Effect of a novel phytase on growth performance, bone ash, and mineral digestibility in nursery and grower-finisher pigs

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ABSTRACT: To compare the effectiveness of 2 phytase enzymes (Phyzyme and Natuphos), growth performance, fibula ash, and Ca and P digestibilities were evaluated in 4 studies. The first 3 studies used 832 pigs (i.e., 288 in the nursery phase, initial BW 8.1 kg; 288 in the grower phase, initial BW 24.2 kg; and 256 in the finisher phase, initial BW 57.8 kg) and were carried out over periods of 28, 42, and 60 d, respectively. Dietary treatments in each study consisted of a positive control [available P (aP) at requirement level]; negative control (Ca remained as in the positive control, and aP at 66, 56, and 40% of the requirement for the nursery, grower, and finisher studies, respectively); negative control plus graded levels of Phyzyme [250, 500, 750, or 1,000; measured as phytase units (FTU)/kg] or Natuphos (250 and 500 FTU/kg for the nursery and grower studies, or 500 and 1,000 FTU/kg for the finisher study) plus a very high dose of Phyzyme (tolerance level, at 10,000 FTU/kg) in the nursery and grower experiments. Across the 3 studies, there was no effect of any dietary treatment on ADFI, but the negative control reduced ADG (10%), G:F (7%), and bone ash (8%) compared with the positive control. In the nursery study, phytase addition increased G:F and bone ash linearly ($P < 0.01$). In the grower study, phytase increased ADG, G:F, and bone ash linearly ($P < 0.01$). In the finisher study, phytase addition increased ADG and bone ash linearly ($P < 0.01$) and increased G:F quadratically ($P < 0.05$); G:F was, on average, 5% greater ($P < 0.05$) with Phyzyme than with Natuphos. The fourth study was conducted to investigate the P-releasing efficacy of the 2 phytases. The apparent fecal digestibility of P, measured with chromic oxide as an external marker in 35 pigs (55.9 kg of BW), showed that aP increased ($P < 0.001$) by 0.17 and 0.06 g (± 0.023) per 100 FTU consumed for Phyzyme and Natuphos, respectively. Also, Phyzyme at 10,000 FTU/kg was not detrimental to animal health or growth performance. At doses intended for commercial conditions, Phyzyme proved to be effective in releasing phytate bound P from diets, with an efficacy superior to a commercially available enzyme.

Key words: bone ash, growth, phosphorus, phytase, pig

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INTRODUCTION

A large portion of P in cereal grains and oilseed meals is in the form of phytic acid (myo-inositol hexaphosphate) commonly called phytate (Erdman, 1979). Soybean and corn phytate represents 1.4 and 0.9% of the DM, respectively, but it binds more than 60% of the total P (Cheryan, 1980). Pigs utilize phytate P poorly because they have limited intestinal phytase, and it is therefore necessary to supplement swine diets with inorganic P sources, resulting in relatively large amounts of P in the manure that contribute to environmental pollution. To mitigate the problem, commercial use of phytases (phosphatase enzymes that cleave P moieties from phytate) has become a generally accepted practice (Lassen et al., 2001).

Economic concerns about use of phytases have encouraged development of new versions of the enzyme (Stahl et al., 2000). *Schizosaccharomyces pombe* has been used to express the appA gene from *Escherichia coli* (Ciofalo et al., 2003) to produce a new 6-phytase (phytases are grouped according to the position of phosphate ester group on the phytate molecule at which hydrolysis is initiated, i.e., as 3- or 6-phytase).

The objective of our research was to evaluate effects of a new phytase enzyme (Phyzyme, produced in *Schizosaccharomyces pombe*; Danisco Animal Nutrition, Marlborough, UK) on growth performance, bone ash, and mineral digestibility compared with a commercial

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phytase enzyme (Natuphos, a 3-phytase produced in *Aspergillus niger*; BASF, Mt. Olive, NJ) with nursery, grower, and finisher pigs fed P-deficient corn-soybean meal diets. An additional objective was to carry out a tolerance test in nursery and grower pigs; the new phytase was fed at 20 times the intended commercial inclusion level.

MATERIALS AND METHODS

Experimental Design and Treatments

Four independent studies were conducted at the Swine Research Center at the University of Illinois. The protocols for the studies were approved by the Institutional Animal Care and Use Committee. The first 3 studies compared the effects of 2 different phytase enzymes (Natuphos and Phyzyme, which is a new enzyme) on growth performance and bone ash. Each study was performed independently and in different phases of production [i.e., nursery (8.2 to 19.3 kg of BW); grower (24.2 to 57.8 kg of BW); and finisher (54.1 to 113.0 kg of BW)]. The fourth study was conducted to compare the effects of the same enzymes on the apparent fecal digestibility of minerals in grower pigs (55.9 to 65.8 kg of BW). A total of 867 pigs [progeny of PIC Line 337 sires mated to C22 dams (PIC, Franklyn, KY)] were used.

The growth performance studies used 9 diets (except for the finisher study, where only 8 diets were used; Table 1) and 2 sexes (gilt and barrow) in a 9×2 factorial arrangement, with a randomized complete block design, with the time of beginning on test being the blocking factor. In each study, there was a positive control, where the available P level was set at the requirement level proposed by the NRC (1998) for the respective growth phase, and a negative-control diet, where the available P was set at 66, 56, or 40% of the requirement for the nursery, grower, or finisher study, respectively. Calcium was set at the same level for the positive- and negative-control diets, creating a more unfavorable (greater) Ca:available P ratio for the negative controls. The Phyzyme treatments consisted of the negative-control diet plus graded levels of the enzyme at 250, 500, 750, 1,000, or 10,000 phytase units (**FTU**)/kg of diet. The latter treatment (10,000 FTU/kg) was omitted in the finisher study. Natuphos treatments in the nursery and grower studies consisted of the negative-control diet plus graded levels of the enzyme at 250 or 500 FTU/kg of diet, whereas in the finisher study Natuphos was supplemented at 500 or 1,000 FTU/kg of diet.

