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Article *in* Journal of Animal Science · January 1993

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Published December 11, 2014

Supplementing Corn-Soybean Meal Diets with Microbial Phytase Linearly Improves Phytate Phosphorus Utilization by Weanling Pigs[1]

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ABSTRACT: Two experiments were conducted with weanling pigs to determine the effectiveness of a dietary supplement of *Aspergillus niger* phytase in improving the availability of phytate-P in corn-soybean meal diets without supplemental inorganic P. Experiment 1 consisted of two P and Ca balance trials and two feeding trials. Twelve pigs (8.18 ± .44 kg BW) were housed individually in stainless steel metabolism cages. Six pigs received 750 phytase units (**PU**)/g of basal diet and the other six pigs received the basal diet without supplemental phytase as control. In Exp. 2, 96 pigs (8.81 ± .75 kg BW) were allotted to 16 partially slotted floor pens and their basal diets were supplemented with either 0, 250, 500, or 750 PU/g for 4 wk. Individual pig weights and pen feed consumption were measured weekly. Blood samples were taken from all pigs at the end of each trial in Exp. 1 and from three pigs per pen weekly in Exp. 2 to measure serum (plasma) inorganic P (P) and Ca concentrations and alkaline phosphatase (**AP**) activities. The results of Exp. 1 indicated that dietary phytase increased P retention by 50% ($P < .0001$) and decreased fecal P excretion by 42% ($P < .0001$). Pigs that received dietary phytase had serum P and Ca concentrations and serum AP activities that were nearly normal, whereas control pigs had values indicative of a moderate P deficiency. Favorable effects of phytase disappeared when the phytase was removed from the diet. The results of Exp. 2 indicated a linear increase in plasma P ($P < .001$), ADG ($P < .07$), and ADFI ($P < .01$) with increased dietary phytase activity. Plasma AP activity decreased linearly with increased dietary phytase activity up to 500 PU/g of diet. Gain/feed and plasma Ca concentration seemed to be unaffected by dietary phytase activity. In conclusion, supplements of *Aspergillus niger* phytase up to 750 PU/g of feed in corn-soybean meal diets of weanling pigs resulted in a linear improvement in utilization of phytate-P.

Key Words: Pigs, Phytase, Phosphorus, Calcium, Phosphorus, Alkaline Phosphatase

J. Anim. Sci. 1993. 71:3359–3367

Introduction

More than 60% of the P in corn and 50% of the P in soybean meal is in the form of phytate, which is poorly available to pigs and other simple-stomached animals (Reddy et al., 1982). Attempts have been made to use microbial phytases to improve the availability of phytate-P in these feeds. Nelson et al. (1971) fed 1-d-old chicks with an acetone-dried preparation of phytase from *Aspergillus niger* and demonstrated an improvement in availability of phytate-P from corn and soybean meal. Recently, positive effects of dietary supplemental phytase produced by the same fungal species on phytate-P utilization in broilers and pigs have been demonstrated by Nasi (1990) and Simons et al. (1990). In contrast, feeding *Saccharomyces cerevisiae* or other phytase-containing yeast cultures to pigs failed to show improvements in phytate-P availability (Cromwell and Stahly, 1978; Chapple et al., 1979; Shurson et al., 1983). However, these researchers used phytase products with undefined activity and(or) used mainly weight gain as the response measure. Therefore, the present two experiments were conducted with weanling pigs to determine 1) the efficacy of supplements of *Aspergillus niger* phytase to a corn-soybean meal diet without supplemental inorganic P on P and Ca balance, serum inorganic P (P) and Ca concentrations, and serum alkaline phosphatase (AP) activity; 2) the possible carryover effects of phytase feeding on serum P and Ca concentrations and AP activity after phytase withdrawal; and 3) the statistical relationship between supplemental dietary phytase activity and the

[1]The authors are grateful to Alko Ltd., Rajamaki, Finland, for providing the phytase and partial financial support of this research project.

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Received May 11, 1992.

Accepted July 21, 1993.

resultant improvement in dietary phytate-P utilization as measured by performance and plasma P status.

