Effects of level of supplemental phytase on ileal digestibility of amino acids, calcium, and phosphorus in dehulled soybean meal for growing pigs.

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Effects of level of supplemental phytase on ileal digestibility of amino acids, calcium, and phosphorus in dehulled soybean meal for growing pigs[1,2,3,4]

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ABSTRACT: Ileally cannulated pigs were used to assess the effects of four dietary levels of microbial phytase (Natuphos) on the apparent and true digestibility of Ca, P, CP, and AA in dehulled soybean meal. Fourteen pigs (25 kg initial BW) were surgically fitted with T-cannulas at the terminal ileum and assigned to diets in a replicated 7 × 7 Latin square design. Following a 14-d recovery, four diets consisting of 30.5% soybean meal with 0, 500, 1,000, or 1,500 units of phytase/kg of diet were fed. Diets 5 (1.05% lysine, 0.90% Ca, and 0.75% P) and 6 (1.05% lysine, 0.90% Ca, and 0.75% P) contained 35.25% soybean meal and 27.0% soy protein concentrate, respectively. Diet 7 (0.37% lysine, 0.03% Ca, and 0.05% P) was a low-CP, casein-based diet used to estimate the nonspecific endogenous losses of Ca, P, CP, and AA in order to estimate the true digestibility of these nutrients. All diets contained cornstarch and dextrose and were fortified with vitamins and minerals. Chromic oxide was used as an indigestible indicator. The diets were fed daily at 9% of metabolic BW ($BW^{0.75}$). Apparent and true ileal digestibility of P increased quadratically ($P < 0.01$) and true digestibility of Ca increased linearly ($P < 0.07$) with increasing levels of phytase. Apparent digestibility of Ca was unaffected ($P = 0.15$) by phytase level. Apparent and true ileal digestibility of CP and most AA increased slightly with the addition of 500 units of phytase/kg of diet, but not at higher levels of phytase supplementation (in most cases, cubic effect, $P < 0.05$). Apparent and true ileal nutrient digestibility coefficients were unaffected by soybean meal source (Diet 1 vs Diet 5), except for arginine and Ca. The apparent and true digestibility coefficients for most of the AA tended ($P < 0.10$) to be lower in diets containing soy protein concentrate vs the common source of soybean meal used in Diet 5, but ileal digestibilities of Ca and P were unaffected ($P = 0.15$). In this study, supplemental microbial phytase did not improve the utilization of AA provided by soybean meal but was an effective means of improving Ca and P utilization by growing swine fed soybean meal-based diets.

Key Words: Amino Acids, Digestibility, Phosphorus, Phytase, Pigs, Soybean Meal

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Introduction

A large portion of the P in cereal grains and oilseed meals is in the form of phytic acid. For example, in soybean meal (**SBM**) approximately 60 to 70% of the P is in this form (Nelson et al., 1968). Phytate P is poorly utilized by nonruminants (Nelson, 1967; Cromwell, 1979) because of insufficient amounts of phytase in the intestinal tract. As a result, diets must be supplemented with highly available sources of inorganic P in order to meet the pig's P requirement. Also, the inefficient degradability of phytate results in excessive fecal excretion of P that can potentially pollute the environment.

Research has shown that phytase addition to grain-oilseed meal diets improves the apparent digestibility

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and relative bioavailability of dietary P (Lei et al., 1992; Jongbloed et al., 1992; Cromwell et al., 1993, 1995). Supplemental phytase has been shown to improve the apparent digestibility of CP and individual AA in diets containing a mixture of feedstuffs in some studies (Officer and Batterham, 1992; Mroz et al., 1994), but not in others (Nasi et al., 1995; Yi et al., 1996; Valaja et al., 1998). Less information is known about the effects of phytase on individual ingredients. In one study, Ketaren et al. (1993) found that supplementing a semipurified SBM diet with phytase did not affect the apparent fecal digestibility of CP, but they did not determine the digestibility of individual AA. Thus, there is no clear consensus regarding the effects of microbial phytase addition on protein or AA digestibility in grain-oilseed meal-based diets, and even fewer data regarding the efficacy of phytase on these measurements in individual ingredients, such as SBM.

The purpose of this research was to assess the effects of various levels of supplemental microbial phytase on the apparent and true ileal digestibilities of CP, AA, Ca, and P in diets containing SBM as the sole source of protein.

Materials and Methods

Animals and Facilities. Crossbred barrows (Hampshire♂ × Yorkshire-Landrace♀) initially averaging 25 kg BW were used in the study. They were individually penned in elevated pens (1.2 × 1.2 m) with mesh floors in an environmentally controlled room during the experiment. Each pen was equipped with a nipple waterer and a rubber feeder for hand-feeding. Room lights were illuminated for 10 to 12 h each day and were turned off during the remainder of each 24-h period. The study lasted 67 d.

