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Effect of Microbial Phytase on Performance, Nutrient Absorption and Excretion in Weaned Pigs and Apparent Ileal Nutrient Digestibility in Growing Pigs

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ABSTRACT : Two experiments were conducted to evaluate the efficacy of *Trichoderma reesei* derived phytase for pigs fed diets with fixed calcium to total phosphorus ratios (1.5:1). In Exp. 1, 280 weaned pigs (initial BW of 10.32±1.94 kg) were allocated to one of five dietary treatments on the basis of weight and gender in a randomized complete block design. Treatments were the low phosphorus (0.6% Ca, 0.4% total P and 0.23% available P) diets supplemented with 0, 250, 1,000, or 2,000 FTU phytase/kg of diet and a positive control diet (PC; 0.85% Ca, 0.58% total P and 0.37% available P). The treatments were applied to seven pens with eight pigs per pen, half male and half female. In Exp. 2, six barrows fitted with ileal T-cannula (initial BW = 35.1±1.6 kg) were assigned to three dietary treatments with a double 3×3 Latin square design. The dietary treatments were the low-phosphorus diet (0.53% Ca, 0.34% total P and 0.14% available P), the low phosphorus diet plus 1,000 FTU phytase/kg and a positive control diet (0.77% Ca, 0.50% total P and 0.30% available P). In Exp. 1, there were linear increases ($p<0.01$) in weight gain, phosphorus absorption, bone strength, calcium and phosphorus content of fat-free dried bone and plasma phosphorus concentrations with increasing dose rate of phytase. The performance of pigs fed the diets with 250, 1,000, or 2,000 FTU of phytase/kg did not differ from pigs fed the PC diet. Pigs fed diets with 1,000 or 2,000 FTU of phytase/kg did not differ from pigs fed the PC diet in bone characteristics. The apparent digestibility of dry matter, crude protein, ash and energy was not affected by dietary treatment. However, pigs fed the PC diet excreted more fecal phosphorus (g/d, $p<0.01$) and fecal phosphorus per BW gain (g/kg) than pigs fed the diets with phytase. Phytase linearly decreased ($p<0.01$) fecal phosphorus excreted per BW gain (g/kg), plasma calcium concentration as well as plasma and bone alkaline phosphatase activity. In Exp. 2, phytase supplementation in the low-P diet increased ($p<0.05$) the apparent ileal digestibility (AID) of Ca, P, leucine, lysine, phenylalanine, alanine and cysteine, tended to AID of crude protein, isoleucine, threonine, asparagine and serine. In conclusion, the novel phytase originated from *Trichoderma reesei* is effective in releasing Ca, P, and amino acids from corn soy based diet for pigs. (**Key Words :** Novel Phytase, Performance, Metacarpal Bone Characteristics, Apparent Ileal Digestibility, Pigs)

INTRODUCTION

The global pig population consumes approximately seven million tons of phosphorus as dicalcium phosphate per year (Selle and Ravindran, 2008) and the global reserves of rock phosphate are not renewable (Abelson, 1999). The combination of dietary surpluses of inorganic phosphorus coupled with poor utilization of phytate-phosphorus contributes to excessive excretion of phosphorus in swine feces (Selle and Ravindran, 2008).

It is well proven that supplementation of corn-soybean meal diets deficient in available phosphorus with phytase increases phosphorus absorption and decreases phosphorus excretion by swine from weaning to finishing without depressing pig performance (Liu et al., 1998; Jendza et al., 2005). Adding 1,000 FTU/kg phytase to corn-soybean meal diets containing no added inorganic phosphorus reduced the daily excretion of water-soluble phosphorus in starter, grower and finisher pigs by 42, 34 and 30%, respectively (Jendza and Adeola, 2009). The dose-response relationship of phytase fits an exponential curve with optimal efficacy up to about 500 FTU/kg of diet (Jendza et al., 2006) with

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further improvement in performance up to 12,500 FTU/kg of diet (Veum et al., 2006). However, an inclusion rate of 500 FTU/kg is commonly recommended for pigs but there are indications that this may be conservative and higher dose rates are justified (Selle and Ravindran, 2008). However, there is limited information on the effect of high dose rate of phytase on performance and nutrient digestion by pigs.

There are many phytases on the market. The traditional phytases are produced from Fungal which can release 0.1% total P for pigs based on DCP (Selle and Ravindran, 2008). These phytases are commonly used in pig feed for many years. The second generation phytase is derived from microbial and is more effective than fungal phytase. At the typical dose rate of 500 FTU/kg feed, this phytase can release at least 20% more P than traditional phytase. With advances in science and discovery, a new phytase is derived from *Trichoderma Reesei*. While the microbial phytase has been assessed in poultry (unpublished), the data on its efficiacy for pigs is urgently required before recommended to the pig industry.