Materials and Methods

Phytase. The microbial phytase used in this study was produced by *Aspergillus niger* (var. *ficuum*). The enzyme product was provided by Alko Ltd. (Rajamaki, Finland) and the specific activity of the product was expressed as phytase units (PU)/gram (one PU is defined as the amount of enzyme that liberates 1 nmol of inorganic P from sodium phytate per minute at pH 5 and 37°C). Actual phytase activity (5×10^5 PU/g) was confirmed by the assay method of Han et al. (1987) before the product was mixed with other feed ingredients in the preparation of the complete diet.

Animals and Treatments. All pigs used in the two experiments were 4-wk-old crossbred (Landrace × Yorkshire × Hampshire) weanling pigs. In Exp. 1, 12 pigs (8.18 ± .44 kg BW) were allotted, based on litter, BW, and sex into two groups receiving supplemental phytase (+ phytase) or no supplemental phytase as control (− phytase). Pigs were housed in individual stainless steel metabolism cages and fed the low-P, basal diet (Table 1) for 2 wk to adjust and deplete P reserves before the formal trials began. Four consecutive trials were then conducted as follows: Trial 1, first P and Ca balance for 7 d; Trial 2, feeding trial (ad libitum access to diets and water) for 10 d; Trial 3, second P and Ca balance for 7 d; and Trial 4, feeding trial (ad libitum access to diets and water) for 14 d. Pigs in the + phytase group received 750, 750, and 687 PU/g of basal diet in Trials 1, 2, and 3, respectively. Supplemental phytase activity in Trial 3 was reduced to approximately 90% of that in Trials 1 and 2 based on the increase in age and in feed intake of pigs. In Trial 4, these pigs were not fed the diet supplemented with phytase but were fed the same basal diet as fed to the control group to determine whether there was any carryover effect of phytase feeding on blood serum P status. In Exp. 2, 96 pigs (8.81 ± .75 kg BW) were split equally into heavy and light blocks based on BW. Within each block, 48 pigs were allotted (based on litter, BW, and sex) to eight pens with six pigs each. Four dietary levels of supplemental phytase activity, 0, 250, 500, and 750 PU/g of basal diet, were assigned randomly to pens twice in each block and four times in the whole experiment. Pigs were reared in environmentally controlled housing (temperature, 22 to 25°C and light:dark cycle, 12 h) with a partially slotted floor and given ad libitum access to feed and water. All pigs were fed the low-P, basal diet for 1 wk to deplete P reserves before the formal trial.

Basal Diets. The basal diets were fortified corn-soybean meal diets without supplemental inorganic P (Table 1). The diets provided adequate levels of all nutrients (NRC, 1988) with the exception of P, Ca, and lysine in Exp. 1 and P and Ca in Exp. 2. Calcium carbonate was added to provide a calculated Ca:P ratio of approximately 1.5 in the basal diets. Concentrations of Ca and P in all experimental diets were analyzed (Table 2). Crystalline lysine was not incorporated into the diets in Exp. 1. Because we were uncertain initially of the effectiveness of phytase in releasing phytate-P from the basal diets, we considered that it might be important to reduce dietary Ca and lysine concentrations proportionately with the low

Table 1. Composition and nutritive values of basal diets

Item	Exp. 1	Exp. 2
	g/kg	
Ingredient		
Ground, shelled corn	780.0	777.4
Soybean meal (44% CP)	200.0	200.0
Calcium carbonate (38% Ca)	10.0	10.0
L-lysine·HCl	—	2.6
Salt (NaCl)	3.5	3.5
Vitamin-trace mineral premix[a]	3.0	3.0
Vitamin E-Se premix[b]	3.0	3.0
Antiobiotic premix[c]	.5	.5
Calculated nutritive values (as-fed)		
ME, MJ/kg	13.8	13.8
CP, g/kg	155.0	155.0
Lysine, g/kg	7.8	10.2
Ca, g/kg	4.4	4.4
P, g/kg	3.2	3.2

[a]Supplied the following amounts per kilogram of diet: vitamin A, 1,980 IU; vitamin D_3, 396 IU; menadione, 3.3 mg; riboflavin, 2 mg; niacin, 11 mg; d-pantothenic acid, 8 mg; choline, 66 mg; vitamin B_{12}, 12 μg; Zn, 45 mg; Fe, 35 mg; Mn, 20 mg; Cu, 6 mg; I, .12 mg.
[b]Supplied 10 IU of vitamin E and .2 mg of Se per kilogram of diet.
[c]Supplied 55 mg of chlortetracycline per kilogram of diet.