In every study, there were 4 blocks, each consisting of 1 pen (4 pigs per pen) of barrows and 1 pen of gilts on each treatment. Within blocks, pigs were randomly allotted to a pen from groups of 9 pigs formed on the basis of litter of origin and BW. Pens were randomly allotted to dietary treatments. Pigs were weighed individually at the beginning of the experiment and every 14 d during the study. Weekly observations of the health status of the animals were recorded by a licensed veterinarian.

Diet Preparation and Feeding

A basal diet containing the major ingredients (corn, soybean meal, vitamins, minerals, and synthetic lysine) was mixed, and phytase, soybean oil, dicalcium phosphate, and limestone were added to aliquots of the basal diet using a ribbon mixer. Phytase supplementation levels were based on an assessment of phytase premix activity carried out before the beginning of the experiment. One FTU was defined as the amount of enzyme required to release 1 μmol of inorganic P/min from 5.1 mmol of sodium phytate at 37°C and pH of 5.5 (Engelen et al., 1994). Pigs had ad libitum access to feed via one 8-space feed hopper per pen in the nursery study and via one single-space feeder per pen for the grower and finisher experiments. Water was continuously available via one nipple drinker in each pen. Fresh feed was added to the feeder twice daily in the nursery study and every day as needed in the grower and finisher studies. Feeders were weighed at the beginning and every 14 d during the studies.

Fibula Ash Content

At the end of the growth performance studies, a total of 208 pigs were harvested (8 pigs/treatment/study). Within pen, the pig that was closest to the mean pen weight was selected. The right fibula was obtained and the adhering tissue was removed mechanically, followed by autoclaving. Cleaned bones were dried in an oven (100°C) for 36 h and then dry-ashed in a muffle furnace (500°C) for 40 h. Fibula ash content was expressed as a percentage of the dry bone weight.

Nursery Study

The study was carried out over a fixed time period of 28 d. Pigs were weaned at 21 ± 3 d of age and moved from the farrowing crates to the nursery facilities. The pens (1×1 m, 0.25 m^2/pig) were on raised decks with perforated metal floors and solid sidewall pen partitions; the facilities had 24-h continuous lighting. Before allotment, pigs were allowed a 1-wk acclimation period, during which they were fed a standard phase I nursery diet (3,410 kcal of ME/kg; 1.23% true digestible Lys; 0.32% available P). A 2-phase dietary regimen was used during the study, with phases II and III being fed from d 1 to 14, and d 15 to 28 of the study, respectively (Table 1).

Grower and Finisher Studies

The studies were carried out in facilities with part-solid, part-slatted concrete floors and vertical, metal bar pen partitions that provided 1.05 m^2/pig of floor space, with 4 pigs per pen. The grower pig study was

Table 1. Composition of diets (%, as-fed basis) used in the growth performance studies[1]

	Nursery						Finisher			
	Phase 2[3]		Phase 3[4]		Grower[2]		Phase 1[5]		Phase 2[6]	
Control	+	−	+	−	+	−	+	−	+	−
Ingredient										
Corn	48.18	48.68	60.18	60.64	70.80	71.17	82.18	82.56	84.52	84.89
Soybean meal, dehulled	23.00	23.00	29.78	29.70	26.09	26.23	15.11	15.18	13.14	13.20
Dried whey	20.00	20.00	5.00	5.00	—	—	—	—	—	—
Soy concentrate meal	3.60	3.50	—	—	—	—	—	—	—	—
Soybean oil	2.94	2.78	2.53	2.37	0.82	0.62	0.71	0.52	0.66	0.49
Limestone	0.95	1.19	0.78	1.10	1.04	1.22	0.74	1.01	0.73	1.01
Dicalcium phosphate	0.57	0.09	0.92	0.38	0.79	0.31	0.73	0.19	0.53	—
Mineral premix[7]	0.35	0.35	0.35	0.35	0.35	0.35	0.30	0.30	0.30	0.30
Vitamin premix[8]	0.20	0.20	0.20	0.20	0.10	0.10	0.10	0.10	0.05	0.05
Salt	0.10	0.10	0.10	0.10	—	—	—	—	—	—
L-Lysine HCl	0.11	0.11	0.16	0.16	0.006	0.006	0.13	0.13	0.06	0.06
Calculated composition										
ME, Mcal/kg	3.41	3.41	3.43	3.43	3.37	3.37	3.38	3.38	3.39	3.39
CP	20.00	20.00	20.00	20.00	18.40	18.40	14.17	14.24	13.36	13.42
Lys, total	1.35	1.35	1.25	1.25	1.00	1.00	0.78	0.78	0.67	0.67
Lys, ileal digestible	1.17	1.17	1.08	1.08	0.84	0.85	0.67	0.67	0.56	0.56
P, total	0.58	0.48	0.57	0.47	0.54	0.44	0.47	0.37	0.43	0.33
P, available	0.32	0.22	0.27	0.17	0.23	0.13	0.19	0.09	0.15	0.05
Ca	0.70	0.70	0.65	0.65	0.62	0.60	0.52	0.50	0.46	0.45
Ca:P, total	1.21	1.46	1.14	1.38	1.15	1.36	1.11	1.35	1.07	1.36
Ca:aP, available	2.19	3.18	2.41	3.82	2.70	4.62	2.74	5.56	3.07	9.00

