchased from Sigma.

Assays. Dietary P was determined colorimetrically (Engelen et al., 1994), and dietary Ca was determined on an atomic absorption spectrophotometer (SpectrAA-30, Varian Techtron Pty., Mulgrave, VIC, Australia) in duplicate samples, which were digested by a wet-ash procedure (Association of Offical Analytical Chemists, 1990). The procedure was validated by including a standard reference material (peach leaves, no. 1547; National Institute of Standards and Technology, U.S. Department of Commerce, Gaithersburg, MD).

***In Vitro* Procedure.** The *in vitro* procedure was designed to simulate the digestive system of swine and consists of two enzymatic digestions: (1) peptic digestion followed by (2) pancreatic digestion with semipermeable membrane dialysis (Figure 1).

Pepsin Digestion. A 1-g (±0.001) sample of finely ground diet (1.0-mm screen) containing 0, 250, or 500 PU/kg of supplemental phytase was mixed with 2 mL of a 0.18 N HCl solution containing a total of 3000 units of pepsin in a 5-mL plastic syringe without the Luer-lock. Pepsin was added at 3000 units/g of feed because Furuya et al. (1979) reported that protein digestibilities *in vitro* were similar with pepsin concentrations of 2000 or 40 000 units/g of diet. The sample was then incubated in a water bath at 39 °C for 75 min. The pH

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Peptic digestion:

(1) Mix 1.0 g of feed sample with 2 mL of a 0.18 N HCl solution containing 1500 U of pepsin/mL; Seal and vortex

⇓

(2) Incubate at 39 ^{0}C for 75 Min

⇓

Pancreatic digestion:

(3) Add 0.65 mL of a 1 M $NaHCO_3$ solution containing 3.7 mg pancreatin/mL, mix

⇓

(4) Transfer slurry to dialysis tubing (12000-14000 MW cut-off), Seal with closure

⇓

(5) Dialyze in 100 mL of a 0.05 M pH 6.0 succinate buffer containing 0.1 M NaCl and 0.02% sodium azide at 39 ^{0}C for 60-240 min.

⇓

(6) Determine P concentration in succinate buffer

Figure 1. *In vitro* procedure with two enzymatic digestions.

of the digesta averaged 2.5 with pH values of 2.1 at the start and 2.7 at the end of peptic digestion.

Pancreatic Digestion with Semipermeable Membrane Dialysis. A preliminary study was conducted to compare the effect of three levels of pancreatin (2.4, 24.0, or 120.0 mg/g of diet sample) on P release from a corn−soybean meal diet containing 500 PU/kg of supplemental phytase. Each concentration of pancreatin was tested in triplicate using a blank to correct for the P contained in the pancreatin extract. Pancreatin concentration did not affect P release in our test when the amount of P added by pancreatin was corrected by the blank. When pancreatin was added in excess to maximize protein digestion *in vitro,* the concentrations ranged from 40 (Drake et al., 1991) to 200 mg/g of feed (Van der Meer and Perez, 1992).

At the end of peptic digestion, the peptic digesta were mixed with a 0.65 mL of a 1 M $NaHCO_3$ solution containing 3.7 mg of pancreatin/mL (2.4 mg/g of diet sample) and transferred into a segment of dialyzing tubing (molecular weight cutoff 12 000−14 000, diameter 1.6 cm; Sigma Chemical Co.). Pancreatic digestion was conducted by placing the segment in 100 mL of a 0.05 M succinate buffer (pH 6.0) containing 0.1 M NaCl and dialyzing in a shaking water bath at 39 °C for 1, 2, 3, 4, or 5 h. To prevent microbial growth, 0.02% sodium azide was added to the buffer. Released P was determined colorimetrically on a spectrophotometer at 415 nm as described by Engelen et al. (1994).