Surgical Procedures. Fourteen pigs were surgically fitted with a simple T-cannula approximately 15 cm anterior to the ileocecal valve. The surgeries were conducted using protocols approved by the Institutional Animal Care and Use Committee of the University of Kentucky. Prior to surgery, pigs were deprived of feed and water for 24 and 12 h, respectively. Pigs were initially anesthetized with 2 mL of Telazol (tiletamine HCl, 50 mg/mL; zolazepam HCl, 50 mg/mL; Fort Dodge Animal Health, Fort Dodge, IA) administered i.m. Atropine (atropine sulfate; Fort Dodge Animal Health) was also injected (2.8 mL i.m.) before surgery to reduce intestinal motility and excessive salivation. A 5% halothane and oxygen combination was used to induce surgical anesthesia via a face mask. Anesthesia then was maintained with a 1% halothane and oxygen mixture in a closed-circuit system administered through nasal inhalation. Following surgery, pigs were given 0.30 mg of Buprenorphine (buprenorphine HCl; Reckitt and Colman Pharmaceuticals, Richmond, VA) every 12 h for 48 h to relieve pain.

Feeding and Sampling. The experiment was conducted in a replicated 7 × 7 Latin square design with pigs randomly allotted to treatments based on initial weight. All pigs were weighed at the start of each weekly period to determine the amount of feed allotted. The feeding level in any given time period was at a daily rate of 9% of the mean metabolic BW ($BW^{0.75}$) of all pigs within each replicate within that specific time period. Feed was placed in the feeders and mixed with a small amount of water (30 to 40% of the weight of feed) to form a thick gruel. The diets were fed twice daily at 0700 and 1700. The pigs averaged 33.3 and 69.9 kg at the initial and final collection periods, respectively.

The pigs were fed and housed in a common manner during the seven adaptation and collection periods. Each experimental period consisted of a 5-d adaptation period followed by a 2-d collection of digesta. Ileal digesta were collected hourly or more frequently if needed for a 12-h period, starting 1 h after the morning feeding. Small balloons were attached to the cannula to collect the digesta. The balloons were changed every hour (or more often if needed) and the samples were placed in an ice bath. At the end of each day's collection, the samples from each pig were pooled and mixed and a 200-mL sample was retained and frozen. Samples from each pig for the 2-d period were pooled, mixed, and freeze-dried.

Experimental Diets. Semipurified diets (Table 1) consisting mainly of sucrose and cornstarch with protein sources of dehulled SBM (two sources), soy protein concentrate, or casein were used in the study. For Diets 1 to 4, dehulled SBM from the University of Kentucky feed processing facility was used. These four diets were formulated to contain 0.95% lysine, 0.50% Ca, and 0.40% P. Phytase (Natuphos, BASF, Mount Olive, NJ) was added to supply 0, 500, 1,000, or 1,500 phytase units/kg to the four diets. Diet 5 contained another source of dehulled SBM (hereafter called "common SBM") and Diet 6 contained soy protein concentrate (**SPC**). These two diets were included as part of a larger regional project such that the results of this experiment could be compared with the results of studies from other stations. These two diets were formulated to contain 1.05% lysine, 0.90% Ca, and 0.75% P. Diet 7 was a low-protein diet (4.4% CP) containing casein that was also part of the regional study. This diet was calculated to contain 0.37% lysine, 0.03% Ca, and 0.05% P and did not have any supplemental Ca or P added. The purpose of this diet was to estimate the nonspecific (endogenous) losses of CP, AA, Ca, and P in order to estimate the corrected (i.e., "true") ileal digestibilities of these nutrients. Use of a low-protein, casein-based diet is a commonly accepted procedure for determining true digestibility of AA, as reviewed by Gabert et al. (2001).