[1]Phytase additions from Phyzyme were made to the negative control diet at 250, 500, 750, 1,000, and 10,000 phytase units (FTU) for nursery and grower; and at 250, 500, 750, and 1,000 FTU for the finisher. Natuphos was added at 250 and 500 FTU for nursery and grower and at 500 and 1,000 FTU for the finisher.
[2]Fed for 42 d during the grower experiment, from 55 d of age and 24 kg of BW.
[3]Fed for the first 14 d of the nursery experiment, from 28 d age and 8 kg of BW (i.e., d 1 to 14).
[4]Fed for the last 14 d of the nursery experiment, from 42 d age and 12 kg of BW (i.e., d 15 to 28).
[5]Fed during the finisher experiment, from 50 to 80 kg of BW.
[6]Fed during the finisher experiment, from 80 to 115 kg of BW.
[7]Each kilogram of mix contained the following: Fe, 25.7 g ($FeSO_4 \cdot H_2O$); Zn, 28.6 g (ZnO); Mn, 5.7 g (MnO); Cu, 2.3 g ($CuSO_4 \cdot 5H_2O$); I, 100 mg (CaI_2); Se, 85.7 mg (Na_2SeO_3); and NaCl, 855 g.
[8]Each kilogram of mix contained the following: retinyl acetate, 1,136 mg; cholecalciferol, 8.25 mg; DL-α-tocopheryl acetate, 44 g; menadione, 2.2 g (menadione sodium bisulfite complex); niacin, 16.5 g; D-Ca-pantothenate, 12.2 g; riboflavin, 4.4 g; vitamin B_{12}, 17.5 mg; and choline chloride, 143 g.

carried out over a fixed time period of 42 d and used a single dietary phase (Table 1). The finisher study was carried out over an average time period of 60 d; a 2-phase dietary regimen was used, with finisher 1 being fed from the beginning (54 kg of BW) to an average BW of 80 kg, and finisher II being fed from 80 to 113 kg of BW.

Mineral Digestibility Study

This study investigated the effects of the 2 phytase enzymes (Phyzyme and Natuphos) on apparent fecal digestibility of Ca and P. A total of 35 pigs were used over an experimental period of 14 d (4-d adaptation to the pen, 5-d adaptation to the diet, and 5-d fecal collection). The index method for estimating digestibility was used, with chromic oxide as an indigestible marker. Five pigs (3 gilts and 2 barrows) were allotted to each treatment according to BW and litter of origin; they were housed in the same facility used for the grower and finisher studies. Pigs (initial BW 55.9 kg) were individually housed at a floor space of 4.2 m^2/ pig, and feed intake was restricted to 90% of the average ad libitum intake measured during the 9-d period before the collection period began. Feed was divided in to 2 equal-size meals, which were given at 0700 and 1900, respectively.

From a basal diet (negative control; 3,375 kcal of ME/kg; 0.74% true digestible Lys; 0.55% Ca; 0.1% available P), 7 treatments were created (Table 2) by addition of potassium monobasic phosphate (KH_2PO_4), arenaceous filler, or phytase as follows: treatments 1 to 3 had, respectively, 40, 70, or 100% of the available P requirement (NRC, 1998), and a Ca:available P ratio of 5.5, 3.4, or 2.6; these treatments were used to create a standard curve. Treatments 4 and 5 had the same available P and Ca:available P ratio as treatment 1, plus 250 or 500 FTU/kg, respectively, from Phyzyme. Treatments 6 and 7 had the same available P and Ca:available P ratio as treatment 1, plus 250 or 500 FTU/kg, respectively, from Natuphos.

During the final 5 d of the study, the floors were cleaned twice daily, and all feces were collected from the floors at least 5 times daily between 0500 and 2300.

Table 2. Composition of diets (%, as-fed basis) used in the mineral digestibility study

	1	2	3	4 – 7
Treatment	0.1% aP[1]	0.16% aP	0.22% aP	0.1% aP + Phytase
Ingredient				
Corn	74.51	74.51	74.51	74.51
Soybean meal	21.98	21.98	21.98	21.98
Soybean oil	1.00	1.00	1.00	1.00
Limestone	1.08	1.08	1.08	1.08
Dicalcium phosphate	0.19	0.19	0.19	0.19
Chromic oxide	0.36	0.36	0.36	0.36
Trace mineral premix[2]	0.35	0.35	0.35	0.35
Vitamin premix[3]	0.10	0.10	0.10	0.10
L-Lysine HCl	0.01	0.01	0.01	0.01
Arenaceous flour	0.50	0.24	0.02	0.50
KH_2PO_4[4]	—	0.26	0.48	—
Phytase	—	—	—	250 to 500 FTU[5]/kg
Calculated composition				
ME, Mcal/kg	3.37	3.37	3.37	3.37
CP	16.75	16.75	16.75	16.75
Lys, total	0.88	0.88	0.88	0.88
Lys, digestible	0.74	0.74	0.74	0.74
P, total	0.40	0.45	0.51	0.39
aP	0.10	0.16	0.21	0.10
Ca	0.55	0.55	0.55	0.55
Ca:P, total	1.37	1.22	1.08	1.41
Ca:aP	5.5	3.44	2.61	5.5

[1]Available P (aP).
[2]Supplied the following per kilogram of complete diet: Fe, 90 mg ($FeSO_4 \cdot H_2O$); Zn, 100 mg (ZnO); Mn, 20 mg (MnO); Cu, 15 mg ($CuSO_4 \cdot 5H_2O$); I, 0.35 mg (CaI_2); Se, 0.3 mg (Na_2SeO_3); and NaCl, 3 g.
[3]Supplied the following per kilogram of complete diet: retinyl acetate, 1,136 μg; cholecalciferol, 8.25 μg; DL-α-L-tocopheryl acetate, 44 mg; menadione, 2.2 mg (menadione sodium bisulfite complex); niacin, 16.5 mg; D-Ca-pantothenate, 12.1 mg; riboflavin, 4.4 mg; vitamin B_{12}, 17.5 μg; and choline chloride, 143 mg.
[4]Reagent grade, 99.5% pure (Fisher Scientific, Agawam, MA).
[5]FTU = phytase units.

