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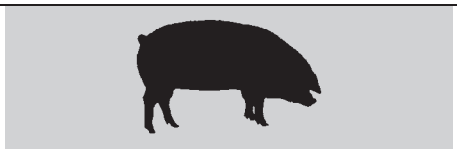

Effects of phytase on amino acid and energy digestibility in corn–soybean meal diets fed to growing pigs[1]

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ABSTRACT

An experiment was conducted to determine whether the effects of phytase on amino acid (AA) and energy digestibility in pigs is influenced by the concentration of dietary P. Fourteen barrows (initial BW: 37.8 ± 4.7 kg) were surgically fitted with a T-cannula in the distal ileum and randomly allotted to a replicated 7 × 7 Latin square design with 7 diets and 7 periods. Six diets were formulated to contain inadequate (0.13%) or adequate (0.23%) concentrations of calculated available P and supplemented with 3 levels (0, 250, or 500 phytase units/kg of diet) of an Escherichia coli phytase (OptiPhos 2000, Enzyvia, Sheridan, IN). A N-free diet was also formulated to measure ileal endogenous AA losses. Standardized ileal digestibility of AA and apparent ileal digestibility and apparent total-tract digestibility of energy and P were measured. Interactions between the effects of available P and phytase on all measured parameters were significant (P < 0.05). In diets containing inadequate available P, phytase supplementation improved (linear, P < 0.05) the standardized ileal digestibility of AA, the apparent ileal digestibility and apparent total-tract digestibility of energy, and the apparent total-tract digestibility of P. In diets with adequate available P, phytase supplementation had a quadratic effect (P < 0.05) on the apparent ileal digestibility of energy and on the standardized ileal digestibility of some AA. In conclusion, OptiPhos phytase may improve the digestibility of AA and energy if dietary P supply is inadequate, whereas in diets containing adequate concentrations of P, no effect of phytase on the digestibility of AA and energy was observed.

Key words: amino acid, digestibility, energy, phytase, pig

INTRODUCTION

Approximately 65 to 70% of the total P in feedstuffs is bound to the inositol ring of phytate (Eeckhout and De Paepe, 1994). Because phytate-bound P is poorly digestible by pigs and poultry, addition of exogenous phytase to cleave the P in phytate and subsequently improve P digestibility (Sands et al., 2001; Kies et al., 2006) is a common industry practice. Phytate may also chelate other nutrients including proteins, starch, and lipids (Selle et al., 2000), which may lead to reduced amino acid (**AA**) and energy digestibility. Therefore, hydrolysis of phytate using exogenous phytases may potentially improve the digestibility of other nutrients in the diet. However, studies in pigs evaluating the effects of phytase on AA and energy digestibility have been inconclusive (Adeola and Sands, 2003; Liao et al., 2005), which may be related to differences in diet composition (Biehl and Baker, 1997; Ravindran et al., 1999) or the type of indigestible marker used in the experiments (Adeola and Cowieson, 2011). The level of P in the diet also appears to affect the efficacy of phytase in diets fed to pigs (Johnston et al., 2004) and poultry (Cowieson et al., 2006; Martinez-Amezcua et al., 2006). We hypothesized, therefore, that effects of microbial phytase on the digestibility of AA and energy may depend on the concentration of available P in the diets. Therefore, the objective of this study was to determine the effects of different levels of OptiPhos (Enzyvia, Sheridan, IN) phytase on AA and energy digestibility in corn–soybean meal diets containing either inadequate or adequate concentrations of available P.

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Table 1. Composition of experimental diets (as-fed basis)

Item	Diet[1]		
	Inadequate P	Adequate P	N free
Ingredient, %			
Ground corn	68.00	68.00	—
Soybean meal, 48% CP	25.00	25.00	—
Soybean oil	3.00	3.00	4.00
Limestone	0.95	1.10	0.90
Monocalcium phosphate	0.35	0.95	1.30
Salt	0.40	0.40	0.40
Chromic oxide	0.40	0.40	0.50
Vitamin–micromineral premix[2]	0.30	0.30	0.30
Corn starch	1.600	0.850	68.10
Solka-floc[3]	—	—	4.00
Sucrose	—	—	20.00
MgO_2	—	—	0.10
K_2CO_3	—	—	0.40
Calculated composition			
CP (N × 6.25), %	17.50	17.50	—
ME, kcal/kg	3,487	3,456	3,770
Ca, %	0.53	0.68	0.56
Total P, %	0.44	0.57	0.28
Available P, %	0.13	0.23	0.23

[1]Two additional diets with inadequate available P and 2 additional diets with adequate available P were formulated and supplemented with phytase (OptiPhos 2000, Enzyvia LLC, Sheridan, IN) at the expense of cornstarch. Phytase was added to these diets at 0.013 and 0.026 to achieve levels of 250 and 500 phytase units, respectively.