The substitution of the soy products was at the expense of cornstarch, and sucrose was held constant at 20% in all diets. Cellulose and fat were included in the casein diet. All of the diets were fortified with adequate levels of vitamins and minerals to meet or exceed NRC (1998) standards, except for Ca and P in Diets 1 to 4

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

Protein source:	Dehulled SBM[a]					SPC[a]	Casein
Item Diet:	1	2	3	4	5	6	7
Ingredient, %							
Cornstarch	44.76	44.68	44.59	44.51	38.10	46.00	65.52
Sucrose	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Cellulose	—	—	—	—	—	—	5.00
Dehulled soybean meal	30.50	30.50	30.50	30.50	35.25	—	—
Soy protein concentrate	—	—	—	—	—	27.00	—
Food-grade casein	—	—	—	—	—	—	5.00
Corn oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Dicalcium phosphate	1.00	1.00	1.00	1.00	3.00	3.00	—
Ground limestone	0.54	0.54	0.54	0.54	0.45	0.45	—
Iodized salt	0.35	0.35	0.35	0.35	0.35	0.35	0.40
Potassium carbonate	—	—	—	—	—	0.35	1.09
Magnesium oxide	—	—	—	—	—	—	0.14
Trace mineral premix[b]	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin premix[c]	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Natuphos-600[d]	—	0.083	0.167	0.250	—	—	—
Calculated analysis							
CP, %	14.64	14.64	14.64	14.64	17.00	17.00	4.44
Lysine, %	0.95	0.95	0.95	0.95	1.05	1.05	0.37
Ca, %	0.50	0.50	0.50	0.50	0.90	0.90	0.03
P, %	0.40	0.40	0.40	0.40	0.75	0.75	0.05
Available P, %	0.24	0.24	0.24	0.24	0.61	0.61	0.05
ME, Mcal/kg	3.80	3.80	3.80	3.80	3.71	3.84	3.68

[a]SBM = soybean meal; SPC = soy protein concentrate.
[b]Provides per kilogram of diet: Fe (ferrous sulfate), 90 mg; Mn (manganous oxide), 5 mg; Cu (copper sulfate), 8 mg; I (potassium iodate), 0.20 mg; Se (sodium selenite), 0.21 mg; Zn (zinc sulfate), 90 mg.
[c]Provides per kilogram of diet: vitamin A, 2,000 IU; vitamin D_3, 300 IU; vitamin E, 20 IU; vitamin K (menadione sodium bisulfate), 4 mg; niacin, 15 mg; riboflavin, 4 mg; pantothenic acid, 12 mg; vitamin B_{12}, 15 μg; pyridoxine, 2 mg; D-biotin, 0.1 mg; folic acid, 0.5 mg; choline, 0.60 g.
[d]Contains 600 phytase units/g; the three levels of the supplement provided 500, 1,000, and 1,500 phytase units/kg of diet.

and Diet 7. The lower levels of Ca and P in Diets 1 to 4 were selected because of the anticipated increase in bioavailability of both minerals resulting from phytase inclusion in the diets. Dicalcium phosphate (24% Ca, 18.5% P), ground calcitic limestone (38% Ca), and iodized salt were used as supplemental sources of Ca, P, Na, and Cl. In addition, potassium carbonate (55% K) and magnesium oxide (15% Mg) were included in the casein diet as supplemental sources of K and Mg, respectively. Chromic oxide (Cr_2O_3) was included as an indigestible indicator in all diets at 0.50% to determine the apparent digestibility of nutrients using the indirect ratio method. All diets were fed in meal form.

Chemical Analysis. All digesta and diet samples were analyzed for CP (N × 6.25), AA, Ca, P, and Cr by the University of Missouri Experiment Station Chemical Laboratory (Columbia, MO) using standard methods (AOAC, 1995). Ileal digesta were lyophilized and ground before analysis. Analysis of Cr and Ca in the diets and ileal digesta samples was by atomic absorption spectrophotometry after ashing. The concentration of P in digesta and diet samples was determined by inductively coupled plasma atomic emission spectroscopy. Amino acids were analyzed using ion-exchange chromatography after acid hydrolysis. Methionine and cystine were oxidized to methionine sulfone and cysteic acid by treatment with performic acid before hydrolysis. Tryptophan was analyzed with ion-exchange chromatography after alkaline hydrolysis. The analyzed composition of the protein sources and the experimental diets is shown in Tables 2 and 3.

Calculation of Digestibility Coefficients. Apparent ileal digestibilities were calculated for each pig using the Cr concentration in feed and digesta as the indigestible marker according to the following equation: AID = 100 – [(Nd/Nf) × (Crf/Crd) × 100], where AID is the apparent ileal digestibility of the nutrient (%), Nd is the nutrient content in the ileal digesta sample, Nf is the nutrient content in the feed, Crf is the Cr content in the feed, and Crd is the Cr content in the ileal digesta sample.