The daily fecal collection from each pig was mixed and homogenized, and a 30-g sample was dried at 105°C for 40 h and then ground and stored. At the end of the study, samples from the 5-d collection for each pig were mixed and used for chemical analyses. Chromic oxide, Ca, and P were measured by inductively coupled plasma, atomic emission spectrometry (AOAC, 2000; Perkin Elmer, Norwalk, CT).

Digestibility Calculations

Apparent fecal digestibility for Ca and P were calculated for individual animals using the index method, which is based on the differential concentrations of chromic oxide (used as an external marker) and the mineral in feed and feces, according to the following formula (Fan et al., 1995):

$$AFD = (100 - [(Mf/Md) \times (Crd/Crf) \times 100]),$$

where AFD is the apparent fecal digestibility of a mineral (%), Mf is the mineral content of the fecal sample (%), Md is the mineral content of the diet (%), Crd is the chromic oxide content of the diet (%), and Crf is the chromic oxide content of the fecal sample (%).

Standard curve methodology (Augspurger et al., 2004) was used to estimate the amount of P liberated by the phytase. Thus, the values for apparent P fecal digestibility were regressed against dietary inorganic P intake (from KH_2PO_4) to establish a standard curve. The P-releasing efficacy of the phytase treatments was estimated from the standard curve, and the results were presented as grams of available P/100 FTU of phytase consumed.

The extra amount of Ca digested by the inclusion of phytase was estimated from the apparent fecal digestibility and the measured concentrations of Ca and phytase in the diet. The difference in the amount of Ca absorbed between the animals fed the control diet and those fed the phytase treatments was divided by the actual phytase intake, and the results were expressed as grams of Ca released per 100 FTU of phytase consumed.

Statistical Analysis

For performance data, the pen was considered the experimental unit; the individual pig was considered the experimental unit for bone ash and mineral digestibility measurements. The PROC UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC) was used to verify normality. The PROC MIXED procedure of SAS was used to analyze the data; the model included the

Table 3. Effect of phytase source and level on growth performance and fibula ash (nursery pig study)

Item	Initial BW,[1] kg	ADFI,[1] g/d	ADG,[1] g/d	G:F,[1,2] g/kg	Fibula ash,[2,3] %
Dietary treatment					
1. Positive-control, adequate P	8.07	723	419	577^{yz}	45.33^{yz}
2. Negative-control, deficient P	8.06	710	371	526^{x}	41.56^{vw}
3. As 2 + Phyzyme 250 FTU[4]/kg	8.11	737	389	525^{x}	43.01^{vwx}
4. As 2 + Phyzyme 500 FTU/kg	8.25	727	401	552^{xy}	43.46^{wxy}
5. As 2 + Phyzyme 750 FTU/kg	8.18	748	415	551^{xy}	45.21^{xy}
6. As 2 + Phyzyme 1,000 FTU/kg	8.17	725	403	553^{xy}	45.89^{yz}
7. As 2 + Phyzyme 10,000 FTU/kg	8.11	698	410	588^{z}	47.70^{z}
8. As 2 + Natuphos 250 FTU/kg	8.21	712	383	536^{x}	40.98^{v}
9. As 2 + Natuphos 500 FTU/kg	8.14	725	399	546^{xy}	42.16^{vw}
Pooled SEM	0.45	35	32	16.9	1.224
Probability[5]	0.59	0.90	0.33	0.005	0.001

$^{v-z}$Within a column, means without a common superscript letter differ ($P < 0.05$).
[1]Data based on 72 pens (8 pens per treatment) with 4 pigs per pen, during a 28-d feeding period.
[2]Indicates a linear response to the phytase addition, including the 2 phytases ($P < 0.01$).
[3]Data are based on 72 pigs (8 pigs per treatment; 1 pig from each pen).
[4]FTU = phytase units.
[5]Probability of a dietary treatment effect.

effects of block (as a random variable), dietary treatment, sex, and the dietary treatment × sex interactions. None of the interactions were significant ($P > 0.05$), and they were removed from the model for the final analysis. To test for curvilinearity in response to phytase inclusion level, linear or quadratic terms were tested by fitting first and second-degree polynomials.

Least squares means were derived for all treatments and were compared using the PDIFF and STDERR options of SAS. When the polynomials were significant, data were reanalyzed using PROC REG procedure of SAS, including linear or quadratic effects of phytase inclusion level. When quadratic responses were detected, the inflexion point was estimated by the first derivative method. In the finishing pig study, bone weight was different among treatments and was, therefore, included as a covariate in the model to analyze bone ash.

RESULTS

There were no interactions between main effects (diet and sex) for any variable in any of the studies, and thus, only means for dietary treatments are presented.

Nursery Pig Study

There was no effect of dietary treatment on ADFI and ADG (Table 3). The G:F was lower ($P < 0.05$) for the negative compared with the positive control and improved linearly ($P < 0.001$) with phytase addition, with an increase of 100 FTU in phytase resulting in an increase in G:F of 5 g/kg [G:F = 538 (± 7) + 0.005 (± 0.003) × phytase level (FTU/kg); $P < 0.02$; $r^2 = 0.20$]. At inclusion rates of 250 and 500 FTU/kg, there were no differences between Natuphos and Phyzyme for G:F. Bone ash percentage was lower ($P < 0.01$) in both the negative control and Natuphos at 250 FTU/kg than in the positive control. Fibula ash percentage increased linearly with increasing dietary Phyzyme [ash = 42.899 (± 0.37) + 0.0005 (± 0.0001) × phytase level (FTU/kg); $P < 0.001$; $r^2 = 0.28$]. When Phyzyme was added at 500 FTU/kg or greater, bone ash was not different ($P > 0.50$) from the positive control diet.