[2]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A, 11,128 IU; vitamin D_3, 2,204 IU; vitamin E, 66 IU; vitamin K, 1.42 mg; thiamin, 0.24 mg; riboflavin, 6.58 mg; pyridoxine, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid, 23.5 mg; niacin, 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

[3]Source of synthetic fiber (Fiber Sales and Development Corp., Urbana, OH).

MATERIALS AND METHODS

Animals, Housing, and Experimental Design

The experimental protocol for this study was approved by the Institutional Animal Care and Use Committee at the University of Illinois. A total of 14 barrows (initial BW: 37.8 ± 4.7 kg) originating from the mating of line 337 boars to C 22 females (Pig Improvement Company, Hendersonville, TN) were allotted to a 7 × 7 replicated Latin square design with 7 periods and 7 diets. Pigs were surgically fitted with a T-shaped stainless-steel cannula in the distal ileum (Stein et al., 1998) and placed in individual pens in an environmentally controlled room (22°C). Pens (1.2 × 1.5 m) were equipped with a fully slatted T-bar floor, a feeder, and a nipple-type drinker. The study was conducted at the Swine Research Center of the University of Illinois.

Diets and Feeding

Three diets were formulated to contain an inadequate quantity of available P (0.13%; NRC, 1998), and each diet was supplemented with an *Escherichia coli* phytase (OptiPhos 2000, Enzyvia, Sheridan, IN) at 0, 250, or 500 phytase units (**FTU**)/kg of diet (Tables 1 and 2). One phytase unit was defined as the amount of enzyme required to release 1 μmol of iP per minute from sodium phytate at 37°C. Three additional diets were formulated to contain an adequate quantity of available P (0.23%; NRC, 1998) and were also supplemented with 0, 250, or 500 FTU/kg of phytase, respectively. A N-free diet based on cornstarch was prepared to measure the basal endogenous CP and AA flow in each pig. In all diets, 0.40% Cr_2O_3 was used as an indigestible marker. Representative samples of each diet were collected and stored at 4°C until analysis. The amount of daily feed allowance was calculated as 3 times the estimated daily ME requirement for maintenance (i.e., 106 kcal of ME/kg of $BW^{0.75}$; NRC, 1998), and this amount was divided into 2 equal meals that were fed at 0800 and 1700 h.

Data and Sample Collection

Ileal and total-tract digestibility data were obtained by collecting ileal and fecal samples from each pig. Each period lasted 7 d. The initial 5 d were an adaptation period to the diet, and ileal digesta samples were collected for 8 h on d 6 and 7. Ileal digesta were collected from the cannulated pigs by attaching 225-mL plastic bags to the uncapped cannula barrel using a cable tie. Filled bags were immediately replaced with a new bag, and the collected digesta were stored at −20°C to prevent bacterial degradation. The digesta collected during each 2-d period from each pig were mixed and comprised a single replicate. At the end of the experiment, the collected ileal samples were thawed, and representative subsamples of ileal digesta were lyophilized and finely ground for chemical analyses. Fecal samples were dried in a commercial oven (50°C), ground, and stored at −20°C until analysis.