The objective of this study was to determine the effect of supplementing corn-soybean meal diets with a novel microbial phytase in a fixed Ca:P ratios on performance, nutrient absorption and excretion, metacarpal bone characteristics and plasma indices of weaned pigs and the apparent ileal digestibility of nutrients for growing pigs.

MATERIALS AND METHODS

Phytase source

The phytase used in this experiment was a novel microbial phytase obtained from *Trichoderma Reesei* (Genencor Bio-products Co., Ltd., Wuxi, China) with an activity of 10,000 FTU/g. This *Trichoderma Reesei* phytase has a pH range of 2.5-5.5 and initiates dephosphorylation of phytate (myoinositol hexakisphosphate).

Before the experiment, the liquid phytase product was sprayed on wheat flour carrier with a target activity of 5,000 FTU/g. The phytase product was analyzed in quadruplicate for phytase activity before mixing into the diets and the actual phytase activity was 5,104 FTU/g of product. One FTU of enzyme activity is defined as the amount of enzyme that liberates 1 umol of inorganic phosphorus per min at 37°C and pH 5.5 (Engelen et al., 2001).

Exp. 1, Growth performance and faecal digestibility

All procedures were approved by the China Agricultural University Institutional Animal Care and Use Committee. In Exp. 1, 280 crossbred piglets (Duroc×Landrace×Large White) with a body weight 10.32±1.94 kg, were selected from the China Agricultural University Research Farm. The piglets were weaned at 28 days of age and were fed a corn-soybean meal diet containing 0.9% calcium and 0.8% total phosphorus for one week before the commencement of the experiment. The piglets were allocated to one of five dietary treatments on the basis of weight and gender in a randomized complete block design (Table 1). The treatments were based on corn and soybean meal and included four low calcium-phosphorus (0.6% Ca, 0.4% total P and 0.23% available P) diets supplemented with 0, 250, 1,000, or 2,000 FTU phytase/kg of diet as well as a positive control diet (PC; 0.85% Ca, 0.58% total P and 0.37% available P). All diets had calcium to total phosphorus ratio of 1.5:1 and contained 0.25% chromic oxide as an indigestible marker.

The available phosphorus content of the experimental diets was calculated by multiplying the analyzed phosphorus values for corn and soybean meal by the bioavailability values for phosphorus published by NRC (1998) to obtain estimated available phosphorus values.

The experimental treatments were applied to seven pens with eight piglets (four barrows and four gilts) per pen. The pens were 2×3 m weaner decks equipped with a woven mesh floor. All pigs had free access to feed and water throughout the 4-week feeding trial. The temperature of pig barn was controlled between 24 and 32°C.

Pigs and feeders were weighed between 0750 and 0950 h on days 0 and 28 in order to calculate weight gain, feed intake and feed efficiency. Fresh fecal grab samples were collected on d 26 to 28 and pooled by pen. Approximately 100 g of fresh feces were collected directly from the floor of each pen into sterile plastic bags and immediately stored at -20°C until analysis.

On day 28, blood samples (about 5 ml) were collected from the jugular vein from one barrow (weighing closest to the average body weight for each pen) per pen, using a 10 ml heparinized blood collection tube (Greiner Bio-One GmbH, Kremsmunster, Austria) after an overnight fast. Blood samples were centrifuged at 3,000×g (Heraeus Biofuge 22R Centrifuge, Hanau, Germany) for 10 min at 4°C and the plasma immediately stored at -80°C for later analysis. At 0830 h on d 29, all of the pigs supplying blood samples were killed by electrical stunning and exsanguination to collect metacarpal bone samples.

The right front foot of each pig was removed and refrigerated at 2°C. The third and fourth metacarpal bones were excised and cleaned of all adhering tissue within 3 d for bone weight measurements and the determination of bone breaking strength and ash weight. Meanwhile, the sixth rib on the right side and duodenum tissue were removed and stored at -80°C for analysis of alkaline phosphatase activity.