Evaluation of the *in Vitro* Procedure with Two Sources of Inorganic P and Graded Levels of Added Inorganic P and Microbial Phytase. Phosphorus release was determined in corn−soybean meal diets containing 0.28% inorganic P from a highly soluble source (reagent grade K_2HPO_4) and 0.20% inorganic P from a less soluble source (feed grade monodicalcium phosphate, Texasgulf Inc., Saltville, VA) after 1−5 h of pancreatic digestion. Samples of a basal corn−soybean meal diet containing 0.32% total P and 0.40% Ca were mixed with graded levels of reagent grade K_2HPO_4 to provide 0.5−3.0 g of inorganic P/kg of diet in 0.50 g/kg increments or with Natuphos, to provide phytase activities of 250−2250 PU/kg of diet in 250 PU/kg increments. The P released was determined by the *in vitro* method with six replicates per assay. The recovery rate of added inorganic P was determined.

Table 1. Ingredients and Chemical Composition of the Basal Diet[a]

item	%	item	%
ingredients		anal. compos	
ground yellow corn	75.59	CP	15.27
dehulled soybean meal (CP 49%)	20.16	P	0.32
soybean oil	2.50	Ca	0.40
ground limestone	0.78		
white salt (NaCl)	0.35		
vitamin premix[b]	0.47		
trace mineral premix[c]	0.15		

[a] For the *in vivo* study, *Aspergillus niger* phytase (Natuphos, BASF) was supplemented at 0, 250, or 500 PU/kg of diet. The diets contained 0.55% Ca after ground limestone was added at 0.24% in substitution of corn. [b] Supplied per kg of diet: vitamin A acetate, 13216 IU; vitamin D_3, 1322 IU; DL-α-tocopheryl, 56.8 IU; menadione sodium bisulfite complex, 4.8 mg; vitamin B_{12}, 36 μg; riboflavin, 9.9 mg; calcium pantothenate, 33.7 mg; niacin, 39.7 mg; choline chloride, 447.6 mg. [c] Supplied (mg/kg diet): Zn as ZnO, 165; Fe as $FeSO_4$, 165; Mn as $MnSO_4$, 33; Cu as $CuSO_4$, 16.5; I as $Ca(IO_3)_2$, 0.3; Se as Na_2SeO_3, 0.3.

Evaluation of the *in Vitro* Procedure with Graded Levels of Dietary Ca. A 2 × 2 × 6 factorial arrangement of treatments with six replicates per assay was used to determine the effect of microbial phytase at 0 or 500 PU/kg of diet, added inorganic P at 0 or 0.18%, and dietary Ca levels at 0.40, 0.45, 0.50, 0.55, 0.60, and 0.65% on P release from corn−soybean meal diets. The basal corn−soybean meal diet contained 0.32% total and 0.06% available P (Table 1). The added P and Ca were provided by reagent grade K_2HPO_4 and $CaCO_3$, respectively.

***In Vivo* Validation of the *in Vitro* Procedure with Growing Pigs.** The *in vivo* experimental procedure and results have been presented by Liu et al. (1997a). Growing barrows (n = 9/treatment, initial BW and age were 18.7 kg and 7 weeks, respectively) were housed individually in elevated pens and fed a basal corn−soybean meal diet supplemented with microbial phytase at 0, 250, or 500 PU/kg of diet for 6 weeks. The basal 15.49% crude protein diet contained 0.06% available P, 0.32% total P, and 0.55% Ca (Table 1). All other nutrients met the requirements of growing pigs suggested by the National Research Council (1988). The pigs were fed to appetite four times daily and had free access to water. Chromic oxide was added to the diets at 0.05% as an indigestible indicator for determination of P digestibility. Pigs were weighed and feed intake was determined weekly. Fecal samples were collected from individual pigs twice daily on days 39−42 of the experiment. Before analysis, the collections were thawed, dried at 55 °C for 48 h, and ground through a 1.0-mm screen. Duplicate samples of diets and feces were digested according to the wet-ash procedure (Association of Official Analytical Chemists, 1990). Fecal P concentration was determined as described for diets.

Statistical Analyses. Data were analyzed using the general linear model procedure of the Statisical Analysis System (1989). The results of *in vitro* factorial design were subject to analysis of variance according to following model

$$X_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + e_{ijk}$$

where μ = overall mean; α_i = phytase effect; β_j = inorganic P effect; γ_k = Ca effect; and e = error contribution with average 0 and variance δ^2; $i = 1 \ldots a$; $j = 1 \ldots b$; $k = 1 \ldots n$.