Chemical Analyses

Samples of ingredients, diets, and ileal digesta were analyzed for DM by

Table 2. Analyzed composition of experimental diets (as-fed basis)

	Inadequate P,[1] FTU/kg			Adequate P,[1] FTU/kg			
Item	**0**	**250**	**500**	**0**	**250**	**500**	**N free**
DM, %	87.7	88.4	88.8	88.5	88.6	88.9	92.2
CP, %	19.5	17.4	17.0	16.8	16.7	15.9	0.64
GE, kcal/kg	4,071	4,037	4,033	4,163	4,180	4,187	3,867
Total P, %	0.49	0.48	0.45	0.60	0.62	0.54	0.40
Indispensable AA,[2] %							
Arg	1.22	1.19	1.10	1.15	1.08	1.00	0.02
His	0.48	0.48	0.45	0.46	0.44	0.41	0.01
Ile	0.82	0.81	0.76	0.74	0.73	0.70	0.03
Leu	1.62	1.63	1.52	1.58	1.48	1.43	0.05
Lys	1.03	1.01	0.94	0.96	0.92	0.86	0.03
Met	0.28	0.28	0.27	0.28	0.26	0.25	—
Phe	0.91	0.90	0.84	0.90	0.81	0.77	0.02
Thr	0.70	0.66	0.61	0.66	0.60	0.56	0.01
Trp	0.24	0.22	0.22	0.21	0.22	0.21	0.04
Val	0.90	0.89	0.83	0.80	0.80	0.76	0.02
Mean	0.82	0.81	0.75	0.77	0.73	0.70	0.02
Dispensable AA, %							
Ala	0.94	0.94	0.88	0.91	0.86	0.83	0.02
Asp	1.83	1.80	1.66	1.73	1.61	1.51	0.02
Cys	0.35	0.34	0.32	0.34	0.32	0.31	—
Glu	3.45	3.42	3.20	3.30	3.12	2.98	0.05
Gly	0.79	0.76	0.71	0.73	0.69	0.65	0.01
Pro	1.05	1.04	0.97	1.02	0.95	0.90	0.02
Ser	0.91	0.82	0.75	0.85	0.74	0.68	0.01
Tau	0.05	0.06	0.05	0.01	0.05	0.05	0.06
Tyr	0.63	0.62	0.58	0.64	0.57	0.54	0.02
Mean	1.11	1.09	1.01	1.06	0.99	0.94	0.02

[1]FTU = phytase units. OptiPhos 2000, Enzyvia LLC, Sheridan, Indiana.

[2]AA = amino acid.

oven drying at 135°C for 2 h (Method 930.15; AOAC International, 2007). Samples were also analyzed for CP (Method 990.03; AOAC International, 2007) using an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ). Gross energy was measured using an oxygen bomb calorimeter (Model 6300, Parr Instrument Co., Moline, IL). Diets and ileal digesta were analyzed for AA [Method 982.30 E (a, b, c); AOAC International, 2007], P by inductively coupled plasma spectroscopy (Method 985.01; AOAC International, 2007) after wet ash sample preparation (Method 975.03; AOAC International, 2007), and for chromium using inductive coupled plasma atomic emission spectrometric method (Method 990.08; AOAC International, 2007), after nitric acid–perchloric acid wet ash sample preparation (Method 968.088D; AOAC International, 2007).

Calculations and Statistical Analysis

The apparent ileal digestibility (**AID**) and the standardized ileal digestibility (**SID**) of CP and AA were calculated as described by Stein et al. (2007). The AID of P and the AID of energy were calculated using the same equation. The apparent total-tract digestibility (**ATTD**) of energy and the ATTD of P were calculated using the concentrations of energy, P, and Cr_2O_3 in the feed and fecal samples, respectively.

Data were analyzed using the MIXED procedure (SAS Institute Inc., Cary, NC) with pig as the experimental unit. Pig and period were the random effects, and diet was the fixed effect. The main effects of phytase supplementation, concentration of available P, and the interaction between phytase and available P were included in the model. The ESTIMATE statement of SAS was also used to determine linear and quadratic effects of increasing levels of phytase in the diet. In all analyses, a probability of $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Pigs remained healthy and readily consumed their diets throughout the experiment. Interactions between the effects of available P and phytase on all measured parameters were significant ($P < 0.05$).