Table 2. Analyzed dietary Ca and P concentrations of experimental diets[a]

Item	Phytase	Ca	P
	PU/g	g/kg	
Experiment 1			
Trial 1			
− Phytase	0	6.8	3.1
+ Phytase	750	5.8	3.2
Trial 2			
− Phytase	0	6.8	3.1
+ Phytase	750	5.8	3.2
Trial 3			
− Phytase	0	7.1	3.5
+ Phytase	687	5.8	3.3
Trial 4	0	8.9	3.7
Experiment 2			
Diet 1, basal	0	5.0	2.8
Diet 2	250	5.5	3.2
Diet 3	500	5.0	3.1
Diet 4	750	5.0	3.3

[a]As-fed basis.

P concentration and to keep the ratios among these three nutrients close to that of NRC (1988).

Measurements and Sample Collection. In Exp. 1, P and Ca balance trials were conducted as previously described (Ilori et al., 1984). The length of the collection period was 4 and 3 d in Trials 1 and 3, respectively. Feces of individual pigs were collected daily and air-dried. Urine was collected into 2-L plastic containers daily, and a 10% well-mixed sample was stored at −20°C for P and Ca analyses. At the end of each trial, including the beginning and the end of the 1st wk of Trial 4, blood samples were taken from each pig and serum was prepared for assay of P and Ca concentrations and serum AP activity. Body weight also was recorded at each bleeding. In Exp. 2, individual pig weights and pen feed consumption were measured weekly. Blood samples were taken weekly from three pigs per pen (selected littermates with uniform BW across treatments) for assay of plasma P and Ca concentrations and plasma AP activity.

Assays. Concentrations of P in feed, feces, urine, and blood serum or plasma were determined by a colorimetric method (Gomori, 1942), and Ca concentrations were determined by flame atomic absorption spectrophotometry (Model IL 951, Instrumentation Laboratory, Wilmington, MA). Serum or plasma AP activity was determined on the day that blood samples were drawn by the method outlined by Sigma Chemical (1987).

Statistics. Differences in blood serum measures and ADG between pigs fed diets supplemented with or without phytase in Exp. 1 were analyzed statistically by simple *t*-test rather than time repeated measurement because of the switch in feeding method and the difference in supplemental phytase activity in the diets between different trials. However, P and Ca balance data in Trials 1 and 3 were pooled and analyzed by time repeated measurements because percentage of improvement and significance level of the improvement by supplemental phytase in the two trials were almost identical. There was also no interaction of trial (time) × treatment. Differences in apparent digestibility of P and Ca and percentage of retention/intake between treatment groups were expressed as absolute values. The results of Exp. 2 were analyzed as a randomized complete block design with four treatments (four phytase levels) in two blocks (heavy and light) with time repeated measurements (Gill, 1986). The pen was the experimental unit. Linear equations of dietary phytase activity with different measures were developed by procedures outlined by Gill (1978). Standard errors of mean difference (SED) and their corresponding approximate df of error were also listed and adjusted for interperiod correlations as described by Gill (1986). Significance level was set as $P < .05$ unless indicated otherwise. All analyses were conducted using SAS (1988) procedures.

Results

Experiment 1

Pooled Balance of Phosphorus and Calcium. With similar daily P intake, pigs fed phytase retained 50% more P daily ($P < .0001$) than control pigs (Table 3).

Table 3. Balance of P and Ca in pigs fed diets with or without supplemental microbial phytase[a].