Linear and quadratic effects on P release were tested with pancreatic digestion times and different levels of inorganic P

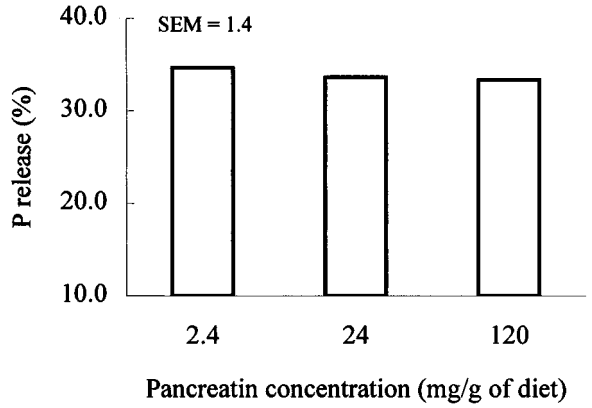

Figure 2. Effect of pancreatin concentration on *in vitro* P release from a corn−soybean meal diet supplemented with phytase at 500 PU/kg. The diets contained 0.32% P and 0.40% Ca. Phosphorus was determined after 4 h of pancreatic digestion. There was no significant effect of pancreatin concentration on net P release ($p > 0.5$).

and phytase. To test the phytase effect in the *in vivo* study, individual pigs were the experimental units. The data were subjected to analysis of variance as a randomized complete block design (Snedecor and Cochran, 1989). Correlation analysis was performed to determine the relationship between *in vitro* P release and *in vivo* P digestibility and growth performance.

RESULTS

Effect of Pancreatin Concentrations. Increasing levels of pancreatin from 2.4 to 24.0 or 120.0 mg/g of diets did not increase ($p > 0.5$) net P release from a corn−soybean meal diet supplemented with microbial phytase at 500 PU/kg (Figure 2). Therefore, a pancreatin concentration of 2.4 mg/g of diet was selected for the *in vitro* procedure.

Effect of Pancreatic Digestion Time. In response to increasing pancreatic digestion time, there was a quadratic increase ($p < 0.05$) in P release from the diet supplemented with phytase at 500 PU/kg and linear increases for the diets supplemented with phytase at 0 ($p < 0.04$) or 250 PU/kg ($p < 0.0001$) (Figure 3). Phosphorus release from the diet with 500 PU/kg of phytase plateaued after 4 h of pancreatic digestion.

Evaluation of the *in Vitro* Procedure with Different Sources and Graded Levels of Added Inorganic P and Phytase. There were quadratic increases in P release from K_2HPO_4 ($p < 0.001$) and mono-dicalcium phosphate ($p < 0.0001$) in response to increasing pancreatic digestion time (Figure 4). After 1 h of pancreatic digestion, 43 and 75% of added inorganic P were released from mono-dicalcium phosphate and K_2HPO_4, respectively. Phosphorus release plateaued after 2 h for added inorganic P from K_2HPO_4 (86%), whereas P release plateaued after 4 h for added inorganic P from mono-dicalcium phosphate (71%).

There was a linear increase in P release in response to increasing inorganic P concentrations in the diets ($p < 0.0001$) (Figure 5). The recovery rate of added inorganic P averaged 98%, ranging from 92 to 114% (SD = 8.31). Thus, the inorganic P was readily dialyzable across the tubing. Dephosphorylation of phytate in the corn−soybean meal diet responded quadratically to increasing levels of microbial phytase ($p < 0.0001$), which can be described by the kinetics of an enzymatically catalyzed reaction (Figure 6).