EXP. 2, Apparent ileal digestibility

Exp. 2 was conducted to determine the effects of

Table 1. Diet composition and nutrients levels, as fed basis

Ingredients (%)	Exp. 1		Exp. 2	
	PC	NC	PC	NC
Corn starch	-	-	-	1.04
Corn	58.20	59.92	66.55	66.55
Soybean meal	24.60	24.30	29.60	29.60
Fishmeal	5.00	5.00	-	-
Whey powder	2.00	2.00	-	-
Corn gluten meal	5.00	5.00	-	-
Limestone	1.05	0.97	1.10	1.06
Dicalcium phosphate	0.90	-	1.20	0.20
Soybean oil	1.60	1.15	-	-
Chromic oxide	0.25	0.25	0.25	0.25
L-lysine-HCl, 98%	0.10	0.11	-	-
Salt	0.30	0.30	0.30	0.30
Premix[1]	1.00	1.00	1.00	1.00
Nutrient levels[2]				
Digestible energy (kcal/kg)	3,400	3,400	3,300	3,300
Crude protein	19.7	20.4	17.73	17.73
Calcium	0.85	0.60	0.77	0.53
Phosphorus	0.58	0.40	0.50	0.34
Available phosphorus	0.37	0.23	0.30	0.14
Lysine	1.26	1.25	1.17	1.13
Digestible lysine	1.07	1.07	0.90	0.90

[1] Premix supplied per kg diet: vitamin A, 11,000 IU; vitamin D_3, 1,503 IU; vitamin E, 44.1 IU; menadione, 4.0 mg; riboflavin, 5.22 mg; pantothenic acid, 20.0 mg; niacin, 26.0 mg; vitamin B_{12}, 0.01 mg; manganese, 35.0 mg; iron, 100.0 mg; zinc, 90.0 mg; copper, 16.5 mg; iodine, 0.30 mg; selenium, 0.30 mg.

[2] Digestible energy, available phosphorus and digestible lysine were calculated while the other nutrients are analyzed values.

phytase supplementation in corn-soybean meal based diets on the apparent ileal digestibility (AID) of nutrients in growing pigs. Six crossbred barrows (Duroc×Landrace× Large White) with an average BW of 35.1±1.6 kg were used in this experiment. Pigs were housed in adjustable metabolic crates with a plastic-covered floor. After a 7-d adaptation period, the pigs were surgically fitted with a simple T-cannula at the distal ileum, as described by Stein et al. (1998). After surgery, pigs were returned to the metabolic crates and allowed a 12-d recovery period. During this period, they were fed increasing amounts of a commercial grower diet twice daily and had unlimited access to water.

After the recovery period, pigs were assigned to one of three treatments according to a double 3×3 Latin square design. The dietary treatments were a low-phosphorus diet (0.53% Ca, 0.34% total P and 0.14% available P), the low P diet plus 1,000 FTU phytase/kg and a positive control diet (0.77% Ca, 0.50% total P and 0.30% available phosphorus). Each period lasted seven days with the first five days used for diet adaptation and the last two days for ileal digesta collection. Pigs were fed the diets at three times their maintenance energy requirement (NRC, 1998) based on their body weight at the beginning of each period. The daily feed allowance was offered in two equal portions at 0800 and 1630 h. Ileal digesta were collected continuously for 12 h from 0800 to 2000 h on days 6 and 7 as described by Stein et al. (1998) and were stored at -20°C until used for analysis.

Chemical analysis of feed, feces and digesta

Feed samples were collected at the start of each trial. Fecal samples were thawed, dried in an oven (65°C) and ground to pass through a 1-mm sieve. Digesta for Exp. 2 were freeze-dried prior to analysis.

Feed and fecal samples were analyzed for dry matter, crude protein (N×6.25), ash, and calcium, according to the methods of AOAC (1990). Gross energy was determined by an automatic adiabatic oxygen bomb calorimeter (Parr 1281 Automatic Energy Analyzer, Moline, IL). Chromium content was analyzed using an atomic absorption spectrophotometer (Hitachi Z-5000 Automatic Absorption Spectrophotometer, Tokyo, Japan) according to Williams et al. (1962). Phosphorus content was analyzed using the UV-visible spectrophotometer (Hitachi, U-1,000 Tokyo, Japan).