Item	− Phytase	+ Phytase	SED[b]	$P <$
	P, mg/d			
Intake	1,801	1,809	95	.93
Fecal	966	561	67	.0001
Urinary	4.0	3.5	1.0	.64
Absorbed	835	1,248	84	.0001
% of Intake	46.4	69.0	2.9	.0001
Retained	831	1,245	84	.0001
% of Intake	46.2	68.8	2.9	.0001
% of Absorbed	99.5	99.7	.2	.09
	Ca, mg/d			
Intake	3,753	3,214	182	.014
Fecal	1,092	526	79	.0001
Urinary	615	460	153	.33
Absorbed	2,661	2,688	172	.88
% of Intake	70.9	83.6	2.0	.0001
Retained	2,046	2,228	250	.48
% of Intake	54.5	69.3	5.1	.02
% of Absorbed	76.9	82.9	5.8	.32

[a]Data presented here were pooled from the two balance trials.
[b]Standard error of difference of two means (df of error = 10).

Daily fecal P output was reduced by 42% ($P < .0001$) in pigs fed phytase. Daily urinary P loss of pigs was extremely small compared with their fecal P loss regardless of dietary phytase supplementation. This resulted in an almost complete retention of absorbed P in both control and phytase-fed pigs and an almost identical increase of 23% ($P < .0001$) in apparent digestibility of P and percentage of P retained/intake in pigs fed phytase.

Daily Ca intake of the control pigs was 14% higher ($P < .01$) than that of pigs fed phytase, due to the somewhat higher Ca concentration of the control diet. But, neither daily Ca absorbed nor daily Ca retained in control pigs was increased. In contrast, pigs fed phytase retained slightly more Ca and had 13% ($P < .0001$) and 14% ($P < .02$) increases in apparent digestibility of Ca and percentage of Ca retained/intake, respectively. Daily fecal Ca output in pigs fed phytase was reduced by 52% ($P < .0001$), whereas daily urinary Ca output was not different ($P < .33$) from that of control pigs. In addition, fecal P and Ca concentrations in pigs fed phytase were reduced by 45% (1.1 vs 2.0%, $P < .0001$) and 50% (1.1 vs 2.2%, $P < .0001$), respectively.

Serum Inorganic Phosphorus and Calcium Concentrations and Alkaline Phosphatase Activity. The initial concentrations of serum P in pigs of the two treatment groups were essentially the same (Table 4). However, serum P concentrations in pigs fed phytase increased to 7.0 mg/dL at the end of Trial 1 and stayed above this level in Trials 2 and 3. In contrast, serum P concentration in control pigs initially increased but then gradually decreased from 6.6 mg/dL at the end of Trial 1 to 4.5 mg/dL at the end of Trial 3. The difference in serum P between the two treatment groups was 2.3 mg/dL at the end of Trial 2 ($P < .0007$) and 3.0 mg/dL at the end of Trial 3 ($P < .0001$). Serum Ca concentration of pigs fed phytase was lower ($P < .001$) than that of the control pigs within these three trials. Serum AP activity of pigs fed phytase was lower ($P < .03$) than that of control pigs at the end of Trial 3.

The significant differences in serum P and Ca concentrations and AP activity between control and phytase-fed pigs disappeared in Trial 4 when phytase-fed pigs were switched to the same low-P, basal diet as that fed to control pigs. Serum P concentrations in the previously phytase-treated pigs decreased and serum Ca increased to the levels of control pigs. Serum AP activity was also similar to that of control pigs after phytase was removed from the diet, but it did not change as much as the concentrations of serum P and

Table 4. Serum inorganic P and Ca concentrations and serum alkaline phosphatase activity of pigs fed diets with or without supplemental microbial phytase in Experiment 1

Trial	− Phytase	+ Phytase	SED[a]	$P <$
	Serum inorganic P, mg/dL			
Initial	5.4	5.2	.54	.84
Trial 1	6.6	7.0	.33	.22
Trial 2	5.1	7.4	.48	.0007
Trial 3	4.5	7.5	.48	.0001
Trial 4				
Wk 1	5.7	5.5	.41	.72
Wk 2	5.8	5.7	.40	.94
	Serum Ca, mg/dL			
Initial	12.3	12.9	.24	.05
Trial 1	12.3	11.0	.30	.001
Trial 2	13.9	10.8	.41	.0001
Trial 3	14.3	11.8	.33	.0001
Trial 4				
Wk 1	17.0	16.2	1.00	.43
Wk 2	14.8	15.0	.83	.86
	Serum alkaline phosphatase, U[b]/dL			
Initial	22.3	23.4	4.03	.78
Trial 1	25.1	22.5	2.24	.27
Trial 2	18.1	16.8	1.54	.44
Trial 3	22.5	16.5	2.37	.03
Trial 4				
Wk 1	17.4	15.4	2.44	.45
Wk 2	17.0	15.9	3.07	.74

[a]Standard error of difference of two means (df for error = 10).
[b]Unit of enzyme activity defined as that which produces 1 μmol of *p*-nitrophenol per minute under the conditions of the assay.