The nonspecific (i.e., endogenous) losses of nutrients were calculated according to the following equation (Moughan et al., 1992): EAL = [Nd × (Crf/Crd)], where EAL is the endogenous loss of the nutrient (mg/kg feed), Nd is the concentration of the nutrient in ileal digesta, Crf is the Cr content in feed, and Crd is the Cr content in ileal digesta. To calculate the true ileal digestibility coefficients, values for apparent ileal digestibility and the endogenous losses were added according to the following equation: TID = AID + (EAL/Nf) × 100, where TID (%) represents the true ileal digestibility coefficient of the nutrient, AID (%) is the apparent ileal digestible

Table 2. Analyzed nutrient composition of soy products and casein (as-fed basis)

Item, %	Dehulled soybean meal in Diets 1 to 4	Dehulled soybean meal in Diet 5	Soy protein concentrate in Diet 6	Casein[a] in Diet 7
CP	48.8	48.2	62.8	92.5
Essential amino acid				
Arginine	3.68	3.56	4.68	3.2
Histidine	1.34	1.29	1.74	2.8
Isoleucine	2.20	2.16	2.94	4.4
Leucine	3.80	3.67	4.97	8.6
Lysine	3.17	2.97	4.08	7.4
Methionine	0.70	0.65	0.90	2.8
Phenylalanine	2.54	2.43	3.24	4.6
Threonine	1.93	1.83	2.55	3.8
Tryptophan	0.69	0.61	0.83	1.8
Valine	2.39	2.31	3.15	6.0
Mineral				
Ca	0.39	0.20	0.36	1.0
P	0.70	0.57	0.75	1.3

[a]Calculated from analyzed diet values.

coefficient, EAL is the endogenous loss (mg/kg feed) of that nutrient after feeding the low-protein casein diet, and Nf is the dietary content of the nutrient (mg/kg feed).

A mean value for endogenous losses estimated by feeding the casein-based diet was used in the calculation of true nutrient digestibility coefficients. The mean endogenous loss value across period (i.e., BW) and animal for each nutrient was used. The mean endogenous value was based on the finding of Stein et al. (1999) that the composition of endogenous protein was unaffected by stage of growth, and that total nutrient intake has a greater effect on endogenous protein and AA losses than either BW or physiological state.

Statistical Analysis. The apparent ileal digestibilities of CP, AA, Ca, and P were statistically analyzed using appropriate statistical methods for a replicated Latin square design (Steele and Torrie, 1980) and the GLM

Table 3. Analyzed nutrient composition of diets (as-fed basis)

Item, %	Dehulled soybean meal Diets 1 to 4[a]	Dehulled soybean meal Diet 5	Soy protein concentrate Diet 6	Casein Diet 7
CP	13.2	15.2	16.0	4.6
Essential amino acid				
Arginine	0.98	1.01	1.20	0.16
Histidine	0.36	0.37	0.46	0.14
Isoleucine	0.58	0.59	0.76	0.22
Leucine	1.02	1.08	1.33	0.43
Lysine	0.84	0.87	1.09	0.37
Methionine	0.20	0.19	0.25	0.14
Phenylalanine	0.67	0.70	0.85	0.23
Threonine	0.52	0.54	0.66	0.19
Tryptophan	0.19	0.25	0.25	0.09
Valine	0.63	0.65	0.83	0.30
Nonessential amino acid				
Alanine	0.59	0.60	0.76	0.14
Aspartic acid	1.52	1.56	1.91	0.32
Cystine	0.21	0.20	0.26	0.02
Glutamic acid	2.53	2.61	3.18	1.02
Glycine	0.57	0.58	0.72	0.09
Proline	0.65	0.66	0.82	0.44
Serine	0.63	0.64	0.78	0.24
Tyrosine	0.42	0.44	0.51	0.19
Mineral				
Ca	0.60	0.84	0.91	0.05
P	0.42	0.71	0.75	0.06

[a]Each value represents the mean of Diets 1 to 4.

procedure of SAS (SAS Inst. Inc., Cary, NC). The model included the effects of period (df = 6), replicate (i.e., square; df = 1), pigs nested within replicate (df = 12), treatment (df = 6), and replicate × treatment interaction (df = 6). The residual mean square (df = 66) was used as the error term to test all effects. Treatment effects were partitioned into the following nonorthogonal contrasts: linear, quadratic, and cubic effects of phytase in Diets 1 to 4, Diet 1 vs Diet 5, Diet 5 vs Diet 6, and the mean of Diets 1 to 6 vs Diet 7. The true digestibilities of the same nutrients were analyzed similarly except that Diet 7 was not included in the analysis because those data were used to calculate the true digestibility coefficients of the nutrients in the other six diets. In all cases, the individually penned pig was considered as the experimental unit.