Grower Pig Study

There was no effect of dietary treatment on ADFI (Table 4). Pigs fed the negative control diet had reduced ($P < 0.01$) ADG compared with those fed the positive control diet. Average daily gain increased linearly ($P < 0.001$) as phytase inclusion increased. At 500 FTU/kg or greater, ADG was greater ($P < 0.05$) for the phytase treatment than for the negative control but was similar ($P > 0.10$) to the positive control. Pigs supplemented with 10,000 FTU/kg grew 7% faster ($P < 0.05$) than pigs fed the positive control. Gain:feed ratio was greater ($P < 0.05$) for the positive than the negative control and was increased linearly ($P < 0.001$) by phytase addition; G:F was 6% greater ($P < 0.05$) at 10,000 FTU/kg compared with the positive control. There were no differences for growth performance between the 2 enzymes at the same inclusion level. Bone ash of pigs fed the negative control diet was 4.4 percentage units lower ($P < 0.05$) than those fed the positive control diet (Table 4). Natuphos at 500 FTU/kg or Phyzyme at all levels gave similar ($P > 0.10$) bone ash values to the positive control (except for the tolerance dose that increased the bone ash over the positive control by 6%). Phyzyme addition increased bone ash linearly by 0.5 percentage units per 100 FTU/kg rela-

Table 4. Effect of phytase source and level on growth performance and fibula ash (grower pig study)

Item	Initial BW,[1] kg	ADFI,[1] g/d	ADG,[1,2] g/d	G:F,[1,2] g/kg	Fibula ash,[2,3] %
Dietary treatment					
1. Positive-control, adequate P	24.15	1,843	831^{y}	453^{xy}	51.05^{xy}
2. Negative-control, deficient P	24.33	1,731	756^{x}	436^{w}	46.67^{v}
3. As 2 + Phyzyme 250 FTU[4]/kg	24.20	1,804	793^{xy}	439^{wx}	51.02^{xy}
4. As 2 + Phyzyme 500 FTU/kg	24.05	1,821	811^{y}	445^{wxy}	49.98^{wx}
5. As 2 + Phyzyme 750 FTU/kg	24.12	1,838	838^{y}	456^{yz}	52.48^{yz}
6. As 2 + Phyzyme 1,000 FTU/kg	24.22	1,820	820^{y}	450^{xy}	52.44^{yz}
7. As 2 + Phyzyme 10,000 FTU/kg	23.97	1,879	890^{z}	474^{z}	54.18^{z}
8. As 2 + Natuphos 250 FTU/kg	23.95	1,741	751^{x}	431^{w}	47.47^{vw}
9. As 2 + Natuphos 500 FTU/kg	24.64	1,831	809^{y}	443^{wxy}	49.44^{wx}
Pooled SEM	1.265	82.5	31.1	9.7	1.048
Probability[5]	0.92	0.44	0.005	0.001	0.001

$^{v–z}$Within a column, means without a common superscript letter differ ($P < 0.05$).
[1]Data are based on 72 pens (8 pens per treatment) with 4 pigs per pen, during a 42-d feeding period.
[2]Indicates a linear effect ($P < 0.001$) of phytase level.
[3]Data are based on 72 pigs (8 pigs per treatment; 1 pig from each pen).
[4]FTU = phytase units.
[5]Probability of a dietary treatment effect.

tive to the negative control [ash = 47.919 (± 0.81) + 0.005 (± 0.0013) × phytase level (FTU/kg); $P < 0.001$; $r^2 = 0.3$].

Finisher Pig Study

There was no effect of dietary treatment on ADFI (Table 5). Pigs fed the negative control diet had reduced ADG ($P < 0.01$) and reduced G:F ($P < 0.05$) compared with those fed the positive control diet (Table 5). Average daily gain did not differ ($P > 0.05$) between sources of phytase at the same inclusion rate and increased linearly [ADG = 0.91 (± 0.02) + 0.00012 (± 0.00003) × phytase level; $P < 0.001$; $r^2 = 0.18$] as phytase level increased (equivalent to an increase of 12 g/d for every 100 FTU/kg added). On average, G:F was 5% greater ($P < 0.02$) for Phyzyme than for Natuphos at the same inclusion level. The response of G:F with increasing phytase level was linear for Natuphos [G:F = 345 (± 9.5) + 0.036 (± 0.015) × phytase level; $P < 0.05$; $r^2 = 0.21$], increasing 3.6 g/kg for every 100 FTU/kg, but with Phyzyme the response was quadratic [G:F = 345 (± 8.2) + 0.143 (± 0.04) × phytase level – 9.68E-5 (± 3.7E-5) × phytase level^2; $P < 0.0001$; $r^2 = 0.40$] with maximum G:F (inflexion point) at 738 FTU/kg.

Table 5. Effect of phytase source and level on growth performance and fibula ash (finisher pig study)

Item	Initial BW,[1] kg	ADFI,[1] kg/d	ADG,[1,2] g/d	G:F,[1,2,3] g/kg	Fibula ash,[2,4] %
Dietary treatment					
1. Positive-control, adequate P	54.08	2.66	995^{yz}	374^{xyz}	53.85^{yz}
2. Negative-control, deficient P	54.11	2.57	884^{x}	346^{w}	50.39^{x}
3. As 2 + Phyzyme 250 FTU[5]/kg	54.11	2.52	938^{xy}	373^{xy}	53.54^{y}
4. As 2 + Phyzyme 500 FTU/kg	54.08	2.54	996^{yz}	393^{yz}	53.96^{yz}
5. As 2 + Phyzyme 750 FTU/kg	54.19	2.57	1,019^{yz}	397^{z}	54.74^{yz}
6. As 2 + Phyzyme 1,000 FTU/kg	54.34	2.68	1,041^{z}	391^{yz}	55.60^{yz}
7. As 2 + Natuphos 500 FTU/kg	53.80	2.78	992^{yz}	361^{wx}	53.76^{yz}
8. As 2 + Natuphos 1,000 FTU/kg	53.97	2.61	991^{yz}	381^{xyz}	56.46^{z}
Pooled SEM	2.159	0.108	30.5	9.9	1.109
Probability[6]	0.955	0.701	0.023	0.001	0.01

$^{w–z}$Within a column, means without a common superscript letter differ ($P < 0.05$).
[1]Data are based on 64 pens (8 pens per treatment) with 4 pigs per pen, during a 60-d feeding period.
[2]Indicates a linear effect ($P < 0.001$) of phytase level.
[3]Indicates a quadratic effect ($P < 0.001$) of phytase level.
[4]Data are based on 64 pigs (8 pigs per treatment; 1 pig from each pen). Bone weight was used as a covariate.
[5]FTU = phytase units.
[6]Probability of a dietary treatment effect.

