

# Effects of a Solid-State Fermented Phytase on Phosphorus and Nutrient Digestibility of Growing Pigs Fed Barley-Soybean Meal Based Diets

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## Story in Brief

A total of 24 barrows were utilized in a 21-d digestibility study to determine the effects of the addition of a solid-state fermented phytase complex (Allzyme SSF; Alltech, Inc) to low available P, barley-soybean meal diets on P and nutrient digestibility. The basal diet, which consisted of barley and soybean meal, was adequate in all nutrients except available P. This diet contained .42% total P (.11% avail. P), provided by barley and soybean meal. Diets 2, 3, and 4 were the basal plus SSF to provide 250, 500, and 1,000 phytase units (PU)/kg, respectively. All diets contained .77% digestible lysine and a Ca:total P ratio of 1.2:1. Pigs were housed individually. Experimental diets were fed at three times maintenance energy requirement with unlimited access to water. Feces, urine, and orts were collected to estimate nutrients digestibility and P excretion. The addition of SSF phytase complex increased average daily gain and gain:feed ratio. Digestibility of phosphorus, dry matter, organic matter, ash, gross energy, and nitrogen increased with the addition of the enzymatic complex (SSF phytase complex). Compared with the basal diet, digestibility of phosphorus for pigs fed 1,000 PU/kg increased by 39%. These results indicate the addition of SSF phytase complex to low P barley-soybean meal diets improved nutrient digestibility of growing pigs with a dramatic decrease in P excretion.

Keywords: Pigs, Phytase, Digestibility

## Introduction

Phytate (myoinositol 1,2,3,4,5,6 hexa, dihydrogen phosphate) is the major form of P in cereal grains and oilseed meals (Reddy et al., 1982). Approximately 70% of the P in cereal grains and oilseed protein supplements is organically bound in the form of phytate, which reduces phosphorus availability to pigs (Kornegay 1996; NRC 1998). Pigs cannot utilize phytate due to the lack of endogenous phytase that hydrolyzes phytic P (Peeler 1972). Improving phytate utilization can reduce the need for inorganic P addition in feed, resulting in reduced P excretion in manure. Addition of microbial phytase to the diet improves P utilization and decreases excretion (O'Quinn et al., 1997). Recently, solid-state fermentation (SSF) has been used to produce microbial phytase. The phytase produced by this system also contains other enzymes ( $\alpha$ -amylase,  $\beta$ -glucanase, protease, xylanase, and cellulase (Filer, 2001), that may improve carbohydrate and protein digestibility. Previous reports from our lab (Park et al., 2003ab) reported that the addition of SSF phytase to low P, corn-soybean based diets improved P utilization, growth performance, and bone traits. However, other nutrients were not affected by the addition of the SSF phytase up to 1,000 PU/kg. Because nutrient digestibility is relatively high in corn-soybean meal diets, the potential to improve digestibility of energy, protein, and dry matter is minimal. On the other hand, barley is known to have a lower feeding value for swine as compared with corn. Thus, there is greater potential to improve nutrient digestibility in barley based diets. Therefore, the objectives of this study were to determine the effects of the addition

of a solid-state fermented phytase complex to low P, barley-soybean meal diets on growth performance, nutrient excretion and digestibility of growing pigs.

### Material and Methods

Twenty-four crossbred barrows with an average BW of 11.0 lb were used in a 21-d study to investigate the effects of addition of SSF phytase complex (Allzyme SSF; Alltech, Inc) on growth performance and nutrient digestibility of growing pigs fed barley-soybean meal-based diets. Pigs were blocked by initial body weight and randomly allotted to one of four dietary treatments in a randomized complete block design. There were six replications per treatment.

The experimental diets (Table 1) were fed in meal form. The basal diet, consisting mainly of barley and soybean meal, was adequate in all nutrients, except available P. This diet contained .42% total P (.11% avail. P), all of which was provided by barley and soybean meal. Diets 2, 3, and 4 were the basal plus SSF phytase to provide 250, 500, and 1,000 phytase units (PU)/kg, respectively. All diets contained .77% digestible lysine and a Ca:total P ratio of 1.2:1.