Results

Composition of Protein Sources and Diets. The concentrations of CP, Ca, P, and essential AA in the two sources of dehulled SBM, SPC, and casein are given in Table 2. The concentrations of nutrients in casein were calculated from the level of casein in the casein diet and the AA analysis of the casein diet. The AA concentrations of the two SBM sources were not greatly different from those listed by NRC (1998). The experimental SBM used in Diets 1 to 4 was slightly higher in lysine (3.17 vs 2.97%) and the other essential AA, compared with the common source of SBM used in Diet 5. The Ca and P concentrations also were higher in the experimental vs common SBM. The levels of CP, essential AA, and P in the SPC were slightly less than those listed by NRC (1998), whereas the Ca level in SPC was similar to NRC (1998). The CP and most of the essential AA in the casein were close to the values listed by NRC (1998); however, Ca and P levels were higher than NRC (1.0 vs 0.83% Ca and 1.3 vs 1.01% P, respectively).

The analyzed concentrations of CP, essential and nonessential AA, Ca, and P in the seven diets are given in Table 3. The assays for Diets 1, 2, 3, and 4 were similar, so the mean analyses of the four diets (used in the calculations of nutrient digestibility) are presented. Most of the analyzed values differed slightly from the calculated values (Table 1) but were within a reasonable range. Lysine was an exception in that the analyzed levels in the experimental SBM were approximately 0.1 percentage point lower than the calculated level, and the difference in analyzed vs calculated lysine in the common SBM was even greater. Nevertheless, the analyzed lysine levels in the SBM diets were sufficient to meet the lysine requirement of pigs possessing a medium lean growth rate (NRC, 1998).

Apparent and True Ileal Digestibility of Amino Acids. Inclusion of phytase in the diet had very little effect on the apparent ileal digestibility of the AA in the experimental SBM (Table 4). There were small numerical increases in apparent ileal digestibility of all of the AA in pigs fed the lowest level of supplemental phytase (500 units/kg), but the improvements were not maintained at the two higher levels of dietary phytase additions (1,000 and 1,500 units/kg). In fact, the apparent ileal digestibilities of most of the AA were no better at the two higher levels of phytase supplementation than in the unsupplemented controls. In some instances, the AA digestibilities at the two higher levels of phytase were numerically less than in the controls. Such was the case for lysine; the apparent ileal digestibility of lysine increased from 89.9% in the control diet to 90.7% when 500 phytase units were added/kg of diet, then decreased to 88.8 and 88.7% when 1,000 and 1,500 phytase units/kg, respectively, were added. In general, this cubic response pattern was relatively consistent for all of the AA, with the trend being significant at $P < 0.05$ for lysine, phenylalanine, tyrosine, and serine and at $P < 0.01$ for tryptophan. A similar cubic response in apparent ileal digestibility from phytase addition was noted for CP ($P < 0.05$).

The apparent ileal digestibility coefficients for CP and AA were not different ($P = 0.15$) for pigs fed the experimental and common sources of SBM without added phytase (Diet 1 vs 5). Apparent ileal digestibilities of methionine, threonine, aspartic acid, and glutamic acid were lower ($P < 0.05$) in pigs fed SPC than in pigs fed the common source of SBM (Diet 5 vs 6). The apparent digestibility of most AA in pigs fed the low-protein casein diet differed ($P < 0.05$) from those of the other diets, but not in a consistent pattern; some of the digestibility coefficients were higher, whereas others were lower. The apparent digestibility values are probably underestimated due to low dietary levels of AA (Gabbert et al., 2001). In some instances, such as for glycine, the low apparent digestibility is due to the high levels of endogenous glycine commonly excreted by pigs.

The endogenous losses of CP and the AA as determined from pigs fed the casein diet are shown in Table 5. These values, expressed as milligrams per kilogram of feed intake, are in close agreement with values reported by Fuller and Cadenhead (1991) and Furuya and Kaji (1992). These losses were used to calculate the true digestibilities of CP and AA at the terminal ileum, shown in Table 6. Although complete absorption of all AA from casein probably does not occur, this method of estimating endogenous AA and true digestibilities of AA is commonly accepted (Gabert et al., 2001).

As expected, the true ileal digestibility coefficients for the various AA (Table 6) were greater than the apparent digestibility coefficients (Table 4); however, both followed similar patterns with respect to the effects of level of supplemental phytase and protein source.