Table 6. Effect of the Ca to available P (aP) ratio or phytase source and level on the digestibility of Ca and P (digestibility study)

	Treatment[1]								
Item	1	2	3	4	5	6	7	SE	*P*-value
Ca digestibility,[2] %	48.48^{wx}	44.98^{w}	48.10^{wx}	57.59^{yz}	59.76^{z}	53.54^{xyz}	50.48^{wxy}	2.362	0.001
Ca absorbed, g/kg of diet	2.71^{wx}	2.51^{w}	2.69^{wx}	3.22^{yz}	3.34^{z}	2.99^{xyz}	2.82^{wxy}	0.132	0.001
Ca released, g/100 FTU consumed				0.17^{z}	0.17^{z}	0.08^{y}	0.03^{y}	0.031	0.05
P digestibility,[3] %	28.90^{v}	34.76^{wx}	39.67^{y}	38.92^{xy}	44.33^{z}	34.09^{w}	36.62^{wxy}	1.639	0.0001
P absorbed,[3] g/kg of diet	1.09^{v}	1.58^{xy}	1.94^{z}	1.47^{wx}	1.67^{y}	1.28^{w}	1.38^{wx}	0.066	0.0001
P released, g/100 FTU consumed				0.16^{y}	0.18^{y}	0.06^{z}	0.06^{z}	0.023	0.005
P released,[4] %				0.051^{yz}	0.067^{z}	0.027^{x}	0.041^{xy}	0.011	0.0001

$^{v–z}$Within a row, means without a common superscript letter differ ($P < 0.05$).

[1]Treatments 1–3 contained P supplementation with Ca:aP ratios of 7.9, 3.9, and 2.9, respectively. The ratio between Ca and aP was accomplished by KH_2PO_4 supplementation. Treatments 4–5 contained Phyzyme with a Ca:aP ratio of 7.9 for both treatments and a phytase concentration of 302 and 377 phytase units FTU/kg, respectively. Treatments 6–7 contained Natuphos with a Ca:aP ratio of 7.9 for both treatments and phytase concentration of 382 and 623 FTU/kg, respectively. Data for phytase concentration are based on the analyses of 8 samples per treatment.

[2]Indicates a linear increase ($P < 0.005$) in Ca digestibility by Phyzyme.

[3]Indicates a linear increase ($P < 0.005$) in P digestibility by the Ca:aP ratio or by the phytase level.

[4]Calculated from the standard curve linear regression equation: Y (P absorbed, g/d) = a + bx (x = P intake from KH_2PO_4, g/d). Phosphorus absorbed, for diets with phytase, was substituted into the standard curve linear regression equation (Y = 2.140 + 0.825 x, $r^2 = 0.80$) to solve for x. This was then divided by daily feed intake to arrive at the P release value.

Bone ash of pigs fed the negative control diet was 3.5% lower ($P < 0.01$) than for pigs fed the positive control diet (Table 5). At the same inclusion rate, Phyzyme and Natuphos gave similar ($P > 0.05$) bone ash values. As phytase inclusion increased, bone ash increased linearly [ash = 60.34 (± 2.94) + 0.798 (± 0.215) × bone weight + 0.0045 (± 0.0012) × phytase level; $P < 0.0001$; $r^2 = 0.3$] by 0.45% units for each 100 FTU/kg of phytase added to the diet.

Mineral Digestibility Study

There was no treatment effect ($P > 0.50$) on any of the growth performance measurements including initial BW (55.9 ± 1.10 kg) or final BW (65.8 ± 1.46 kg), ADFI (2.0 ± 0.13 kg), ADG (825 ± 71 g), and G:F (0.412 ± 0.026).

The effect of sex on the apparent fecal digestibility of minerals was significant only for Ca, where gilts had greater values than barrows (53.73 vs. 49.97 ± 1.25%; $P < 0.05$). There was no effect ($P > 0.05$) of Ca:available P ratio or Natuphos enzyme level on the quantity of Ca absorbed (Table 6). In contrast, Ca absorption increased in response to Phyzyme additions ($P < 0.001$). The estimated Ca release per 100 FTU averaged 0.17 g for Phyzyme ($P < 0.05$) and 0.05 for Natuphos ($P > 0.10$).

Feeding the low-P control diet resulted in a lower ($P < 0.001$) P digestion coefficient than when feeding diets with additional P from KH_2PO_4. Digestibility coefficients and absorbed *P* values increased linearly ($P < 0.001$) as phytase was added to the negative control diet. Furthermore, P digestion coefficients for pigs fed the phytase-supplemented diets were similar or even greater (at the greatest dose of Phyzyme; $P < 0.05$) than those of the adequate P control diet. The standard curve developed from the additions of KH_2PO_4 [i.e., P absorbed = 0.468 (± 0.24) + 0.843 (± 0.075) × daily P intake; $P < 0.001$, $r^2 = 0.90$] was used to estimate the efficiency of the enzymes in liberating P from the diet. Phosphorus absorption increased linearly ($P < 0.001$) as phytase intake increased, and the increase was greater for Phyzyme (0.167 g per 100 FTU) than for Natuphos (0.061 g per 100 FTU). With the use of phytase, the apparent fecal digestibility of P improved over the control by 44% for Phyzyme and 22% for Natuphos ($P < 0.001$).

