

## Supplementation of Low-Calcium and Low-Phosphorus Diets with Phytase and Cholecalciferol

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### Introduction

Supplementation of pig diets with microbial phytase has been shown to improve calcium, copper, phosphorus, and zinc utilization, and consequently reduce the excretion of these minerals in the manure (Adeola, 1995; Adeola et al., 1995). The level of Ca in diets may also affect the utilization of phytic acid-P through the formation of insoluble calcium phytate and/or reduction of phytase activity. Cholecalciferol (Vitamin D) plays a role in Ca and P absorption, and therefore influences their utilization. Mohammed et al. (1991) reported that cholecalciferol supplementation of poultry diets increases phytic acid-P utilization. Metabolites of cholecalciferol may or may not improve phytic acid-P utilization in poultry or pigs. Because Ca, cholecalciferol, and phytase affect phytic acid-P utilization and subsequent pig performance, the objectives of this study were to evaluate the effects of dietary Ca and phytase supplementation on performance of growing pigs, and to determine whether phytase and cholecalciferol supplementation would improve the growth performance of growing pigs fed a low-Ca, low-P corn-soybean meal diet.

### Materials and Methods

#### *Pigs and Housing*

Crossbred pigs were blocked by weight and sex and assigned to diets. Pigs were assigned to individual pens equipped with stainless-steel feeders and nipple drinkers in a totally enclosed facility with slatted concrete floors. The temperature in the facility was maintained at 68°F with a 12-hour light-dark cycle. Pigs were given free access to feed and water for 28 days, and pig weights and feed intake were monitored weekly. Blood samples were obtained at the beginning and end of the experiments.

#### *Experiment 1*

This experiment investigated the effects of reducing calcium levels from .6 to .3% in a low-phosphorus (no inorganic phosphorus) diet with or without phytase supplementation (720 units/lb) on growth performance and plasma concentrations of calcium and phosphorus. A basal diet (Table 1) was formulated to be adequate in energy and all nutrients for the 45-lb pig except for calcium and phosphorus. The low-calcium, low-phosphorus basal diet contained .3% Ca and .34% P. The basal diet was supplemented with 0, .15, or .3% Ca, and 0 or 720 phytase units/lb, in a 3 x 2 factorial arrangement. A positive control calcium- and phosphorus-adequate diet (.6% Ca and .54% P) was included as the seventh diet (Table 1). Twenty-eight barrows and 28 gilts in two groups of 14 barrows and 14 gilts each were assigned to the seven diets in a randomized complete block design, using initial weight and sex as the basis for blocking.

### *Experiment 2*

Experiment 2 was similar in every respect to the first experiment except that the basal diet was formulated to contain .25% Ca (Table 1) and was supplemented with 0, .05, or .1% Ca, and 0 or 360 phytase units/lb.

### *Experiment 3*

The third experiment was conducted to investigate the effect of phytase and cholecalciferol supplementation of low-calcium, low-phosphorus diet on growth performance. The low-Ca (.35%), low-P (.34%) diet which supported the best performance in experiment 2 was used as the basal diet in experiment 3 (Table 1). The basal diet was supplemented with 0 or 360 phytase units/lb, and with 0 or .8 ppm cholecalciferol. The same positive control diet as in experiment 1 was used. The five diets were fed to 30 barrows and 30 gilts in a randomized complete block design.

## **Results**

### *Experiment 1*

Results for Experiment 1 are given in Table 2. When pigs were fed low-P corn-soybean meal diets, there was a linear reduction ( $P < .05$ ) in weight gain as dietary Ca increased from .3 to .6%. The linear reduction in weight gain from increased dietary Ca was offset by supplementing the diets with phytase at 720 units/lb. The positive control diet (diet #7) supported similar weight gain as the phytase-supplemented diets. Weight gain and feed intake were greater ( $P < .05$ ) in the positive control diet than in the low-P diets that were not supplemented with phytase. Gain:feed ratio was higher ( $P < .05$ ) in pigs that received the phytase-supplemented low-P diets than in those whose low-P diets were not supplemented with phytase. In pigs fed the diets without phytase, the gain:feed ratios were lower ( $P < .05$ ) than in pigs that received the positive control diet. The concentration of P in plasma was higher ( $P < .05$ ) in pigs that received either the positive control or the phytase-supplemented low-P diets than in pigs that received the diets not supplemented with phytase. Regardless of phytase supplementation, there was a linear effect ( $P < .05$ ) of increasing dietary Ca level on plasma P concentration in pigs that received the low-P diets. Plasma Ca concentration was higher ( $P < .05$ ) in pigs that received the positive control diet than in those that received the low-P diets without phytase.