Evaluation of the *in Vitro* Procedure with Graded Levels of Dietary Ca. The effect of dietary

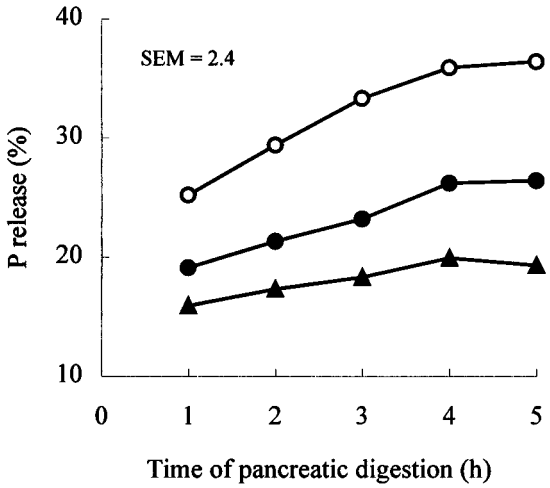

Figure 3. Effect of pancreatic digestion time on P release from corn−soybean meal diets supplemented with microbial phytase at 0 (▲), 250 (●), or 500 (○) PU/kg. In response to increasing pancreatic digestion time from 1 to 5 h, there was a quadratic increase in P release from the diet supplemented with phytase at 500 PU/kg ($Y = -0.61x^2 + 6.54x + 19.12$, $r^2 = 0.812$, $p < 0.05$) and a linear response in P release from the diets with no phytase ($Y = 0.95x + 15.30$, $r^2 = 0.217$, $p < 0.04$) or 250 PU/kg ($Y = 1.96x + 17.4$, $r^2 = 0.665$, $p < 0.0001$).

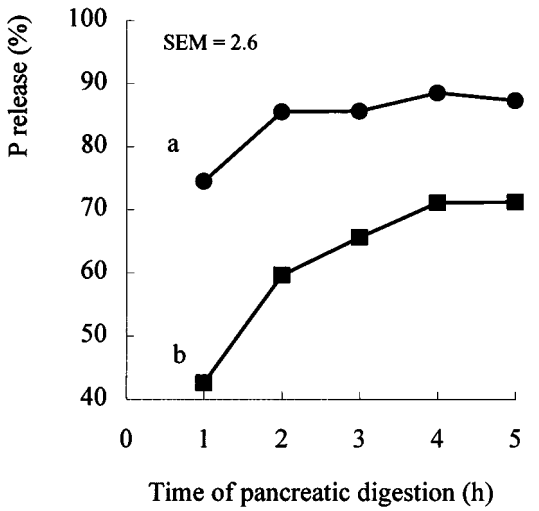

Figure 4. Effect of pancreatic digestion time on *in vitro* P release from (a) reagent grade K_2HPO_4 and (b) feed grade mono-dicalcium phosphate. In response to increasing pancreatic digestion time from 1 to 5 h, there were quadratic increases in P release from K_2HPO_4 ($Y = -1.54x^2 + 12.07x + 64.99$, $r^2 = 0.765$, $p < 0.001$) and feed grade mono-dicalcium phosphate ($Y = -2.54x^2 + 21.61x + 24.44$, $r^2 = 0.938$, $p < 0.0001$).

Ca level on *in vitro* P release from diets supplemented with or without inorganic P and microbial phytase is presented in Figure 7. There were significant interactions of inorganic P × Ca ($p < 0.0001$) and phytase × inorganic P × Ca ($p < 0.05$) on P release from the diets. These interactions suggest that the effect of dietary Ca level on P release was dependent on dietary P concentration when dietary Ca ranged from 0.40 to 0.65%. Dietary Ca concentration had no significant effect ($p > 0.05$) on P release with or without phytase when the diet did not contain added inorganic P. When the diets contained 0.18% inorganic P with or without phytase, high dietary Ca levels decreased ($p < 0.05$) P release from the diets.

***In Vivo* Validation of the *in Vitro* Procedure with Growing Pigs.** The results of the *in vivo* study

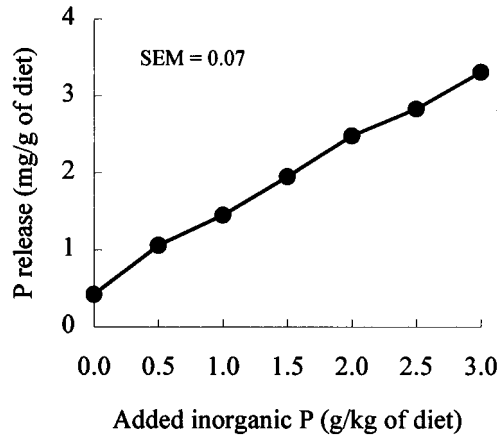

Figure 5. Effect of inorganic P concentrations in a corn−soybean meal diet on P release determined by the *in vitro* procedure. The diet contained 0.40% Ca. The P release responded linearly to increasing levels of added inorganic P ($Y = 30.481x + 16.506$, $r^2 = 0.995$, $p < 0.0001$).