Ca. Compared with that at the end of Trial 3, serum AP activity and serum P concentration in control pigs in Trial 4 decreased by approximately 5 units (U)/dL and increased by 1 mg/dL, respectively. These changes may have been due to the switch from restricted feed intake in Trial 3 to ad libitum access to diets in Trial 4 and to an increase in age.

Weight Gain. Pigs fed phytase had a greater ADG ($P < .06$) than control pigs in Trial 2, when all pigs were allowed to consume their diets on an ad libitum basis (Table 5). The ADG of pigs and the difference between control and treated groups was relatively small in the two balance trials. In the final week of Trial 4, control pigs grew faster than pigs previously fed phytase, probably due to compensatory growth. However, the ADG of pigs was really measured only to indicate that pigs in both groups were in good health and were gaining weight in the experiment.

Experiment 2

Plasma Inorganic Phosphorus and Calcium Concentrations and Alkaline Phosphatase Activity. Plasma P concentrations increased linearly with increase in dietary phytase activity (Table 6). The responses could be represented by the following four linear equations:

Wk 1. $Y = 4.10 + .00292X$ ($P < .001$, $r = .97$);
Wk 2. $Y = 4.02 + .00322X$ ($P < .001$, $r = .98$);
Wk 3. $Y = 3.69 + .00377X$ ($P < .001$, $r = .96$);
Wk 4. $Y = 3.23 + .00346X$ ($P < .001$, $r = .99$);

where X = dietary phytase activity (PU/g) and Y = plasma P concentration (mg/dL). There was no maximum break point of plasma P concentrations among the three phytase-supplemented groups of pigs. Compared with suggested normal values (7 to 8 mg/dL, Ullrey et al., 1967), plasma P concentrations of pigs receiving no phytase were < 50% of normal, and those of pigs receiving the highest phytase activity (750 PU/g) were slightly below normal.

There was no effect of dietary phytase activity on plasma Ca concentration of pigs.

Plasma AP activity of pigs receiving no supplemental phytase increased from wk 1 to 4 and eventually exceeded ($P < .05$) that of the three groups of pigs receiving supplemental phytase at wk 4. Plasma AP activity decreased linearly ($P < .06$) with increase in dietary phytase activity from 0 to 750 PU/g of feed at wk 3 but only up to 500 PU/g of feed at wk 4. The relationships between plasma AP activity (Y, U/dL) and dietary phytase activity (X, PU/g) fit the following equations:

Wk 3. $Y = 21.68 - .0008X$ ($P < .06$, $r = .94$);
Wk 4. $Y = 26.94 - .0383X + .0000326X^2$
($P < .05$, $R^g = .99$).

Weight Gain, Feed Intake, and Gain/Feed. Pigs that received supplemental phytase grew faster ($P < .05$) than pigs that received no phytase at wk 3 and 4 (Table 7). There was no significant difference in ADG among the three groups of pigs fed phytase, but a linear increase in ADG with increase in dietary phytase activity was evident. The relationships between ADG (Y, g/d) in wk 4 and over the entire period and dietary phytase activity (X, PU/g) can be described by the following linear equations:

Wk 4. $Y = 358 + .159X$ ($P < .06$, $r = .98$);
Overall. $Y = 327 + .112X$ ($P < .07$, $r = .95$).

Feed intake (Y, g/d) increased linearly as dietary phytase activity (X, PU/g) increased, and the responses at wk 3 and wk 4 can be described by the following linear equations:

Wk 3. $Y = 867 + .253X$ ($P < .01$, $r = .95$);
Wk 4. $Y = 999 + .369X$ ($P < .0001$, $r = .97$).