DISCUSSION

The objective of the growth performance studies was to compare the effect of 2 different phytase enzymes when added to available P-deficient diets during 3 different phases of pig growth (i.e., nursery, grower, and finisher). The reduction of available P compared with the requirement (NRC, 1998) in the negative control diets were 34, 44, and 60% for the nursery, grower, and finisher studies, respectively, and the length of the restriction lasted for 28, 42, and 60 d, respectively. Differences in available P in the positive and negative-control diets were created mainly from additions of limestone and dicalcium phosphate to keep a constant Ca level. This approach resulted in different Ca:available P ratios than in the negative-control diets that would exacerbate the effects of the available P deficiency (Vipperman et al., 1974; Liu et al., 1998; Brady et al., 2002). The negative-control diet had no effect on feed intake but reduced ($P < 0.05$) growth rate (grower and finisher studies only), G:F, and bone ash content compared with the positive-control diet.

Nursery Pig Study

Feed intake and BW gain were not different between the positive and negative control diets. Previous stud-

ies with nursery pigs that showed reduced feed intake and growth rate for the negative compared with the positive control generally used a lower level of available P relative to requirement than in the current trial. Yi et al. (1996) reported that the effect of phytase supplementation on pig performance depended on the degree of available P restriction, being greater at low dietary available P concentration and not apparent when the diet is supplemented with 50% or greater of the available P requirement.

In our study, phytase inclusion to a P-deficient diet positively affected feed efficiency and bone ash. Phyzyme addition at 500 FTU/kg or greater improved feed efficiency, and the high dose (10,000 FTU/kg) was not different from the positive control. Similarly, Augspurger et al. (2004) reported that in weanling pigs, despite a linear growth response to phytase addition, the response was never better than that of the positive control, except for bone ash, which continued to respond linearly to enzyme addition above the level of the positive control.

Bone criteria are more sensitive and reliable indicators of P bioavailability than growth performance (Koch and Mahan, 1985); furthermore, storage of Ca and P in bone continues even after the dietary need for other functions have been met (Vipperman et al., 1974; Mahan, 1982). Phytase addition linearly increased bone ash percentage. The average bone ash percentage in pigs fed the negative control diets is in agreement with values previously reported for piglets (Mahan, 1982; Armstrong et al., 2000) and grower-finisher pigs (Brady et al., 2002). Also, pigs fed Phyzyme at 10,000 FTU/kg (tolerance dose treatment) deposited 15% more bone ash than those fed the negative control diet and 5% more than those fed the positive control diet, which indicates an increase in available P. This extra available P may not manifest in improved performance because gain and feed efficiency in nursery pigs are maximized at approximately 0.1% less P than that required for maximum bone ash (Mahan, 1982).

From the weanling pig study, we conclude that the addition of Phyzyme was effective in improving growth performance and bone ash and that the tolerance dose was not detrimental.

Grower and Finisher Pig Studies

Differing from the nursery study that lasted only 28 d, the length of the grower and finisher pig studies were 42 and 60 d, respectively. This longer time period obviously allowed more time for the expression of negative effects due to available P deficiency. Both grower and finisher pigs fed the positive-control diet performed considerably better than those fed the negative-control diet, with on average across the studies 11% faster gain, 6% better feed efficiency, and 8% more bone ash. At 10,000 FTU/kg, Phyzyme increased BW gain, G:F, and bone ash by 17.7, 8.7, and 24.6%, respectively, relative to the negative control.

The poor performance of finisher pigs fed the negative control diets in our experiment contrast with the results of Mavromichalis et al. (1999), who found that omitting two-thirds of the supplemental P did not impair growth performance of late-finishing pigs (87 to 120 kg). Moreover, Peter et al. (2001) removed both inorganic P and some trace minerals from a diet for pigs ranging from 80 to 123 kg of BW and did not find any deleterious effect on growth performance. In our study, however, pigs were on trial from 53 ± 2.2 to 113 ± 4.3 kg of BW, and negative responses to P-deficient diets were observed during this longer feeding period.

In general, increasing the inclusion of each of the phytase enzymes in the diet resulted in linear increases in not only ADG and feed efficiency but also bone ash. One explanation for the increased feed efficiency could be that the extra P liberated by the phytase enzyme might have allowed a greater rate of protein deposition in the pigs. Vipperman et al. (1974) fed various levels of Ca and P and observed that increasing dietary P resulted in increased nitrogen retention. In growing pigs, greater rates of nitrogen retention are associated with greater rates of feed efficiency because of the associated water deposition (Just, 1984).

Different feed efficiency responses between the phytase enzymes could be related to their specific efDifferent feed efficiency responses between the Sands, 2003; Johnston et al., 2004). Deleterious effects of the phytate molecule have been known for more than 30 yr. Davies and Nightingale (1975) showed a negative effect of phytate on mineral availability. Unfortunately the relationships between phytate and the macronutrients (i.e., energy and protein) have not been well established. However, much debate has been generated by contradictory findings. The biochemical characteristics of the phytate molecule (myoinositol 1,2,3,4,5,6 hexa kis dihydrogen phosphate) make it strongly negatively charged at all pH values normally encountered in feeds, and this creates the potential for binding positively charged molecules like proteins (Cheryan, 1980). Those binding effects are stronger in the presence of high dietary Ca concentrations (Adeola and Sands, 2003). Although some studies have shown an effect of phytase in increasing the digestibility of protein, amino acids, and nitrogen (with growing pigs, Johnston et al., 2004; male broilers, Ravindran et al., 2000; lactating sows, Baidoo et al., 2003), others have not (with growing pigs, Traylor et al., 2001; in young chicks, Peter and Baker, 2001, and Augspurger and Baker, 2004).

The greater growth rates such as those observed with the high (10,000 FTU/kg) Phyzyme level could be explained by the fact that the phytase enzyme promoted a greater availability of nutrients other than P. In support of this theory, Ravindran et al. (2000) reported that supplemental phytase increased the apparent metabolizable energy of the diet. Also, John-

ston et al. (2004) reported a similar effect with ileal-cannulated pigs whereby phytase when combined with diets low in Ca and P may increase the average ileal digestibilities of amino acids, starch, gross energy, and DM.