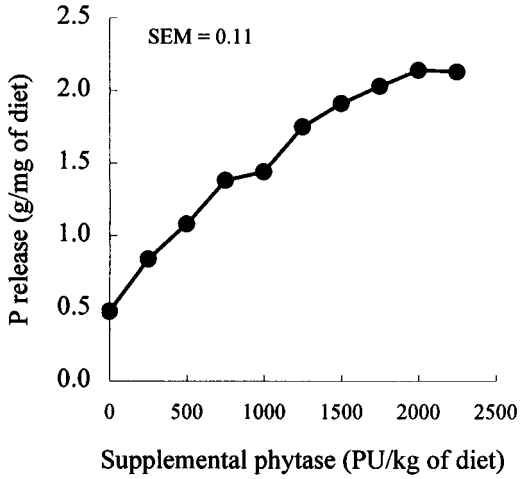

Figure 6. Effect of microbial phytase (EC 3.1.3.8) concentrations in a corn−soybean meal diet on P release determined by the *in vitro* procedure. The diet contained 0.40% Ca. The P release responded quadratically to increasing levels of microbial phytase ($Y = -0.0000047x^2 + 0.039x + 16.362$, $r^2 = 0.923$, $p < 0.0001$).

have been reported by Liu et al. (1997a). Supplementation with microbial phytase linearly increased average daily gain ($p < 0.05$) and gain/feed ratio ($p < 0.01$) of growing pigs (Table 2). Both *in vitro* P release and *in vivo* P digestibilities responded linearly to phytase supplementation at 0, 250, and 500 PU/kg of diet ($p < 0.05$ and 0.06, respectively) (Figure 8).

A simple correlation model was tested to determine the ability of the *in vitro* procedure to predict *in vivo* responses of growing pigs to phytase supplementation at 0, 250, and 500 PU/kg of diet. *In vitro* P release after 1, 2, 3, 4, or 5 h of pancreatic digestion was correlated ($p < 0.05$) with *in vivo* P digestibility and growth performance. For example, *in vitro* P release determined after 4 h of pancreatic digestion was positively correlated with average daily gain ($r = 0.999$, $p < 0.01$), average daily feed intake ($r = 0.998$, $p < 0.04$), gain/feed ratio ($r = 0.999$, $p < 0.02$), and apparent P digestibility ($r = 0.999$, $p < 0.02$) of growing pigs (Table 3).

DISCUSSION

We developed an *in vitro* procedure for swine to predict P release from corn−soybean meal diets by simulating the digestive conditions in the stomach and small intestine of growing swine. A similar approach was used by Zyla et al. (1995) to develop an *in vitro* procedure to predict P release from corn−soybean meal diets for poultry. Because phytate is dephosphorylated by microbial phytase mainly in the stomach (Jongbloed et al., 1992), simulation of the digestive conditions of the stomach is essential for development of a valid *in*

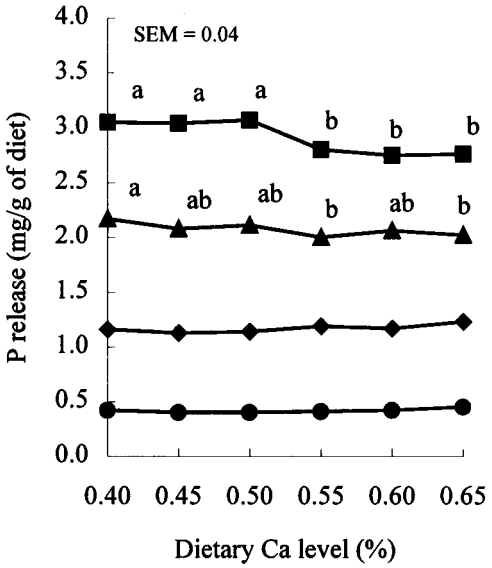

Figure 7. Effect of dietary Ca concentrations on P release from corn−soybean meal diets either with or without inorganic P or microbial phytase: (●) no added P and no phytase; (◆) 500 PU/kg; (▲) 0.18% added P; (■) 0.18% added P and 500 PU/kg. [ab]Means with unlike superscript letters within the same line differ ($p < 0.05$).