Apparent and True Ileal Digestibility of Calcium and Phosphorus. The apparent and true ileal digestibility of P responded in a positive manner to the various levels of supplemental phytase (Tables 4 and 6). Apparent digestibility of P increased quadratically ($P < 0.01$) with increasing level of phytase, with the majority of the

Table 4. Apparent ileal digestibility of crude protein, amino acids, Ca, and P in experimental diets[a]

Item	Diet:	1	2	3	4	5	6	7	
	Protein source:	SBM	SBM	SBM	SBM	SBM	SPC	Casein	
	Phytase, units/kg:	0	500	1,000	1,500	0	0	0	SE
		%							
Essential amino acid									
Arginine[b]		93.2	93.7	93.1	93.1	92.8	92.3	81.6	0.53
Histidine[b]		89.4	89.8	88.6	88.9	89.2	87.8	90.9	0.51
Isoleucine[b]		87.9	88.8	87.6	87.9	88.0	87.4	90.3	0.49
Leucine[b]		86.4	87.6	86.1	86.6	86.9	86.1	90.0	0.55
Lysine[bc]		89.9	90.7	88.8	88.7	89.6	88.8	92.4	0.59
Methionine[bd]		90.2	90.6	89.0	89.4	89.4	87.6	95.2	0.45
Phenylalanine[bc]		82.5	83.4	80.9	82.2	82.4	82.0	79.1	0.83
Threonine[be]		80.5	81.3	78.7	79.8	81.8	79.2	76.0	0.91
Tryptophan[bf]		89.3	90.9	87.7	88.9	89.7	89.1	85.8	0.76
Valine[b]		85.8	86.6	85.1	85.5	86.5	85.3	89.7	0.60
Nonessential amino acid									
Alanine[b]		84.0	84.6	82.4	83.4	84.8	83.2	75.3	0.95
Aspartic acid[bd]		87.4	88.1	86.4	87.1	86.9	83.5	84.4	0.63
Cystine[b]		82.2	82.7	79.0	80.7	82.4	78.7	23.0	2.19
Glutamic acid[bd]		91.5	92.1	90.5	91.3	90.8	89.0	93.3	0.54
Glycine[b]		77.5	79.4	75.7	76.7	81.1	76.3	11.9	3.70
Proline[b]		83.8	84.9	82.7	84.1	84.9	82.1	70.4	1.72
Serine[c]		86.0	87.0	85.3	86.0	85.8	84.6	85.1	0.59
Tyrosine[c]		85.6	86.8	84.8	85.7	86.5	86.0	86.8	0.62
CP[bc]		82.5	83.4	80.9	82.2	82.4	82.0	79.0	0.83
Mineral									
Ca[b]		63.5	67.5	68.9	69.2	57.7	57.2	30.2	3.09
P[bg]		49.8	64.3	66.9	69.8	53.8	54.8	74.7	1.66

[a]Means based on 14 observations per treatment. SBM = soybean meal; SPC = soy protein concentrate. Diets 5 and 6 were included as part of a larger regional study.
[b]Diet 7 vs mean of others ($P < 0.01$).
[c]Cubic effect of phytase level ($P < 0.05$).
[d]Diet 5 vs Diet 6 ($P < 0.01$).
[e]Diet 5 vs Diet 6 ($P < 0.05$).
[f]Cubic effect of phytase level ($P < 0.01$).
[g]Quadratic effect of phytase level ($P < 0.01$).

improvement occurring with the first increment of added phytase and lesser increases with further additions of phytase. True ileal digestibility coefficients for P followed a similar trend (quadratic, $P < 0.01$). There were no differences ($P = 0.15$) in P digestibility with respect to the two SBM sources or between SBM and SPC.

Phytase supplementation tended to improve the apparent digestibility of Ca from 63.5% in the basal diet to 69.2% at the highest level of phytase addition, but not significantly ($P = 0.15$). True digestibility of Ca, however, increased linearly ($P < 0.07$) from 69.3% in the basal to 75.0% at the highest level of phytase supplementation. Additionally, pigs fed the common SBM source had lower ($P < 0.05$) true digestibility coefficients of Ca than those fed the experimental SBM. True Ca digestibilities were similar for pigs fed common SBM and SPC diets.

Discussion

The purpose of this research was to assess the effects of various levels of supplemental microbial phytase on the ileal digestibility of CP, AA, Ca, and P in diets containing SBM as the sole source of protein. Approximately 61% of the P in SBM is in the form of phytate (Nelson et al., 1968). Mroz and Jongbloed (1998) proposed that the presence of phytate in phytate-rich diets interferes with optimal AA utilization from intact protein by 1) formation of indigestible protein-phytate complexes, 2) inhibition of digestive enzymes, and 3) depressed absorption of nutrients from the small intestine. In vitro studies have shown that phytate inhibits many proteolytic enzymes because of the formation of protein-phytate complexes (Caldwell, 1992). Because pigs have very little active phytase in the gut, they must either be fed ingredients containing endogenous phytase (e.g., wheat or wheat by-products) or be fed a supplemental source of the enzyme in order to utilize the P in high-phytate diets.