In Exp. 2, amino acids in diets and digesta were assayed

Table 2. Effect of phytase supplementation on performance of weaned pigs[1] (Exp. 1)

	Phytase FTU/kg of diet[2]				PC diet	SEM	p-value		
	0	250	1,000	2,000			Treatment	Linear	Quadratic
Weight gain (g/d)	488[c]	527[b]	564[a]	556[ab]	543[ab]	10	<0.01	<0.01	0.04
Feed intake (g/d)	855	904	914	905	896	23	0.40	0.33	0.51
Feed conversion ratio	1.75[a]	1.71[ab]	1.61[c]	1.63[c]	1.65[bc]	0.03	<0.01	<0.01	0.04

[abc] Means in the same row with different superscripts are significantly different (p<0.05).
[1] Data represents mean of seven pens with eight pigs per pen.

using ion-exchange chromatography with an automatic amino acid analyser (L-8800 Hitachi Automatic Amino Acid Analyzer, Tokyo, Japan) after hydrolyzing with 6 mol/L HCl at 110°C for 24 h. Cystine was determined as cysteic acid and methinonine as methionine sulfone after preoxidation with performic acid and precolumn derivation using phenylisothiocyanate (L-8800 Hitachi Automatic Amino Acid Analyzer, Tokyo, Japan). Tryptophan was determined after hydrolyzing with 4 mol/L NaOH at 110°C for 22 h using phenylisothiocyanate (Model 76337, Agilent Technologics, Waldbronn, Germany).

Metacarpal bone characteristics analysis

The breaking strength of the fresh bones was determined using an Instron testing machine (Model TML, Instron Corp., Canton, MA) similar to the procedure described by Veum and Ellersieck (2008). Force was applied to the center of the bone, which was held by two supports spaced 25 mm apart. After determination of breaking strength, the bones were wrapped with cheesecloth, boiled in deionized water for 2 h, dried at 55°C for 24 h and extracted with ethyl ether for 4 days. Ash content was determined after the fat-free bones were dried at 55 and 100°C for 18 and 2 h, respectively and then ashed in a muffle furnace at 600°C for 16 h.

Alkaline phosphatase activity and plasma calcium and phosphorus analysis

The 6th rib and duodenum tissues were minced and homogenized (10% w/v) in ice-cold sodium-potassium phosphate buffer (0.01 M, pH 7.4) containing 0.86% NaCl. The homogenate was centrifuged at 3,000×g for 10 min at 4°C. The resultant supernatant was used for the determination of alkaline phosphatase activity. Assay kits for the alkaline phosphatase activity and plasma urea nitrogen, calcium and phosphorus concentration were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Prepared sample supernatants and plasma were analyzed.

Statistical analysis

All data were processed using the SAS (SAS inst. Inc., Cary, NC). In Exp. 1, all data were analyzed by ANOVA as a randomized complete block design. Statistical differences among treatments are separated by Student Neuman Keul's multiple range test. Significance was taken at p≤0.05, with a trend being between p≥0.06 and p≤0.10. The preplanned single df comparisons were the linear and quadratic responses for the 0, 250, 1,000 and 2,000 FTU/kg phytase. Pens were the experimental units. In Exp. 2, data were analyzed using the Proc-Mixed procedure of SAS. The statistical model for the digestibility values had treatment and period as fixed effects and pig as a random effect. When the ANOVA was significant, differences between treatments were tested using least square means (t-tests).

RESULTS

Performance

There were linear increases (p<0.01) in weight gain with increasing dose rate of phytase (Table 2) in the diet. Pigs fed the PC diet and diets with 1,000 or 2,000 FTU/kg phytase had a greater (p<0.05) weight gain than pigs fed the low phosphorus diet (0 FTU/kg phytase) while no difference was observed among diets with phytase supplementation and the PC diet. Pigs fed diets with 1,000 or 2,000 FTU/kg phytase had a better (p<0.05) feed efficiency compared with pigs fed the low phosphorus diet, whereas feed intake was not affected by treatment.

Apparent total tract absorption and excretion of calcium, phosphorus and nutrient digestibility

The apparent digestibility of dry matter, crude protein, ash and energy were not affected by treatment (Table 3). The intake of calcium and phosphorus were greater (g/d, p<0.01) for pigs fed the PC diet than for pigs fed the low-phosphorus diets with or without phytase (Table 4). There was a linear increase (g/d, p<0.01) in the absorption of phosphorus and linear decreases (g/d, p<0.01; g/kg, p<0.01) in the total excretion of phosphorus and fecal phosphorus excreted per BW gain with increasing dietary dose rate of phytase. The apparent absorption of phosphorus was greater (g/d, p<0.05) for pigs fed the PC diet than for pigs fed low phosphorus diets with or without phytase and pigs fed the PC diet excreted more (g/d, p<0.05) phosphorus than pigs

Table 3. Effect of phytase supplementation on the apparent total tract digestibility of nutrients for weaned pigs[1] (Exp. 1)

Item (%)	Phytase FTU/kg of diet				PC diet	SEM	p-value		
	0	250	1,000	2,000			Treatment	Linear	Quadratic
Dry matter	86.0	87.1	86.3	85.3	86.1	0.60	0.30	0.12	0.08
Crude protein	81.9	83.4	82.7	83.5	83.4	0.90	0.69	0.39	0.417
Energy	84.5	84.9	84.3	83.5	84.9	0.70	0.38	0.10	0.08
Ash	56.8	60.8	61.7	58.7	61.0	1.94	0.40	0.79	0.87

[abc] Means in the same row with different superscripts are significantly different ($p<0.05$).
[1] Data represents mean of seven pens with eight pigs per pen.