<b>Table 1. Composition of experimental diets, as fed basis</b>				
	Dietary treatments			
Total P, %	.47	.47	.47	.47
Available P, %	.11	.11	.11	.11
Phytase, PTU/kg	0	250	500	1,000
Barley	76.51	76.51	76.51	76.51
Soybean meal	21.57	21.57	21.57	21.57
Corn Starch	.10	.08	.05	-
Limestone	1.07	1.07	1.07	1.07
Sodium chloride	.25	.25	.25	.25
Vitamin premix <sup>a</sup>	.15	.15	.15	.15
Trace mineral premix <sup>a</sup>	.15	.15	.15	.15
Antibiotic <sup>b</sup>	.20	.20	.20	.20
SSF Phytase <sup>c</sup>	-	.025	.05	.10
Calculated analysis				
ME, kcal/kg	2,960	2,959	2,958	2,956
Lysine, % (App. Dig.)	.77	.77	.77	.77
Ca, %	.50	.50	.50	.50
Total P, %	.42	.42	.42	.42
Available P, % <sup>d</sup>	.11	.11	.11	.11
Ca:Total P	1.2:1	1.2:1	1.2:1	1.2:1
Added phytase activity, PTU/kg of diet	0	250	500	1,000
<sup>a</sup> Provided the following per kg of diet: 5,506 IU of vitamin A, 551 IU of vitamin D, 33 IU of vitamin E, 3.6 mg of vitamin K (as menadione), 221 µg of biotin, 137 mg of choline, 33.04 mg of niacin, 24.78 mg of pantothenic acid (as d-pantothenate), 5.51 mg of riboflavin, 27.55 µg of vitamin B <sub>12</sub> , 1.66 mg of folacin, 100 mg of Zn, 2 mg of Mn, 100 mg of Fe, 10 mg of Cu, .30 mg of I, and .30 mg of Se				

<sup>b</sup> Provided 55 mg of chlortetracycline per kilogram of diet

<sup>c</sup> Solid-state fermented phytase complex (Allzyme® SSF; Alltech, Inc) contains 1,000 PU/g of product

<sup>d</sup> Analyzed total P were .44, .44, .44, and .43, respectively

Pigs were housed individually in metabolic chambers in an environmentally-controlled room. The chambers were specially designed for the total, but separate collection of feces, urine, and wasted feed. Experimental diets were fed at three times maintenance energy requirements with unlimited access to water. During a 7-d collection period (d 14 to 21), feces, urine, and wasted feed were collected to estimate nutrient digestibility and P excretion. The pigs were weighed weekly to estimate ADG and gain:feed ratio. Feed intake was determined by the NRC equation (NRC, 1998).

Data were analyzed as a randomized complete block design using procedures described by Steel et al. (1997), with initial BW as the blocking criterion. The model included the effects of block (rep), treatment, and block x treatment (error). The effects of phytase supplementation were tested for linearity and curvilinearity using orthogonal polynomial contrasts. For the four levels of phytase, polynomial coefficients for unequally spaced treatments were generated using the ORPAL matrix function of the IML procedure of SAS. In addition, a nonorthogonal contrast was used for comparisons between diets containing SSF phytase and control diet. In all cases, pig served as the experimental unit.

## Results and Discussion

The addition of SSF phytase complex increased (linear,  $P < .05$ ) ADG and gain:feed ratio (Table 2). Compared with the basal diet, pigs fed 1,000 PU/kg had 19 and 26% greater ADG and gain:feed, respectively. Feed intake was not affected ( $P > .10$ ) by dietary treatment.

	Dietary treatments				
	0	250	500	1,000	SE
Total P, %	.47	.47	.47	.47	
Available P, %	.11	.11	.11	.11	
Phytase, PTU/kg	0	250	500	1,000	SE
ADG, lb <sup>b</sup>	1.22	1.21	1.41	1.46	.07
ADFI, lb	2.88	2.67	2.83	2.61	.09
Feed/gain, <sup>bc</sup>	2.63	2.31	2.22	2.10	.03

<sup>a</sup> Least squares means for 6 pigs/trt

<sup>b</sup> Linear effect of SSF phytase ( $P < .05$ )

<sup>c</sup> None vs SSF Phytase ( $P < .05$ )

During the 7-d collection period, feed intake increased with increasing (linear,  $P < .01$ ) levels of SSF phytase complex. Total phosphorus intake (Table 3) was linearly increased ( $P < .01$ ) with SSF phytase levels due to the higher feed intake. Daily fecal phosphorus excretion was decreased (linear,  $P < .01$ ) by 16.8% with SSF phytase, resulting in increased phosphorus

digestibility. Pigs fed 1,000 PU/kg had approximately 63% greater phosphorus digestibility compared with that of pigs fed the diet without SSF phytase. Also, ash digestibility was increased (linear,  $P < 0.01$ ) by the addition of SSF phytase. Pigs fed the diet supplemented with 1,000 PU/kg SSF phytase complex had much greater ash digestibility (63.8%) compared with pigs fed the diet without SSF phytase complex (51.9%).