The results of this experiment indicate that supplemental phytase has very little, if any, effect on the apparent or true digestibility of CP in dehulled SBM. Our results agree with those reported by Nasi et al. (1995), who found only a 0.8 percentage unit improvement in the apparent total tract digestibility of CP when

pigs were fed a barley-rapeseed meal-based diet supplemented with 1,000 units of phytase. Yi et al. (1996) investigated the effectiveness of phytase supplementation in young pigs fed a semipurified diet consisting of soybean meal as the sole protein source and also found that phytase supplementation, ranging from 0 to 1,400 units/kg of diet, resulted in a small and variable change in apparent total tract digestibility of CP. Ketaren et al. (1993) observed that the fecal digestibility of CP was unaffected by supplementing a semipurified SBM diet with 1,000 units of microbial phytase/kg of diet. However, our results are in contrast to those of Mroz et al. (1994), who reported a 2.3% improvement in digestibility of CP when pigs were fed a corn-tapioca-SBM-based diet supplemented with 800 units of microbial phytase. Others researchers (Ketaren et al. 1993; Mroz et al., 1994) have found that protein deposition and(or) protein retention were increased in pigs fed diets supplemented with microbial phytase compared with an unsupplemented basal diet.

In our study, there was a slight numerical improvement in apparent and true ileal digestibility of most of the AA when 500 units of phytase was added per kilogram of diet. This improvement was 0.8 of a percentage unit when averaged across the 10 essential AA. Further additions of phytase (1,000 or 1,500 phytase units/kg of diet) failed to maintain the slight improvement in AA digestibility. In most instances, the higher levels of dietary phytase resulted in digestibility coefficients that were no better, or even slightly reduced, compared with those of the controls. Our results with dehulled SBM are not consistent with other researchers who have evaluated the effects of phytase addition to combinations of protein-supplying ingredients in diets. For example, in three studies with growing-finishing pigs, Officer and Batterham (1992) and Mroz et al. (1994) found that apparent ileal digestibilities of lysine, methionine, cystine, and threonine were improved by 5.1, 3.0, 5.4, and 4.4 percentage units, respectively, when 800 to 1,000 units of microbial phytase was added to a mixed ingredient diet. Others researchers (Radcliffe et al., 1999; Zhang and Kornegay, 1999) have shown that the apparent ileal digestibility of selected AA linearly increased when 250 or 500 units of microbial phytase/kg of diet was added to corn-SBM based diets. Perhaps our results differ because we evaluated a single protein source (dehulled SBM) in a semipurified diet whereas others used diets with cereal grains and other protein sources.

Table 5. Endogenous losses of crude protein, amino acids, Ca, and P in growing pigs fed the casein diet[ab]

Item	Endogenous loss, mg/kg of feed	SE
Essential amino acid		
Arginine	294	20.7
Histidine	127	9.2
Isoleucine	223	15.8
Leucine	431	31.8
Lysine	281	22.7
Methionine	68	5.3
Phenylalanine	482	32.2
Threonine	457	30.2
Tryptophan	100	7.7
Valine	320	23.1
Nonessential amino acid		
Alanine	345	25.5
Aspartic acid	498	35.2
Cystine	154	10.7
Glutamic acid	689	47.3
Glycine	793	84.5
Proline	1,303	190
Serine	359	21.8
Tyrosine	264	20.1
CP	9,694	650
Mineral		
Ca	349	30.3
P	177	20.8

[a]Means are based on 14 observations.
[b]These endogenous losses are based on the assumption that at these low dietary intakes of CP, Ca, and P in pigs fed the casein diet, 100% of the amino acids, Ca, and P are absorbed.

The apparent ileal digestibility values for all essential AA (except phenylalanine) in the dehulled SBM in our study were approximately 3 percentage units or more higher than those listed by NRC (1998) or than mean values for the AA in SBM in a review by Southern (1991). When adjustments were made for endogenous secretions of AA, the true digestibility coefficients for AA in dehulled SBM were approximately 4 percentage units greater than those listed by NRC (1998) or mean values for SBM compiled by Southern (1991). However, our apparent and true digestibility coefficients were within the upper range of values cited by Southern (1991). In contrast, the apparent and true digestibility AA coefficients for SPC in our study averaged 5 to 6 percentage units lower than those listed by NRC (1998).