Table 4. Effect of phytase supplementation on the apparent total tract absorption and excretion of calcium and phosphorus of weaned pigs[1] (Exp. 1)

	Phytase FTU/kg of diet				PC diet	SEM	p-value		
	0	250	1,000	2,000			Treatment	Linear	Quadratic
Feed intake (g)	855	904	914	905	896	23	0.40	0.33	0.51
Phosphorus									
Intake (g/d)	3.14^{c}	3.46^{bc}	3.62^{b}	3.48^{bc}	5.01^{a}	0.14	<0.01	0.49	0.54
Absorbed (g/d)	1.17^{d}	1.80^{c}	2.18^{b}	2.26^{b}	2.77^{a}	0.11	<0.01	<0.01	<0.01
Excreted (g/d)	1.98^{a}	1.66^{a}	1.43^{ab}	1.23^{c}	2.24^{a}	0.10	<0.01	<0.01	<0.01
Excretion/ADG (g/kg)	4.06^{a}	3.14^{b}	2.55^{c}	2.26^{c}	4.14^{a}	0.15	<0.01	<0.01	<0.01
Absorbed/intake (%)	37.0^{d}	51.8^{c}	60.2^{ab}	64.9^{a}	55.5^{bc}	1.95	<0.01	<0.01	<0.01
Excreted/intake (%)	63.0^{a}	48.2^{b}	39.8^{cd}	35.1^{d}	44.5^{bc}	1.95	<0.01	<0.01	<0.01
Calcium									
Intake (g/d)	4.77^{c}	5.49^{b}	5.73^{b}	5.49^{b}	7.45^{a}	0.21	<0.01	0.39	0.38
Absorbed (g/d)	3.06^{c}	3.74^{b}	4.09^{b}	3.86^{b}	5.16^{a}	0.18	<0.01	0.16	0.11
Excreted (g/d)	1.71^{b}	1.75^{b}	1.63^{b}	1.63^{b}	2.29^{a}	0.15	0.03	0.65	0.90
Absorbed/intake (%)	63.9	67.9	71.8	70.6	69.5	2.38	0.21	0.07	0.05
Excreted/intake (%)	36.1	32.1	28.2	29.4	30.5	2.38	0.21	0.07	0.05

[abcd] Means in the same row with different superscripts differ ($p<0.05$). [1] Data represents mean of seven pens with eight pigs per pen.

in other treatments.

There were no linear or quadratic responses due to phytase supplementation in the apparent absorption and excretion of calcium (Table 4). Pigs fed the PC diet had the highest intake and absorption of calcium while pigs fed the unsupplemented low phosphorus diet had the lowest intake and absorption of calcium. Values for pigs fed the three phytase supplemented diets were intermediate. Calcium excretion was significantly higher ($p<0.05$) for pigs fed the PC than for the four low phosphorus diets regardless of whether they were supplemented or unsupplemented with phytase.

Metacarpal bone characteristics

For both the third and fourth metacarpal bone, there were linear ($p<0.01$) and quadratic ($p<0.01$) increases in metacarpal bone breaking strength, as well as fat-free dried bone calcium and phosphorus with increasing dose rate of phytase (Table 5). However, fresh bone weight and fat-free dry weight were not affected by dietary treatment.

Pigs fed the PC diet had a greater ($p<0.01$) bone breaking strength than pigs fed the NC diet for the third and fourth metacarpal bone, whereas the bone breaking strength of pigs fed the PC diet or diets with 1,000 or 2,000 FTU/kg did not differ. Similar results were observed in for fat-free dried bone calcium and phosphorus for the third and fourth metacarpal bone.

Alkaline phosphatase activity and plasma indices

There were linear increases ($p<0.01$) in plasma phosphorus concentration and linear decreases ($p<0.01$) in plasma calcium concentration, plasma alkaline phosphatase activity and bone alkaline phosphatase activity with increasing dose rate of phytase (Table 6). Pigs fed the PC diet had a lower ($p<0.01$) plasma calcium concentration, plasma alkaline phosphatase activity and bone alkaline phosphatase activity than pigs fed diets with 0 or 250 FTU/kg phytase. In contrast, pigs fed the PC diet had a greater ($p<0.01$) plasma phosphorus concentration than pigs fed the low phosphorus diets. No difference was observed for plasma urea nitrogen concentration or duodenum alkaline phosphatase activity.