	Dietary treatments				
Total P, %	.47	.47	.47	.47	
Available P, %	.11	.11	.11	.11	
Phytase, PTU/kg	0	250	500	1,000	SE
<b>Phosphorus</b>					
Intake, g/d <sup>bde</sup>	4.43	5.05	5.73	5.52	.22
Feces, g/d <sup>bd</sup>	2.91	2.71	2.61	2.43	.09
Absorbed, g/d <sup>bde</sup>	1.53	2.35	3.12	3.09	.19
Digestibility, % <sup>bde</sup>	34.28	45.86	54.43	55.96	1.97
<b>Ash</b>					
Intake, g/d <sup>bde</sup>	62.4	72.9	83.7	82.4	3.2
Feces, g/d	30.0	31.1	31.0	29.8	1.0
Absorbed, g/d <sup>bde</sup>	32.5	41.8	52.8	52.6	2.9
Digestibility, % <sup>bde</sup>	51.93	56.79	62.87	63.76	1.65
<sup>a</sup> Least squares means for 6 pigs/trt					
<sup>b</sup> Linear effect of SSF phytase ( $P < 0.01$ )					
<sup>c</sup> Linear effect of SSF phytase ( $P < 0.05$ )					
<sup>d</sup> Quadratic effect of SSF phytase ( $P < 0.05$ )					
<sup>e</sup> None vs SSF phytase ( $P < 0.05$ )					

Dry matter intake (Table 4) was increased (linear,  $P < 0.01$ ) by the addition of SSF phytase. Dry matter excretion of pigs fed SSF phytase complex was higher ( $P < 0.05$ ) than that of pigs fed control diet due to higher feed intake. However, dry matter digestibility was increased (linear,  $P < 0.01$ ) by the addition of SSF phytase complex. When 1,000 PU/kg was added to diet, dry matter digestibility increased by 3.2% compared with the diet without SSF complex.

Organic matter intake (Table 4) increased (quadratic,  $P < 0.05$ ) with SSF phytase complex level. Due to the increased organic matter intake, the fecal excretion of organic matter increased (quadratic,  $P < 0.01$ ) with SSF phytase. However, digestibility of organic matter increased (linear,  $P < 0.01$ ) by the addition of SSF phytase complex. Pigs fed the diet containing SSF phytase complex (1,000 PU/kg) had 2.6% greater organic matter digestibility, compared with pigs fed the control diet.

Digestibility of gross energy and nitrogen (Table 4) were improved ( $P < .01$ ) by the addition of SSF phytase complex. Compared with pigs fed the diet without SSF phytase complex, pigs fed 1,000 PU/kg had 2.6% and 4.1% greater digestibility of energy and nitrogen, respectively. Digestible energy was 73.5 kcal/kg greater for the diet containing 1,000 PU/kg compared with control diet (Table 4).