P-Inadequate Diets

Supplementation of phytase in diets with inadequate concentration of available P linearly improved ($P < 0.05$) the AID of CP and all AA except Pro (Table 3). The mean AID of indispensable AA was 80.9, 83.6, and 85.6% for pigs that were fed diets containing 0, 250, or 500 FTU/kg of phytase, respectively. Among the indispensable AA, the increase in AID values at supplemental phytase of 250 FTU/kg ranged from 1.2 (Arg, 87.4 vs. 88.6%) to 3.7 (Thr, 70.4 vs. 74.1%) percentage units. When supplementing with 500 FTU/kg of phytase, the increase in AID ranged from 2.6 (Arg, 87.4 vs. 90.0%) to 6.6 (Thr, 70.4 vs. 77.0%) percentage units. Supplementation of phytase in diets with inadequate concentration of available P also improved (linear, $P < 0.05$) the SID of CP and all AA except Pro (Table 4). The mean SID of indispensable AA was 83.7% in diets without added phytase, and 87.2 and 88.9% in diets containing 250 and 500 FTU/kg of phytase, respectively. Among the indispensable AA, the increase in SID of AA in diets containing 250 FTU/kg of phytase ranged from 1.3 (Arg, 90.9 vs. 92.2%) to 4.1 (Thr, 75.7 vs. 79.8%) percentage units, whereas in diets containing 500 FTU/kg of phytase, the increase in SID of AA ranged from 2.9 (Arg, 90.9 vs. 93.8%) to 7.5 (Thr, 75.7 vs. 83.2%) percentage units. The primary basis of the antinutritional properties of phytate is its poly-anionic structure, which results in very high chelating capacity (Angel et al., 2002). Under acidic conditions, phytate can bind proteins and AA (O' Dell and de Borland, 1976; Knuckles et al., 1985). Selle et al. (2000) proposed that phytate-protein/AA complexes are inherent in plant ingredients and that phytate may also form complexes with intact protein or free AA after protein digestion in the gut. Singh and Krikorian (1982) also reported that phytate has the ability to inhibit the activity of proteolytic enzymes such as pepsin and trypsin under gastrointestinal conditions.

Increasing supplementation of phytase in diets with inadequate concentration of available P also improved (linear, $P < 0.05$) the AID and the ATTD of energy, as well as the ATTD of P (Table 5). The increase in AID of energy from phytase supplementation was 3.4 and 6.8 percentage units (71.6 vs. 75.0 and 78.4%) for 250 and 500 FTU/kg of supplemental phytase, respectively. The ATTD of energy increased by 0.8 and by 3.2 percentage units (85.4 vs. 86.2 and 88.6%) when diets were supplemented with 250 and 500 FTU/kg of phytase, respectively. The increase in ATTD of P was 12.9 and 27.6 percentage units (24.7 vs. 37.6 and 52.0%) when diets were supplemented with 250 and 500 FTU/kg of phytase, respectively. Phytate may also negatively affect starch (Plaami, 1997) and fat digestibility (Johnston et al., 2004), which can potentially reduce the energy value of plant feedstuffs. Kornegay (2001) suggested that phytate reduces starch digestibility by directly forming phosphate linkages between phytic acid and starch, combining with digestive enzymes required for starch digestion, or binding Ca, which is a catalyst for enzyme activities. It was also thought that phytate may reduce fat digestibility because of evidence that it directly binds lipids (Cosgrove, 1966) or forms insoluble metallic soaps in the gut lumen when Ca–phytate complexes with fatty acids (Ravindran and Bryden, 1999). It is firmly established that phytase hydrolyses phosphate bonds in phytate (Selle and Ravindran, 2008), which, theoretically, would release not only P but also Ca, protein, AA, starch, and fat that are bound to phytate and consequently improve the use of all these nutrients by pigs.

P-Adequate Diets

Increasing levels of phytase in diets with adequate concentration of available P increased (quadratic, $P < 0.05$) AID and SID of Arg, His, Trp, Val, and Glu but had no effects on the AID and SID of any other AA. The AID of energy also increased (quadratic, $P < 0.05$) from 78.5 to 81.2 and 78.4% when 0, 250, and 500 FTU/kg of phytase, respectively, were added to the diets. However, supplemental phytase had no effect on the ATTD of energy in diets with adequate available P. Similarly, the ATTD of P was not affected by adding phytase to these diets. These observations indicate that OptiPhos phytase improves the digestibility of P, CP, AA, and energy in growing pigs but only when diets are inadequate in available P. Previously, Fan et al. (2005) evaluated the effects of adding 500 FTU/kg of an *Aspergillus niger* phytase in growing pigs fed diets containing either inadequate (0.18%) or adequate (0.23%) concentrations of available P and observed similar results. In their study, the AID and ATTD of P and energy and the AID of CP were significantly improved only in pigs fed diets with inadequate concentration of available P. The same effect was observed for the AID and SID of Ile, Lys, and Thr but none for the rest of the AA. Mroz et al. (1994) also demonstrated that microbial phytase significantly improved the ATTD of CP and AA in diets that were deficient in available P. Likewise, Kerr et al. (2010) observed linear increases in ATTD of energy in finishing pigs fed diets with only 0.06% available P and supplemented with increasing levels of OptiPhos phytase, but none to variable effects were observed for other phytases. On the other hand, Liao et al. (2005) reported no effects of microbial phytase when supplemented to diets that were adequate in available P, which is also in agreement with our results. Yi et al. (1996) also observed that phytase supplementation improved N digestibility only in diets that were deficient in P (0.05 and 0.16% available P), but phytase did not improve the digestibility of N in the diet containing 0.32% available P. These observations are in agreement with results from this experiment, suggesting that the concentration of available P in diets may have an influence on the effects of phytase in improving the digestibility of protein, AA, and en-