Table 5. Effect of phytase supplementation on the metacarpal bone characteristics of weaned pigs[1] (Exp. 1)

	Phytase FTU/kg of diet				PC diet	SEM	p-value		
	0	250	1,000	2,000			Treatment	Linear	Quadratic
Third metacarpal bone									
Breaking strength (N)	237[b]	270[b]	313[a]	306[a]	309[a]	12	<0.01	<0.01	<0.01
Fresh wt (g)	9.16	9.18	9.33	9.39	9.38	0.32	0.97	0.65	0.90
Fat-free dry wt (g)	2.98	3.02	3.25	3.30	3.37	0.13	0.16	0.12	0.27
Ash wt (g)	1.47	1.54	1.63	1.59	1.60	0.05	0.32	0.09	0.04
Fat-free dried bone calcium (%)	18.71[b]	19.10[b]	19.91[a]	20.04[a]	20.21[a]	0.21	<0.01	<0.01	<0.01
Fat-free dried bone phosphorus (%)	8.69[b]	8.95[ab]	9.55[a]	9.56[a]	9.42[a]	0.20	0.02	<0.01	<0.01
Forth metacarpal bone									
Breaking strength (N)	244[c]	281[b]	324[a]	326[a]	320[a]	12	0.01	0.01	0.01
Fresh wt (g)	9.21	9.47	9.67	9.84	9.81	0.28	0.49	0.29	0.55
Fat-free dry wt (g)	3.15	3.19	3.45	3.46	3.41	0.11	0.20	0.14	0.28
Ash wt (g)	1.50[b]	1.55[ab]	1.62[ab]	1.65[a]	1.56[ab]	0.04	0.18	0.07	0.16
Fat-free dried bone Ca (%)	18.48[b]	18.96[b]	20.17[a]	20.32[a]	20.46[a]	0.34	<0.01	<0.01	<0.01
Fat-free dried bone P (%)	8.71[b]	8.94[b]	9.49[a]	9.66[a]	9.78[a]	0.18	<0.01	<0.01	<0.01

[abc] Means in the same row with different superscripts differ ($p<0.05$). [1] Data represents mean of seven pigs.

Apparent ileal digestibility of dry matter, crude protein, calcium, phosphorus and AA

Pigs fed the low-P diet with 1,000 FTU/kg phytase had a greater ($p<0.01$) AID of Ca and P than pigs fed diets without phytase (Table 7). Furthermore, phytase supplementation significantly improved AID of leucine, lysine, phenylalanine, alanine and cysteine and tended to increase AID of crude protein, total AA, Isoleucine, threonine, asparagine and serine. However, No statistical differences (Table 7) were observed for AID of DM and some other AA between treatments.

DISCUSSION

The results obtained from the present experiment demonstrate that phytase supplementation in the low-phosphorus containing corn and soybean meal-based diet, at levels of 250 FTU/kg phytase and above, produced growth and feed efficiency similar to those shown by the positive control and significantly increased weight gain compared with the non-supplemented low-phosphorus diet. This observation confirms the well-documented improvement in weight gain of pigs fed phytase-supplemented, low-phosphorus diets (Adeola et al., 1995; Augspurger et al., 2003). The optimal dose of phytase was 1,000 FTU/kg as this treatment produced the highest weight gain, best feed conversion and highest bone breaking strength. Our results suggest that 1,000 FTU/kg phytase can successfully replace all of the inorganic phosphorus in diets fed to weaned pigs. Adeola et al. (2004) reported that including an E. *coli*-

Table 6. Effect of phytase supplementation on the alkaline phosphatase activity in various tissue and plasma indices of weaned pigs[1] (Exp. 1)

	Phytase, units/kg of diet				PC diet	SEM	p-value		
	0	250	1,000	2,000			Treat	Linear	Quadratic
Alkaline phosphatase									
Bone (unit[2]/g)	354[a]	313[b]	267[c]	254[cd]	236[d]	8.11	<0.01	<0.01	<0.01
Duodenum (unit[2]/g)	42	48	43	41	43	3.24	0.61	0.36	0.62
Plasma (unit[2]/ml)	145[a]	134[a]	111[b]	113[b]	107[b]	5.93	0.01	<0.01	<0.01
Other plasma indices (mmoL/L)									
Phosphorus	1.60[c]	2.09[b]	2.35[b]	2.37[b]	2.84[a]	0.09	<0.01	<0.01	<0.01
Calcium	3.65[a]	3.06[b]	2.44[c]	2.43[c]	2.10[c]	0.12	<0.01	<0.01	<0.01
Plasma urea nitrogen	4.65	4.65	4.09	4.11	4.20	0.42	0.76	0.28	0.50

[abcd] Means in the same row with different superscripts differ ($p<0.05$).