Table 2. Effect of Phytase Supplementation on Growth Performance of Growing Pigs[a]

	microbial phytase			
item	0, PU/kg	250, PU/kg	500, PU/kg	LSD
daily feed intake, kg	1.69	1.75	1.81	0.12
daily gain,[b] kg	0.58	0.66	0.76	0.07
gain/feed ratio[b]	0.35	0.38	0.42	0.03

[a] Nine replicates of one pig per pen. [b] Linear effect of phytase level on daily gain ($p < 0.05$) and gain/feed ratio ($p < 0.01$).

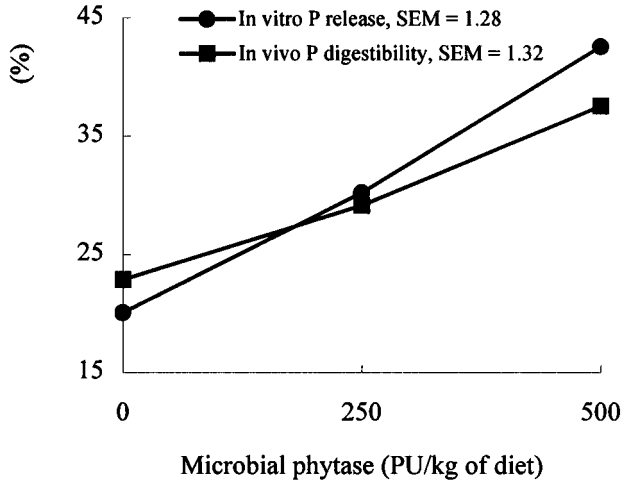

Figure 8. Effect of adding microbial phytase at 0, 250, or 500 PU/kg of diet on *in vitro* P release and *in vivo* P digestibility of growing pigs fed a corn−soybean meal diet. The diet contained 0.32% total P and 0.55% Ca. Phytase linearly increased *in vitro* P release from the diet ($Y = 0.045x + 19.702$, $r^2 = 0.997$, $p < 0.05$) and *in vivo* P digestibility of growing pigs ($Y = 0.029x + 22.503$, $r^2 = 0.993$, $p < 0.06$).

Table 3. Correlation Coefficient among *in Vitro* P Release, *in Vivo* P Digestibility, and Growth Performance of Growing Pigs[a]

parameter	*in vitro* P release	P digestibility	daily gain	daily feed intake	gain/feed
in vitro P release		0.999 (0.02)	0.999 (0.01)	0.998 (0.04)	0.999 (0.02)
P digestibility	0.999 (0.02)		0.999 (0.01)	0.996 (0.05)	1.000 (0.002)
daily gain	0.999 (0.01)	0.999 (0.01)		0.997 (0.04)	0.999 (0.01)
daily feed intake	0.998 (0.04)	0.996 (0.05)	0.997 (0.04)		0.996 (0.05)
gain/feed	0.999 (0.02)	1.000 (0.002)	0.999 (0.01)	0.996 (0.05)	

[a] The observation numbers were 9 and 3 for the *in vivo* and *in vitro* studies, respectively. Figures in parentheses are the corresponding *p* values.

vitro procedure for studying P release. Absorption of minerals takes place in the complex environment of the small intestine, and simulation of conditions in the small intestine is the second most critical step for an *in vitro* procedure designed to predict availability of minerals (Wolters et al., 1993).