### *Experiment 2*

The basal diet in the first experiment was formulated to contain Ca at .3%, and .15 or .3% Ca was added to the basal diet. Based on pig performance in the first experiment, the Ca level in the basal diet was reduced to .25%, and 0.05 or .1% Ca was added to the basal diet to narrow the Ca:P ratio. Furthermore, phytase was reduced from 720 to 360 units/lb. Growth performance and plasma P and Ca concentrations are given in Table 3. Supplementing the low-Ca, low-P diets with phytase improved ( $P < .05$ ) weight gain and gain:feed ratio. Plasma P and Ca concentrations in pigs that received the phytase-supplemented diets were higher ( $P < .05$ ) than in pigs that received the low-Ca, low-P diets without phytase supplementation. Weight gain, gain:feed ratio, and plasma P and Ca concentrations

were greater ( $P < .05$ ) in pigs that received the positive control diet (diet #7) than in pigs that received the diets without phytase, but similar ( $P > .10$ ) to those in pigs that received the phytase-supplemented diets.

### *Experiment 3*

The objective of the third experiment was to evaluate the effects of phytase or cholecalciferol supplementation on the growth performance of pigs when fed the low-Ca, low-P diet that supported optimum performance of pigs in the second experiment. Results are in Table 4. When compared with the pigs that received the positive control diet (.6% Ca and .54% P), weight gain was lower ( $P < .10$ ), feed intake was higher ( $P < .05$ ), and gain:feed ratio was lower ( $P < .05$ ) in pigs that received the low-Ca (.35%), low-P (.34%) diet. Supplementation of the low-Ca, low-P diet with phytase and/or cholecalciferol reduced ( $P < .05$ ) feed intake and improved ( $P < .05$ ) gain:feed ratio. The growth performance of pigs that received the positive control diet was similar ( $P > .10$ ) to those that received the phytase- or cholecalciferol-supplemented low-Ca, low-P diet.

### **Discussion**

The basal corn-soybean meal diet used in the first experiment was severely deficient in P, and the Ca level was set at a level of .3% to achieve a Ca:P ratio of 0.9:1. We knew from studies reported previously that the addition of Ca without inorganic P supplementation would depress performance. Increasing the Ca:P ratio of the basal diet from 0.9:1 to 1.8:1 led to a predictable reduction in performance of growing pigs, including a 20% reduction in weight gain and a 10% reduction in feed efficiency. Over 65% of the total P in corn and soybean meal is bound in phytic acid, the utilization of which is influenced by a variety of factors. The high-Ca-induced reduction in performance of pigs receiving a low-P diet in the first experiment may be a result of either decreased phytic acid-P utilization, or decreased utilization of non-phytic acid-P through the formation of insoluble Ca phytate or Ca phosphate precipitates. Other possibilities include the direct depression of phytase activity arising from extra Ca competing for phytase active sites, or increased intestinal pH resulting from increased dietary Ca which reduces the soluble mineral fractions, hence limiting their availability for absorption (McCuaig et al., 1972; Wise, 1983; Shafey and McDonald, 1991).

In the first experiment, the Ca-induced 20% and 10% reductions in weight gain and feed efficiency, respectively, were offset by supplementing the diets with microbial phytase. Supplementing the diets with 720 phytase units/lb increased weight gain by 19% (1.32 to 1.57 lb/day) and improved feed efficiency by 15% when compared with the low-P diets that were not supplemented with microbial phytase. The results of the second experiment illustrate that a narrow Ca:P ratio (0.7:1 to 1:1) is not depressive to growth performance as is a wider ratio (0.9:1 to 1.8:1, experiment 1). Furthermore, microbial phytase improved growth performance by 10%, and the growth performance of pigs that received low-P, low-Ca, phytase-supplemented diets were similar to those that received adequate-P, adequate-Ca diets.