[1] Data represents mean of seven pigs.

[2] Unit: one unit alkaline phosphatase activity is defined as the amount of alkaline phosphatase in 100 ml plasma that liberates 1 mg of hydroxybenzene from disodium phenyl phosphate per quarter at 37°C.

Table 7. Effect of phytase supplementation on apparent ileal nutrient digestibility in growing pigs[1] (Exp. 2)

Item (%)	PC	NC	NC+1,000	SEM	p-value
Apparent ileal digestibility					
Dry matter	77.1	75.8	79.0	1.2	0.20
Crude protein	79.8	77.7	82.3	1.3	0.09
Calcium	65.7[b]	51.9[c]	72.6[a]	2.1	<0.01
Phosphate	55.7[b]	32.5[c]	70.2[a]	3.7	<0.01
Indispensable					
Arginine	89.2	89.4	90.6	1.0	0.61
Histidine	85.2	84.6	87.5	1.0	0.15
Isoleucine	83.2	81.3	87.0	1.5	0.07
Leucine	85.3[ab]	84.0[b]	88.2[a]	1.1	0.05
Lysine	86.2[b]	85.0[b]	89.2[a]	0.5	<0.01
Methionine	90.4	88.7	91.6	1.6	0.43
Phenylalanine	83.5[b]	82.9[b]	88.2[a]	1.2	0.02
Threonine	77.3	73.7	81.0	1.8	0.06
Tryptophan	84.0	83.5	88.0	1.5	0.12
Valine	82.2	80.7	84.4	1.3	0.19
Dispensable					
Alanine	80.0[ab]	75.3[b]	83.2[a]	1.7	0.03
Asparagine	82.3	80.6	85.1	1.1	0.06
Cysteine	72.1[ab]	69.1[b]	77.8[a]	1.8	0.02
Glutamine	84.8	83.6	87.5	1.2	0.11
Glycine	69.2	63.4	71.8	3.0	0.20
Proline	80.5	77.2	83.5	1.9	0.12
Serine	83.0	81.3	85.4	1.1	0.09
Tyrosine	83.5	82.6	86.9	1.5	0.15
Total amino acid	83.0	81.2	85.9	1.2	0.07

[abc] Means in the same row with different superscripts differ ($p<0.05$). [1] Data represents mean of six pigs.

derived phytase at 500 and 1,000 FTU/kg improved ADG by 20 and 30%, ADFI by 1 and 10% and G:F by 18 and 17%, respectively, in 20-kg pigs fed a P-deficient corn-soybean meal diet. Other experiments with pigs fed corn-SBM diets deficient in available P also showed that high phytase dose rate (450 FTU/kg) were required to obtain bone strength or P absorption values comparable with those of pigs fed the PC diet, whereas lower does rate of phytase (250 FTU/kg) were adequate for growth performance (Veum and Ellersieck, 2008).

The lack of significant response in total tract digestibility of DM, CP and GE in the present and other studies (Jendza et al., 2006; Radcliffe et al., 2006) in the literature may be due to interference of microbial degradation in the hindgut. The results of the current study indicated 38.5 and 42.9% increases in phosphorus digestibility with the addition of 1,000 or 2,000 FTU phytase/kg diets compared with the unsupplemented, low-phosphorus diet was within the range of values reported in other experiments with young pigs that were group-fed corn-soybean meal diets deficient in available phosphorus (Omogbenigun et al., 2003).

An estimate of the % digestible P released by phytase in the starter low-P diets in Exp. 1 may be calculated utilizing the data in Table 4 as described by Veum and Ellersieck, (2008). Using this method of calculation, the digestible P released by phytase in diets NC+250, NC+1,000 and NC+2,000 is estimated at 0.062, 0.102 and 0.113%, respectively. The estimated digestible P provided by dicalcium phosphate in the PC diet is 0.172%, which is greater than the % digestible P released by phytase in diet NC+1,000 and NC +2,000. However, pigs fed diets containing 1,000, or 2,000 FTU phytase/kg obtained similar performance compared to pigs fed PC diets, this indicated that phytase may influence growth performance via mechanisms other than phytate-P liberation. In one balance study, Adeola et al. (2004) found that phytases derived from *A. niger* and *E. coli* released 0.038% and 0.042% P, respectively, in weaned pigs offered corn-soybean diets at a dose rate of 750 FTU/kg. Augspurger et al. (2003) reported the P-release value was 0.081, 0.043, and 0.108% for *A. niger* phytase, *P. lycii*, and the *E coli-derived* phytase, respectively, and attributed the superior efficacy of *E. coli*-derived phytase to a greater resistance to pepsin, and lower, wider pH optima. The