A number of studies have clearly shown that microbial phytase additions to grain-oilseed meal-based diets markedly improves the digestibility and relative bioavailability of dietary P and reduces the excretion of P via manure into the environment. Cromwell et al. (1993) reported that 1,000 units of phytase/kg of diet improved the bioavailability of P from 15% in an unsupplemented corn-SBM diet to 45% in a similar diet containing phytase. Previous studies from our laboratory (Pierce et al., 1994) demonstrated that phytase supplementation reduces P excretion by 30 to 40%, which is in close agreement with studies reviewed by Kornegay et al. (1998). Using purified diets similar to those in our study, Ketaren et al. (1993) found that supplementing a semipurified, SBM diet with 1,000 units of microbial phytase/kg of diet improved apparent digestibility of P from 42 to 69%. Similar improvements have been reported by others (Lei et al., 1992; Jongbloed et al., 1992; Cromwell et al., 1995). Inclusion of phytase in our diets increased the true digestibility of Ca and P in SBM, with most of the improvement resulting from the first increment (500 phytase units/kg) of supplemental phytase.

Table 6. Effects of phytase level on true ileal digestibility of crude protein, amino acids, Ca, and P in soybean meal[a]

Item	Diet:	1	2	3	4	5	6	SE
	Protein source:	SBM	SBM	SBM	SBM	SBM	SPC	
	Phytase, units/kg:	0	500	1,000	1,500	0	0	
		%						
Essential amino acid								
Arginine[b]		96.2	96.7	96.1	96.1	95.3	94.7	0.28
Histidine[c]		93.0	93.4	92.1	92.4	92.2	90.6	0.50
Isoleucine		91.8	92.7	91.4	91.7	91.3	90.3	0.46
Leucine		90.6	91.8	90.3	90.8	90.3	89.2	0.54
Lysine[d]		93.2	94.0	92.1	93.0	92.4	91.3	0.60
Methionine[ef]		93.7	94.0	92.5	92.9	92.6	90.4	0.47
Phenylalanine[d]		89.7	90.6	88.0	89.4	88.3	87.8	0.71
Threonine[cd]		89.3	90.1	87.5	88.6	89.3	86.2	0.80
Tryptophan[g]		94.5	96.1	92.9	94.2	94.4	93.4	0.76
Valine		90.9	91.7	90.2	90.6	90.7	89.1	0.58
Nonessential amino acid								
Alanine		89.9	90.5	88.3	89.3	89.7	87.7	0.73
Aspartic acid[f]		90.6	91.4	89.7	90.4	89.7	86.0	0.54
Cystine[df]		89.5	90.0	86.4	88.0	89.1	84.7	0.87
Glutamic acid[c]		94.2	94.8	93.2	94.1	93.1	91.0	0.55
Glycine[f]		91.4	93.3	89.5	90.5	92.9	87.7	1.23
Proline[df]		104.0	105.1	102.8	104.3	102.3	98.8	0.75
Serine[d]		91.7	92.8	91.0	91.8	91.0	89.4	0.54
Tyrosine[d]		91.9	93.1	91.1	92.0	91.7	91.1	0.58
CP[d]		89.8	90.8	88.2	89.6	88.7	87.9	0.71
Mineral								
Ca[be]		69.3	73.3	74.8	75.0	61.6	61.0	2.26
P[h]		54.0	68.5	71.1	74.0	56.2	57.2	1.48

[a]Means based on 14 observations per treatment. SBM = soybean meal; SPC = soy protein concentrate.
[b]Diet 1 vs Diet 5 ($P < 0.05$).
[c]Diet 5 vs Diet 6 ($P < 0.05$).
[d]Cubic effect of phytase level ($P < 0.05$).
[e]Linear effect of phytase level ($P < 0.07$).
[f]Diet 5 vs Diet 6 ($P < 0.01$).
[g]Cubic effect of phytase level ($P < 0.01$).
[h]Quadratic effect of phytase level ($P < 0.01$).

In summary, the results of this research indicate that phytase addition to a semipurified diet in which all of the AA are provided by dehulled SBM improves the apparent and true digestibility of P and the true digestibility of Ca in the SBM but does not improve the apparent or true ileal digestibility of the AA in SBM. The failure of phytase to improve AA digestibility in this protein source is not in agreement with general assumptions in the feed industry based on research with grain-SBM and other diets containing a mixture of feed ingredients. Further research should be conducted to more clearly delineate the efficacy of microbial phytase on AA utilization in diets for swine.

Implications

The results of this study with ileally cannulated pigs clearly demonstrate that microbial phytase addition to semipurified diets containing dehulled soybean meal as the sole source of amino acids improves the digestibility of calcium and phosphorus but does not consistently improve the digestibility of amino acids in soybean meal. The improved utilization of soybean meal phosphorus means that less phosphorus is excreted into the environment. Additional definitive research is needed to more clearly establish whether the digestibilities of amino acids in various protein sources are uniformly affected by microbial phytase supplementation.

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