Table 3. Apparent ileal digestibility (AID, %) of amino acids (AA) in diets with inadequate (0.13%) or adequate (0.23%) concentration of available P without or with supplemental phytase fed to growing pigs[1]

								P-value						
	Inadequate P,[2] FTU/kg			Adequate P,[2] FTU/kg							Inadequate		Adequate	
Item	0	250	500	0	250	500	SEM	P level	Phytase	P level × phytase	Linear	Quad.	Linear	Quad.
CP, %	73.4	73.6	79.3	76.9	80.5	79.1	1.40	<0.01	<0.01	0.02	<0.01	0.09	0.22	0.10
Indispensable AA, %														
Arg	87.4	88.6	90.0	90.0	91.2	89.3	0.66	<0.01	0.09	0.01	<0.01	0.93	0.46	0.02
His	81.9	85.5	87.0	86.0	88.6	86.2	0.85	<0.01	<0.01	<0.01	<0.01	0.24	0.90	0.01
Ile	81.0	83.1	85.7	84.2	86.2	84.7	0.82	<0.01	<0.01	0.01	<0.01	0.73	0.60	0.06
Leu	81.7	84.2	86.3	85.9	87.2	86.0	0.75	<0.01	<0.01	0.01	<0.01	0.81	0.97	0.14
Lys	82.3	84.1	86.9	85.3	86.9	85.5	0.84	0.02	<0.01	0.01	<0.01	0.65	0.84	0.10
Met	83.7	85.9	88.2	87.9	88.9	88.1	0.69	<0.01	<0.01	0.01	<0.01	0.92	0.81	0.28
Phe	81.2	84.2	85.9	85.7	86.6	84.9	0.71	<0.01	<0.01	0.01	<0.01	0.47	0.41	0.10
Thr	70.4	74.1	77.0	76.7	77.7	75.6	1.30	<0.01	0.06	0.01	<0.01	0.79	0.53	0.30
Trp	78.0	80.8	84.2	81.8	85.7	83.7	1.08	<0.01	<0.01	0.01	<0.01	0.76	0.17	0.01
Val	75.5	78.6	81.5	78.9	82.0	79.9	1.12	0.04	<0.01	0.03	<0.01	0.94	0.52	0.04
Mean	80.9	83.6	85.6	84.7	86.4	84.6	0.76	<0.01	<0.01	<0.01	<0.01	0.68	0.92	0.06
Dispensable AA, %														
Ala	74.8	77.3	80.9	79.7	80.9	80.4	1.37	0.01	0.03	0.08	<0.01	0.70	0.71	0.58
Asp	75.4	80.5	82.4	81.7	83.3	81.1	1.06	<0.01	<0.01	<0.01	<0.01	0.16	0.69	0.10
Cys	69.9	76.2	78.1	78.2	80.9	79.1	1.40	<0.01	<0.01	0.02	<0.01	0.14	0.59	0.13
Glu	78.5	84.6	85.5	84.7	87.1	84.6	1.06	<0.01	<0.01	<0.01	<0.01	0.02	0.93	0.03
Gly	60.0	63.0	68.4	66.9	71.2	67.2	2.82	0.01	0.13	0.09	0.01	0.68	0.92	0.13
Pro	70.7	57.3	72.0	69.5	70.7	72.3	6.24	0.26	0.17	0.21	0.83	0.01	0.68	0.97
Ser	78.3	80.1	81.7	81.9	82.6	80.3	1.02	0.06	0.44	0.04	0.02	0.94	0.28	0.22
Tyr	81.1	84.5	86.0	86.0	86.8	85.1	0.73	<0.01	<0.01	<0.01	<0.01	0.24	0.39	0.12

[1]Each value is the least squares means of 14 replicate pigs. Quad. = quadratic.