current study observed a similar or better P-release value for the novel microbial phytase. Hoppe and Schwarz (1993) reviewed the available data and concluded that 500 FTU/kg phytase was equivalent to 1 g (0.1%) P as monocalcium phosphate or 0.8 g (0.08%) digestible P, in corn-soybean diets for pigs and this finding has been generally accepted by the industry (Selle and Ravindran, 2008). In agreement, the current study indicated that 1,000 FTU/kg *Trichoderma Reesei* phytase was equivalent to 1.80 g inorganic P as dicalcium phosphate based on the P absorbed and weaned pig performance.

In the current study, increased plasma calcium and decreased plasma phosphorus in pigs fed unsupplemented low phosphorus diet compared with those pigs fed the PC diet confirmed that the phosphorus-inadequate pigs presented a profile of phosphorus deficiency rather than calcium deficiency. Similar features were reported in phosphorus-deficient pigs (Létourneau et al., 2010). Meanwhile, pigs fed NC diet had increased alkaline phosphatase activity in plasma and bone compared with those fed the PC diet, which confirmed that the activity of alkaline phosphatase in bone is more sensitive than that in plasma in a situation of phosphorus deficiency. The activity of alkaline phosphatase in bone has been shown to increase in response to a lack of circulating phosphorus for bone mineralization (Boyd et al., 1983), which may have resulted in an increase of alkaline phosphatase activity in plasma, as plasma alkaline phosphatase is mainly derived from skeletal and non-skeletal sources (Broadus et al., 1981). The phytase dose-dependent (linear and quadratic) increase in plasma P and decrease in plasma calcium, alkaline phosphatase activity and bone alkaline phosphatase activity, indicates that phytase facilitated digestion of dietary phytate-phosphorus, preventing excessive bone resorption or demineralization.

The improvement in the AID of Ca and P by phytase supplementation was expected and in concurrence with other studies (Paditz et al., 2004; Lindberg et al., 2007). In the current experiment, pigs fed low-P diet with 1,000 FTU phytase had increased the AID of Ca (40%) and P (116%) compared to pigs fed NC diet. Phytase supplementation numerical improved the AID of N and all of AA in a corn-SBM diet fed to growing pigs compared to diets without phytase. The degree of improvement was modest for some amino acids, such as threonine, where AID increased by 2-4% with phytase supplementation. However, for some amino acids the increase in AID was significant, such as phenylalanine. Furthermore, an estimated of contribution for the ileal absorbed P was calculated as 0.128% (the difference of 70.2% and 32.5% multiplied 0.34%) utilizing the data in Table 7. The contribution for ileal absorbed Ca, CP, total AA, lysine, methionine, cysteine, threonine, tryptophan and valine by 1,000 FTU/kg phytase were 0.110, 0.826, 0.829, 0.047, 0.009, 0.028, 0.052, 0.008 and 0.030%, respectively. Johnston et al. (2004) suggested that phytase addition along with a reduction in dietary calcium and phosphorus allowed a reduction of dietary concentrations of amino acids and energy. These findings indicated considerable economic benefits.

In a comprehensive literature review involving 281 cannulated pigs, Selle and Ravindran (2008) reported that phytase improved AID of AA by an average of 2.12% in a range of 1.01 to 3.93% compared with pigs with non-phytase control. In agreement, we observed 4.7% and 2.9%, respectively, higher AID of total AA in pigs fed 1,000 FTU/kg phytase compared to that of pigs fed the NC diet and PC diet. The numerical improvement in the AID of nitrogen and AA may partly explain that phytase influence growth performance via mechanisms other than phytate-P liberation in Exp. 1.

IMPLICATIONS

The results of the present study clearly demonstrate that the supplementation of microbial phytase improves phytate phosphorus utilization in young pigs and improved AID of AA, Ca and P in growing pigs. These findings suggest that feeding low-phosphorus diets supplemented with phytase could provide both economic and environmental benefit in the same package. However, further research will be need to determine the exact inorganic phosphorus equivalents of the novel microbial phytase and compare with other phytases.

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