The pH value of the incubation medium for an *in vitro* method is usually chosen to match the enzyme requirements (Gauthier et al., 1986). A pH of 2.5 was chosen for the *in vitro* stomach simulation in our procedure because that is within the range of pH in the gastric contents of growing pigs (Moore and Tyler, 1955a,b; Lawrence, 1972; Argenzio and Southworth, 1975) and also is one of the two pH optimums for microbial phytase (Simons et al., 1990). A pH of 6.0 was chosen for the dialysis medium because it is approximately the small intestine digesta pH reported for growing pigs (Moore and Tyler, 1955a,b; Hartman et al., 1961; Argenzio and Southworth, 1975).

A valid *in vitro* procedure for determining the release of P from diets should have an acceptable recovery rate of added inorganic P, which averaged 98% in the present study. Phosphorus release determined according to the *in vitro* procedure in the present study increased linearly with increasing concentrations of added inorganic P. Therefore, inorganic P is readily dialyzable across the cellulose tubing, and this procedure can be used to predict P release from corn−soybean meal diets.

Because microbial phytase linearly increases *in vivo* P utilization by pigs, a valid *in vitro* procedure should respond to microbial phytase. This effect was demonstrated with graded levels of microbial phytase in the present study. The quadratic response to P release with increasing levels of phytase to 2250 PU/kg followed the kinetics of an enzymatically catalyzed reaction (Zyla et al., 1995). Thus, our procedure predicts the effectiveness of microbial phytase on dephosphorylation of phytate in corn−soybean meal diets.

Calcium addition has been shown to inhibit phytate hydrolysis by inhibiting phytase activity (Ranhotra, 1973). Calcium has also been shown to inhibit P absorption by forming insoluble Ca−P complexes in the intestine (Guyton, 1986). Therefore, an effective *in vitro* procedure for studying P release from diets should also be relatively sensitive to dietary Ca concentration. The effect of dietary Ca level, within the range of the Ca requirement of growing pigs, on the release of P from corn−soybean meal diets was demonstrated in the present study. Decreasing dietary Ca level below 0.60% [requirement for growing pigs suggested by the National Research Council (1988)] increased P release in the present study when the diets were supplemented with inorganic P with or without phytase supplementation. The *in vitro* results confirmed the *in vivo* studies which showed that decreasing dietary Ca concentrations in low P diets increased P absorption (Liu et al., 1997b) and improved growth performance of pigs by increasing P utilization (Lei et al., 1994; Liu et al., 1996).

In vitro P release from corn−soybean meal diets was affected by the source of added inorganic P. Added inorganic P from K_2HPO_4 was more dialyzable than that from mono-dicalcium phosphate, indicating that P from K_2HPO_4 is more available than P from mono-dicalcium phosphate. The time of pancreatic digestion also affected *in vitro* P release in the present study. *In vitro* P release from a corn−soybean meal diet supplemented with 0, 250, and 500 PU/kg of phytase determined after 1, 2, 3, 4, and 5 h of pancreatic digestion, respectively, was significantly correlated with *in vivo* P digestibility and growth performance in the present study. A significant advantage of this *in vitro* procedure is that it has scientific validity when completed within 3 h and is valid for ranking purposes after 1 h of pancreatic digestion. However, if the objective is to determine maximum P release, a 4 h pancreatic digestion is recommended.

An *in vitro* procedure must be validated with *in vivo* results before it can be utilized to predict *in vivo* responses. Supplementation with microbial phytase significantly improved both *in vitro* P release and *in vivo* swine growth performance in our experiments. The *in vitro* P release significantly correlated with growth performance ($r > 0.998$) and P digestibility ($r = 0.999$) of growing pigs fed 0.32% total P diets supplemented with microbial phytase at 0, 250, or 500 PU/kg of diet. Therefore, our *in vitro* procedure to measure the dephosphorylation of phytate in corn−soybean meal diets can be used to predict *in vivo* P absorption and growth performance responses of pigs fed low P diets supplemented with phytase.

In conclusion, the *in vitro* procedure described to predict P availability simulates the digestive conditions of the gastrointestinal tract of pigs. The procedure is sensitive to dietary Ca concentration and predicts P availability in corn−soybean meal diets supplemented with graded levels of inorganic P or microbial phytase.

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