[2]FTU = phytase units. OptiPhos 2000, Enzyvia LLC, Sheridan, Indiana.

Table 4. Standardized ileal digestibility (SID, %) of amino acids (AA) in diets with inadequate (0.13%) or adequate (0.23%) concentration of available P without or with supplemental phytase fed to growing pigs[1]

								P-value						
	Inadequate P,[2] FTU/kg			Adequate P,[2] FTU/kg							Inadequate		Adequate	
Item	0	250	500	0	250	500	SEM	P level	Phytase	P level × phytase	Linear	Quad.	Linear	Quad.
CP, %	80.6	81.7	87.2	85.1	88.4	87.2	1.40	<0.01	<0.01	0.03	<0.01	0.16	0.24	0.13
Indispensable AA, %														
Arg	90.9	92.2	93.8	93.7	95.2	93.6	0.66	<0.01	0.02	0.01	<0.01	0.82	0.94	0.03
His	84.0	87.6	89.3	88.3	91.0	88.7	0.85	<0.01	<0.01	<0.01	<0.01	0.27	0.70	<0.01
Ile	83.4	85.5	88.4	86.8	88.9	87.6	0.82	<0.01	<0.01	0.01	<0.01	0.68	0.51	0.06
Leu	83.7	86.3	88.5	88.0	89.4	88.3	0.75	<0.01	<0.01	<0.01	<0.01	0.88	0.80	0.14
Lys	84.8	86.8	89.7	88.0	89.8	88.6	0.84	<0.01	<0.01	<0.01	<0.01	0.60	0.62	0.11
Met	85.2	87.4	89.7	89.3	90.5	89.8	0.69	<0.01	<0.01	0.01	<0.01	0.90	0.67	0.27
Phe	83.5	86.5	88.4	88.0	89.2	87.6	0.71	<0.01	<0.01	<0.01	<0.01	0.52	0.68	0.09
Thr	75.7	79.8	83.2	82.4	83.9	82.3	1.30	<0.01	<0.01	0.01	<0.01	0.82	0.98	0.28
Trp	81.6	84.7	88.1	85.9	89.6	87.8	1.08	<0.01	<0.01	0.01	<0.01	0.88	0.16	0.01
Val	79.3	82.5	85.6	83.2	86.3	84.4	1.12	0.01	<0.01	0.02	<0.01	0.99	0.42	0.05
Mean	83.7	87.2	88.9	88.6	89.7	88.2	0.73	<0.01	<0.01	<0.01	<0.01	0.27	0.70	0.10
Dispensable AA, %														
Ala	79.6	82.1	86.1	84.7	86.1	85.9	1.38	<0.01	0.01	0.09	<0.01	0.63	0.52	0.56
Asp	78.1	83.3	85.3	84.5	86.3	84.4	1.06	<0.01	<0.01	<0.01	<0.01	0.18	0.93	0.10
Cys	73.0	79.5	81.5	81.4	84.4	82.7	1.40	<0.01	<0.01	0.02	<0.01	0.15	0.48	0.12
Glu	80.5	86.7	87.7	86.9	89.3	87.0	1.06	<0.01	<0.01	<0.01	<0.01	0.02	0.93	0.03
Gly	76.1	79.9	86.5	84.5	89.9	87.1	2.82	<0.01	0.02	0.09	<0.01	0.62	0.92	0.13
Pro	116.1	103.5	121.8	116.7	121.4	126.0	6.24	0.04	0.04	0.13	0.37	<0.01	0.68	0.97
Ser	82.2	84.4	86.4	86.0	87.3	85.5	1.02	0.02	0.11	0.05	<0.01	0.93	0.28	0.22
Tyr	83.7	87.2	88.9	88.6	89.7	88.2	0.73	<0.01	<0.01	<0.01	<0.01	0.27	0.39	0.12

[1]Each value is the least squares means of 14 replicate pigs. Quad. = quadratic.