Overall ADFI also responded linearly to the increase in dietary phytase activity: $Y = 812 + .208X$ ($P < .01$, $r = .97$). However, this equation should be interpreted with caution because of the interaction ($P < .001$) of treatment with time on ADFI.

Gain/feed was not significantly improved by the increase in dietary phytase level ($P < .29$).

Table 5. Daily gains of pigs fed diets with or without supplemental microbial phytase in Experiment 1[a]

Trial	− Phytase	+ Phytase	SED[b]	$P <$
Initial	65	71	20	.80
Trial 1	205	219	18	.48
Trial 2	370	436	32	.06
Trial 3	212	252	35	.28
Trial 4				
Wk 1	338	354	57	.79
Wk 2	411	275	101	.21

[a]Unit: grams per day.
[b]Standard error of difference of two means (df for error = 10).

Table 6. Plasma inorganic P and Ca concentrations and plasma alkaline phosphatase activity of pigs receiving graded dietary levels of supplemental microbial phytase activity in Experiment 2

Time	Phytase, PU/g of diet			
	0	250	500	750
	Plasma inorganic P, mg/dL			
Initial	5.2	5.0	5.4	5.6
Wk 1[a]	4.1	5.0	5.2	6.5
Wk 2	3.9	5.1	5.5	6.4
Wk 3	3.4	5.1	5.4	6.5
Wk 4	3.2	4.1	5.0	5.9
	(SED[b] = .32, df of error = 33)			
	Plasma Ca, mg/dL			
Initial	11.6	12.2	12.0	11.6
Wk 1	12.3	12.4	12.5	11.9
Wk 2	13.0	12.6	12.4	12.4
Wk 3	12.6	12.2	12.3	11.8
Wk 4	12.6	12.4	12.2	11.9
	(SED = .45, df of error = 22)			
	Plasma alkaline phosphatase, U[c]/dL			
Initial	18.4	20.7	16.6	18.8
Wk 1	16.6	20.6	16.6	18.1
Wk 2	29.7	21.2	18.9	17.4
Wk 3[d]	21.3	20.8	16.6	16.0
Wk 4[e]	26.9	19.7	15.7	16.6
	(SED = 2.25, df of error = 40)			

[a]Linear response in all 4 wks ($P < .001$).
[b]Standard error of difference of means between any two dietary levels of phytase activity at a given week and the approximate df of error were adjusted for interperiod correlations.
[c]Unit of enzyme activity defined as that which produces 1 μmol of *p*-nitrophenol per minute under the conditions of the assay.
[d]Linear response ($P < .06$).
[e]Quadratic response ($P < .05$).

For all the measures taken in Exp. 2, there was no interaction between dietary phytase activity and block (weight). Consequently, block effect was not included in Tables 6 and 7.

Discussion

Results of the balance studies strongly indicate that addition of *Aspergillus niger* phytase at 750 PU/g to a corn-soybean meal diet improved phytate-P utilization in weanling pigs. Given the similar daily intake of P, pigs that received supplemental phytase had a 23% greater ($P < .0001$) apparent digestibility of P than pigs that received no supplemental phytase. Correspondingly, daily fecal P excretion was reduced by 42% ($P < .0001$) in these pigs. Simons et al. (1990) have reported that addition of *Aspergillus niger* phytase at 1,000 PU/g of feed (phytase units are converted to the unit as defined in our study when other studies are discussed) to diets of growing pigs (35 to 70 kg) increased apparent digestibility of P by 24% and decreased the amount of P in the feces by 35%. Nasi (1990) and Leunissen and Young (1992) demonstrated that supplements of the same phytase as used in this study at 500 PU/g of diets increased apparent digestibility of P to the same level as that of their positive control diets. However, the increase over the diet unsupplemented with phytase was 11% in the study of Leunissen and Young (1992) and accounted for only 50% of that obtained in studies of Nasi (1990) and Simons et al. (1990) and in this study. The relatively small improvement may be explained by the relatively high dietary concentration of total P (.55% with .04% calcium phosphate supplementation) and high apparent digestibility of P in the basal diet (60%). In addition, both Nasi (1990) and we have shown the same amount of increase in percentage of P retained/intake as that of apparent digestibility of P. An increase of 10% in the percentage of P retained/absorbed due to 500 PU/g of diet was found in the study of Nasi (1990). A marginal effect of phytase on P retained/absorbed was also demonstrated in this study, but the improvement may be too small to consider. Addition of phytase to diets has also been shown to increase bone strength and P concentration (Cromwell et al., 1991; Leunissen and Young, 1992). Therefore, *Aspergillus niger* phytase seems to increase phytate-P utilization as well as digestion.