	Dietary treatments				
	0	250	500	1,000	SE
Total P, %	.47	.47	.47	.47	
Available P, %	.11	.11	.11	.11	
Phytase, PTU/kg	0	250	500	1,000	SE
<b>Dry matter</b>					
Intake, g/d <sup>bde</sup>	902.9	1037.4	1150.7	1139.5	45.7
Feces, g/d <sup>e</sup>	216.6	240.4	243.1	237.6	9.2
Absorbed, g/d <sup>bde</sup>	686.3	796.6	907.6	901.9	41.5
Digestibility, % <sup>ce</sup>	75.97	76.54	78.84	79.16	.87
<b>Organic Matter</b>					
Intake, g/d <sup>bde</sup>	840.5	964.2	1066.9	1057.1	42.4
Feces, g/d <sup>e</sup>	186.6	209.4	212.1	207.8	8.3
Absorbed, g/d <sup>bde</sup>	653.8	754.8	854.9	849.3	38.7
Digestibility, % <sup>c</sup>	77.76	78.04	80.09	80.36	.83
<b>Gross Energy</b>					
Intake, kcal/d <sup>bde</sup>	3,968.2	4,564.6	4,982.2	4,947.7	200.1
Feces, kcal/d <sup>e</sup>	996.3	1128.1	1138.9	1115.5	46.1
Absorbed, kcal/d <sup>be</sup>	2971.9	3436.4	3843.3	3832.2	179.1
Digestibility, % <sup>b</sup>	74.86	75.01	77.09	77.47	.95
<b>Digestible Energy</b>					
kcal/kg	3290.1	3301.6	3337.9	3363.6	41.5
<b>Nitrogen</b>					
Intake, g/d <sup>bde</sup>	30.38	36.40	40.24	40.60	1.6
Feces, g/d <sup>de</sup>	7.86	9.50	9.40	8.88	.48
Absorbed, g/d <sup>bde</sup>	22.52	26.91	30.84	31.72	1.43
Digestibility, % <sup>b</sup>	74.07	73.69	76.51	78.18	1.23
<sup>a</sup> Least squares means for 6 pigs/trt					
<sup>b</sup> Linear effect of SSF phytase ( $P < 0.01$ )					
<sup>c</sup> Linear effect of SSF phytase ( $P < 0.05$ )					
<sup>d</sup> Quadratic effect of SSF phytase ( $P < 0.05$ )					
<sup>e</sup> None vs SSF phytase ( $P < 0.05$ )					

The phytase used in this experiment was produced by a solid-state fermentation (SSF) process. The SSF phytase contains significant activity of  $\alpha$ -amylase,  $\beta$ -glucanase, protease, xylanase and

cellulose. Phytic acid is able to inhibit  $\alpha$ -amylase, trypsin, tyrosinase and pepsin (Nair et al., 1991). Therefore, the degradation of phytate by the addition of dietary phytase complex could enhance digestibility of other nutrients as well as phosphorus. In a previous study from our lab, the addition of SSF phytase complex to low P, corn-soybean meal diets dramatically decreased fecal excretion of phosphorus, resulting in an increase in digestibility of phosphorus, but there was no improvement in digestibility of other nutrients by SSF phytase complex. Due to the high digestibility of corn-soybean meal diets, the potential for improvement in the digestibility of other nutrients by enzymes might be minimal.

Previous studies in our lab (Park et al., 2003ab) have shown no beneficial effect of SSF phytase on dry matter digestibility of pigs fed corn-soybean meal based diets or corn-soybean meal diets containing 20% wheat middlings. However, the beneficial effects of SSF phytase complex were observed in this study with barley. These results indicate that the addition of SSF phytase complex is more beneficial for energy and protein in relatively low quality feed (low available P, low energy, high fiber content, etc.). A similar response was also observed in organic matter excretion and digestibility. However, previous studies showed that the addition of phytase did not improve digestibility of organic components in barely, corn-soybean meal or tapioca, hominy feed-soybean meal diets (Jongbloed et al., 1992; Nasi and Helander, 1994; Simons et al., 1990). The enzyme used in those studies contained only phytase. However, Nasi et al. (1995) reported that the addition of 1,000 PU/g of phytase complex to a barley-rapeseed meal diet significantly improved ash digestibility (55%) compared with the unsupplemented diet (52%). The phytase used in their study contained protein, starch, and pectin-degrading enzymes. These authors also found improved protein digestibility.

Digestibility of energy and nitrogen were improved by the addition of SSF phytase complex. Similar results were found by Nasi et al. (1995) who reported that the addition of phytase complex improved crude protein digestibility of barley-rapeseed meal diets. However, a previous study from our lab suggested that the addition of SSF phytase to low P, corn -soybean meal diets did not improve digestibility of gross energy or nitrogen. These results indicate the response and magnitude of phytase effect varies with different types of feedstuffs.

### **Implications**

The addition of a solid-state fermented phytase complex to low P, barley-soybean meal based diets improved feed efficiency and nutrient digestibility by growing pigs. Digestibility of total P was dramatically increased by the addition of SSF phytase complex (up to 1,000 PU/kg). Also, dry matter, energy and protein digestibility were increased by the addition of SSF phytase complex. However, such improvements were not observed in previous studies utilizing corn-soybean meal diets. Therefore, these data indicate that the addition of a solid-state fermented phytase complex improves nutrient digestibility as well as P utilization of pigs fed barley-soybean meal diets.

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