Mineral Digestibility Study

The objective of this study was to investigate the Ca- and P-releasing efficacy of the 2 phytase enzymes used in the previous performance studies. Whereas dietary levels of Ca were kept at the requirement (NRC, 1998) for all the treatments, available P concentration was increased by the use of a highly available source of P (KH_2PO_4) or by the use of phytase.

The average digestibility values for Ca and P (48.5 and 29%, respectively) in pigs fed the negative control diet are in agreement with values previously reported for growing pigs (Yi et al., 1996; Kemme et al., 1997). However, there are some discrepancies between the apparent fecal digestibility coefficients reported here and those by other authors (O'Quinn et al., 1997; Harper et al., 1997; Baidoo et al., 2003). These differences among studies can be explained not only by the dissimilar concentration and sources of total and available P in the diets but also by the different Ca:P ratios. This is because an adverse effect of Ca on the digestibility of P has been reported when diets are formulated with wide Ca:P ratios (Mahan, 1982; Qian et al., 1996), and that effect is even greater at low available P levels (Hall et al., 1991; Cromwell et al., 1995). Fernández (1995) demonstrated that variation in digestibility coefficients can be related to differences in absorption between different P sources (Fernández, 1995) and also to the physiological stage of the animals because age rather than live weight determines the absorption capacity of the pig (Fernández, 1995; Kemme et al., 1997).

Under normal intake conditions, dietary Ca absorption is regulated at the level of the digestive tract (Fernández, 1995), and with greater availability, the absorption and net balance of Ca will increase. Our results show that Ca absorption did not change with the increase in available P from KH_2PO_4, but it increased by 7 and 21% with supplemental Natuphos and Phyzyme, respectively. Even though some reports have shown no effect of phytase addition on Ca digestibility (Yi et al., 1996; Harper et al., 1997), others have shown that phytase can increase Ca digestibility in growing pigs but not in sows (Kemme et al., 1997). Moreover, Johnston et al. (2004) found that phytase increased the ileal digestibility of Ca.

Excess Ca is known to affect the performance of pigs by interfering with P absorption and usage (Vipperman et al., 1974; Mahan, 1982). It is also known that the optimum Ca:P ratio varies with the level of Ca and P in the diet (Vipperman et al., 1974) and that this ratio is even more relevant at low P levels (Hall et al., 1991). Qian et al. (1996) reported that narrowing the Ca:P ratio from 2 to 1.2 led to a 16% increase in phytase efficiency; similar results were produced in low P diets (Liu et al., 1998). Also, an interaction between Ca:P and phytase has been reported where P digestibility was improved by phytase only at low Ca:P ratios (Brady et al., 2002). The phytase-induced increase in Ca digestibility, particularly with Phyzyme, has important implications in diet formulation. Because of the abundant supply and low cost of limestone, overages of Ca occur in commercial feeds (Hall et al., 1991). If phytase is included in those diets, there will be an overload of Ca that can artificially increase the P requirement and reduce the beneficial effects of the enzyme.

Compared with the requirement (NRC, 1998), the available P reduction in the basal diets was 55%. With phytase, P digestibility was improved over the negative control by 44% for Phyzyme and 22% for Natuphos. Thus, fecal excretions would be reduced for the phytase-supplemented diets, showing that even with the use of P-deficient diets, it is possible for phytase to reduce the excretion of P in the fecal material.

Our estimates of apparent fecal digestibility of P due to Natuphos are lower than others have reported. Augspurger et al. (2003) showed an increase in available P from the use of Natuphos by 0.16 g per 100 FTU (based in fibula ash concentration). Also, Harper et al. (1997) and Yi et al. (1996), based in apparent fecal digestibility, found an increase in available P of 0.17 and 0.14 g/100 FTU, respectively. However, the growth trials carried out as part of this study showed a greater response in growth performance and bone ash relative to the negative control for Phyzyme than for Natuphos supplementation, which supports the results of the digestibility study reported here.

The *E. coli* and *A. niger* phytases are known as specific enzymes for phytic acid. Whereas *E. coli* produces 6-phytases (i.e., those that preferentially yield L-myo-inositol 1,2,3,4,5-pentakisphosphate) as the first intermediates, *A. niger* produces 3-phytases, which give rise to D-myo-inositol 1,2,4,5,6-pentakisphosphate. Both phytase enzymes do not have broad substrate specificity but are specific for phytic acid (Wyss et al., 1999). The differences observed between Phyzyme and Natuphos in both performance and bone ash response are probably explained by differences in digestive effects of the enzymes on phytic acid. Thus, the 2 enzymes have different pH optima and also different affinity for the derivates of phytate (because the rate of hydrolysis is reduced differently for phytate intermediates containing less phosphate groups; Lim et al., 2000). It is also possible that both enzymes may be affected differently by the Ca:available P ratio, Natuphos being less active than Phyzyme when greater levels of Ca are present (Qian et al., 1996; Liu et al., 1998).

Phyzyme is derived from *Schizosaccharomyces pombe*, where a variety of plasmids have been developed to facilitate the molecular manipulation (Siam,

et al., 2004). Previous research (Ciofalo et al., 2003) has shown that Phyzyme is safe for animal use because it does not induce any in vitro genotoxicity or in vivo toxicity in rats. This was confirmed in our study because in the nursery and grower experiments, Phyzyme at 10,000 FTU/kg had no negative effects, demonstrating that Phyzyme is both safe and efficacious in pigs.

Apparently, Phyzyme has the ability to replace inorganic P supplementation, and its efficacy is as good as or better than Natuphos. The use of Phyzyme at a level of 10,000 FTU/kg was not detrimental, and at doses of 500 FTU or greater Phyzyme showed positive effects by releasing P bound to phytate from corn-soybean diets.

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