[2]FTU = phytase units. OptiPhos 2000, Enzyvia LLC, Sheridan, Indiana.

Table 5. Apparent ileal (AID, %) and total tract (ATTD, %) digestibility of energy and ATTD of P in diets with inadequate (0.13%) or adequate (0.23%) concentration of available P without or with supplemental phytase fed to growing pigs[1]

	Inadequate P,[2] FTU/kg			Adequate P,[2] FTU/kg				*P*-value						
										Inadequate		Adequate		
Item	0	250	500	0	250	500	SEM	P level	Phytase	P level × phytase	Linear	Quad.	Linear	Quad.
AID of energy, %	71.6	75.0	78.4	78.5	81.2	78.4	1.15	<0.01	<0.01	0.03	<0.01	0.99	0.95	0.03
ATTD of energy, %	85.4	86.1	88.6	88.3	88.2	88.0	0.44	<0.01	<0.01	<0.01	<0.01	0.12	0.60	0.87
ATTD of P, %	24.7	37.6	52.0	51.8	52.9	54.8	2.41	<0.01	<0.01	0.03	<0.01	0.78	0.36	0.87

[1]Each value is the least squares means of 14 replicate pigs. Quad. = quadratic.
[2]FTU = phytase units. OptiPhos 2000, Enzyvia LLC, Sheridan, Indiana.

ergy. In contrast, Brady et al. (2002) also fed growing-finishing pigs with barley-wheat-soybean meal-based diets containing inadequate (0.14%) or adequate (0.23%) concentrations of available P supplemented with 750 FTU/kg of *Peniophora lycii* phytase but did not observe any interactive effects between available P level and phytase in the diet on CP and energy digestibility. However, this same study showed an interaction on P use, where phytase only improved P digestibility and retention in pigs fed the adequate-P diet but had no effects in pigs fed the diet with inadequate P. Regardless of the available P level, Johnston et al. (2004) observed significant increases on AID and ATTD of P and Ca for finishing pigs fed phytase-supplemented diets; however, no differences were observed for CP or GE digestibility.

The lack of effect of OptiPhos phytase in diets with adequate concentrations of P may be explained by the buffering effect of Ca. Possibly, the different concentrations of monocalcium phosphate (**MCP**) in diets in this experiment created a difference in the pH of these diets, where diets that had greater concentration of MCP (adequate P) possessed a higher pH. This hypothesis is in agreement with Zhang and Nancollas (1994), who reported that in a closed system, dicalcium phosphate dissolution would lead to an increase in pH. This increase in pH is closely related to the formation of binary phytate–protein complexes (Adeola and Sands, 2003). Apparently, the optimum condition for the formation of these complexes is at low pH when proteins have a net positive charge and phytate has a net negative charge. Therefore, diets that were inadequate in P may not have changed gastric pH, which may have created a favorable environment (low pH) for phytate to form complexes with proteins. Once formed, the phytate–protein complexes were then hydrolyzed by microbial phytase, and therefore, digestibility of protein and AA was improved. Conversely, diets that were adequate in P likely increased gastric pH, which may have inhibited the formation of phytate-protein complexes. Because these complexes were not formed, microbial phytase did not have enough substrate to be efficiently used, and this could be the reason why we did not observe improvements in protein and AA digestibility when phytase was added to diets that were adequate in P. This may also be the case for other negatively charged nutrients that form complexes with phytate. Moreover, the possible changes in pH caused by the concentration of MCP in the adequate-P diets may have an effect on phytase activity. The phytase used in this experiment has a pH range from 2.5 to 3.5 for optimum activity (Rodriguez et al., 1999). If the pH in the stomach was increased above this range, phytase activity would be likely reduced. This may also explain the lack of improvement in protein, AA, and energy digestibility as a response to added microbial phytase to the adequate P diets.

IMPLICATIONS

Results from the present experiment indicate that OptiPhos phytase improves the digestibility of P, CP, AA, and energy when supplemented in corn–soybean meal diets that are deficient in P. The fact that the concentration of MCP in the diets may influence gastric pH and, therefore, inhibit the beneficial effects of microbial phytase on use of protein and AA needs further investigation. The inconsistency of the published data regarding the effects of phytase on protein and AA use may be a result of differences in the concentrations of available P among diets used in different experiments.

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