Table 7. Daily gain, feed intake, and gain/feed of pigs receiving graded dietary levels of supplemental microbial phytase activity in Experiment 2

Time	Phytase, PU/g of diet			
	0	250	500	750
	Daily gain, g			
Initial	108	139	133	156
Wk 1	242	295	307	294
Wk 2	300	326	365	365
Wk 3	371	452	436	462
Wk 4[a]	351	411	433	475
Overall[b]	316	371	385	405
	(SED[c] = 29, df of error = 38)			
	Feed intake, g/d			
Wk 1	557	666	679	667
Wk 2	762	784	842	835
Wk 3[d]	872	985	984	1,084
Wk 4[e]	992	1,122	1,144	1,292
Overall[f]	796	890	912	961
	(SED = 54, df of error = 19)			
	Gain/feed, g/kg			
Wk 1	433	435	448	458
Wk 2	390	423	433	435
Wk 3	424	463	443	433
Wk 4	355	368	380	383
Overall	403	418	420	425
	(SED = 28, df of error = 44)			

[a]Linear response ($P < .06$).
[b]Overall mean from wk 1 to 4 and linear response ($P < .07$).
[c]Standard error of difference of means between any two dietary levels of phytase activity at a given week and the approximate df of error were adjusted for interperiod correlations. For overall mean comparisons, SED was 17, 47, and 15 for ADG, ADFI, and gain/feed, respectively (df of error = 11).
[d]Linear response ($P < .01$).
[e]Linear response ($P < .001$).
[f]Linear response ($P < .01$), but with an interaction ($P < .001$) of time × treatment on ADFI.

Supplemental microbial phytase seems to increase Ca utilization in addition to P utilization because apparent digestibility of Ca and percentage of Ca retained/intake were both increased by 13% in pigs fed supplemental phytase. However, this increase may be confounded with an effect of the lower daily Ca intake. Nevertheless, comparable increases in digestion and utilization of Ca by dietary phytase have been previously demonstrated (Nasi, 1990; Simons et al., 1990). Dietary phytase may increase Ca utilization indirectly by increasing P utilization because dietary Ca will be well utilized for skeletal growth only as dietary P is simultaneously utilized.

The effectiveness of phytase in improving phytate-P availability has also been shown by the responses in performance and measures of blood P status. In Exp. 1, pigs that received supplemental phytase maintained serum P and Ca concentrations and serum AP activity near to the normal range (Miller et al., 1964; Ullrey et al., 1967), whereas pigs that received no supplemental phytase developed a moderate P deficiency. In Exp. 2, plasma P concentration, ADG, and ADFI increased linearly as dietary phytase activity increased. More convincingly, favorable effects of dietary phytase on serum P and Ca concentrations and AP activity in pigs fed phytase in the first three trials of Exp. 1 disappeared when phytase was withdrawn from the diet. This rapid and consistent change not only indicates no carryover effect of phytase feeding on the measures of blood P status, but also confirms the effectiveness of phytase on phytate P utilization. The ineffectiveness of yeast phytase on phytate P availability in pigs (Cromwell and Stahly, 1978; Chapple et al., 1979; Shurson et al., 1983) may be attributed to 1) the possibility that activity of the yeast phytase products may have been insufficient to produce a response and(or) 2) a possible incompatibility of these yeast phytases with the low pH in the stomach of pigs. The *Aspergillus niger* phytases used in this study and by other researchers who obtained positive results (Nasi, 1990; Simons et al., 1990; Cromwell et al., 1991; Leunissen and Young, 1992) are acid- and heat-resistant over a broad pH and temperature range (pH 2.0 to 6.0, and up to 60°C). Therefore, we may reasonably expect these enzymes to be active and to function in the stomach of pigs under physiological conditions. This hypothesis has been confirmed by Jongbloed et al. (1992). Using two

simple T-cannulas in the duodenum and terminal ileum of pigs, they demonstrated that substantial degradation of phytate by supplemental dietary *Aspergillus niger* phytase (1,500 PU/g) took place in the gastro-duodenal region. Subsequently, liberated orthophosphates were absorbed in the small intestine, and fecal P excretion was greatly reduced. Hence, supplemental phytase may not only improve utilization of phytate-P in cereal-plant protein diets, but it may also alleviate or eliminate P pollution by reducing P in swine manure applied to the land, a severe problem facing the swine industry in some parts of the world today (Cromwell, 1991).

Plasma P concentration seems to be the most sensitive and convenient measure of dietary phytase on phytate-P utilization in this study. Supplements of dietary phytase at 750 PU/g of feed produced comparable improvement in P concentrations and AP activities in serum of pigs in Exp. 1 and in plasma of pigs in Exp. 2. However, significant differences in serum Ca concentrations between pigs that received no phytase and 750 PU/g of diet shown in Exp. 1 was not seen in plasma Ca concentrations in Exp. 2. This inconsistency may be partly accounted for by the differences in dietary Ca concentrations in the two studies.

The linear effects of dietary phytase activity up to 750 PU/g of diet on most of the measures of phytate-P utilization taken in this study were very consistent. This was reflected by the extremely high repeatability of the strong correlations and significance shown in the developed orthogonal polynomials. Similar response patterns in chicks to supplemental dietary phytase activity have been reported by Nelson et al. (1971) and Schöner et al. (1991). However, phytase dose-related responses in pigs seem to vary in different studies. A linear relationship ($P < .01$) between supplementing dietary phytase at 0, 500, and 1,000 PU/g of diet and ADG and bone strength in growing pigs was shown in one study (Cromwell, 1991), but the higher level of supplemental dietary phytase (1,000 PU/g) was more effective in improving only bone strength in another study (Cromwell et al., 1991). Similarly, there was no further beneficial effect on any of the response criteria by doubling supplemental phytase activity from 500 to 1,000 PU/g of diet in weanling pigs in the study of Leunissen and Young (1992). In comparison to the results of this study, the lack of further improvement in phytate-P utilization by dietary phytase activity > 500 PU/g of diet in the studies discussed above may be explained by the fact that 1) the growing-finishing pigs used by Cromwell et al. (1991) had a lower P requirement than the weanling pigs we used and 2) the basal diet used by Leunissen and Young (1992) had a higher total and available P concentration than our basal diets, due to .04% supplemented calcium phosphate and different feed ingredients, as mentioned above. Lower P requirement and higher dietary available P concentration would reduce the amount of phytate-P needed to meet the requirement of P in pigs, thereby making the higher dietary phytase activity unnecessary to release just this portion of P from phytate. Cromwell et al. (1991) did not observe any effect of phytase supplemented in the diets with adequate inorganic P. In addition, inorganic P is a strong inhibitor of phytase in vitro (Shieh and Ware, 1968; Gibson and Ullah, 1988).

Among all the measures made in this study, it seems that only the response of plasma AP activity was maximized at wk 4 of Exp. 2 at dietary phytase activity of 587 PU/g of diet. However, this level of phytase activity may not be the optimal dose that could maximize the improvement of phytate-P utilization because other response measures still showed linear increases up to 750 PU/g of diet. Even 750 PU/g of diet may still be inadequate for pigs to maintain normal plasma P concentrations (Ullrey et al., 1967). Therefore, higher dietary levels of phytase activity should be tested in future studies to determine the phytase activity at which response measures are maximum.

Implications

The *Aspergillus niger* phytase used in this study was effective in improving the availability of phytate-P in a corn-soybean meal diet for weanling pigs. Therefore, inorganic P supplementation, the third-largest expense in cereal grain-plant protein swine diets, would be greatly reduced. More importantly, fecal P excretion in pigs fed phytase was reduced by almost half. This will greatly alleviate or eliminate swine manure P pollution to the environment.

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