The concentrations of Ca and P in the plasma were measured in the first two experiments as putative indicators of the utilization of these minerals. Increasing the Ca:P ratio from 0.9:1 to 1.8:1

resulted in a linear reduction in plasma P concentration that parallels the linear reduction in weight gain. When the dietary Ca level in the low-P diet was reduced from .6 to .3%, there was a significant improvement in the prevailing hypophosphatemia. Supplementation of diets with microbial phytase did offset the Ca-induced decrease in plasma P concentrations, which also parallels growth. The increase in plasma P concentration in response to supplemental microbial phytase presumably arises from increased hydrolysis of phytic acid during intestinal transit and the resultant absorption of the P that is released.

The results of the third experiment illustrate the effects of microbial phytase and cholecalciferol supplementation on growth performance, and allow the evaluation of the efficacy of these compounds in a low-P, low-Ca diet relative to an adequate-P, adequate-Ca diet. The results clearly confirm that either microbial phytase or cholecalciferol supplementation of a low-P, low-Ca diet improves growth performance of growing pigs and gives a similar performance as an adequate-P, adequate-Ca diet. The results also show that the effect of the combination of microbial phytase and cholecalciferol in a low-P, low-Ca diet on growth performance are neither additive nor synergistic.

### **Implications**

The data from this study shows that for growing pigs (42 to 88 lb body weight), a low-P (.34%), low-Ca (.35%) diet supplemented with microbial phytase (360 units/lb) or cholecalciferol (.8 ppm) gives a similar growth performance as a Ca- and P-adequate (.6 and .54%, respectively) diet. The use of phytase- or cholecalciferol-supplemented low-Ca, low-P diets would have an environmental benefit of reducing phosphorus concentration of pig manure. Because the pigs used in the current experiments were not monitored to market, it is not known whether feeding the phytase- or cholecalciferol-supplemented low-Ca, low-P diet from 42 to 88 lb body weight has any effects at heavier weights to market size.

### **References**

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Table 1. Composition of diets.

Ingredient	Concentration, g/kg			
	Basal Diet			Positive Control Diet Experiments 1,2 and 3
	Experiment 1	Experiment 2	Experiment 3	
Yellow corn	780.0	780.0	780.0	780.0
Soybean meal (dehulled)	190.0	190.0	190.0	190.0
Sodium chloride	3.5	3.5	3.5	3.5
Lysine•HCl	3.0	3.0	3.0	3.0
Vitamin Premix <sup>z</sup>	1.3	1.3	1.3	1.3
Trace mineral premix <sup>y</sup>	0.5	0.5	0.5	0.5
Selenium premix <sup>x</sup>	0.5	0.5	0.5	0.5
Antioxidant <sup>w</sup>	0.5	0.5	0.5	0.5
Tylan-40 <sup>®v</sup>	1.25	1.25	1.25	1.25
Dicalcium phosphate	----	----	----	11.0
Calcium carbonate	6.0	4.60	7.4	8.0
Corn starch <sup>u</sup>	13.45	14.90	12.1	0.45
Total	1,000.00	1,000.00	1,000.00	1,000.00
Calculated energy and nutrients <sup>t</sup>				
CP (%)	15.7 (16.1)	15.7 (15.2)	15.7 (16.3)	15.7 (16.0)
Lysine (%)	1.02	1.02	1.02	1.02
Total Ca (%)	.30 (.35)	.25 (.23)	.35 (.37)	.60 (.65)
Supplemental Ca (%)	.23	.17	.28	.52
Total P (%)	.34 (.37)	.34 (.31)	.34 (.38)	.54 (.58)
Supplemental P (%)	----	----	----	.20

<sup>z</sup> Vitamin premix supplied the following per kilogram of diet: 3175 IU vitamin A; 315 IU vitamin D<sub>3</sub>; 12 IU vitamin E; 815 ug menadione sodium bisulfite; 16.2 ug vitamin B<sub>12</sub>; 3.2 mg riboflavin; 11.7 mg d-pantothenic acid; and 18.2 mg niacin.

<sup>y</sup> Trace mineral premix supplied the following per kilogram of diet: 90 mg Fe; 30 mg Mn; 75 mg Zn; 8.8 mg Cu; and 1 mg I.

<sup>x</sup> Selenium premix supplied 100 ug Se per kilogram of diet.

<sup>w</sup> Antioxidant was dry polyanox containing ethoxyquin, methyl anhanilate, butylated hydroxytoluene, butylated hydroxyanisole, propylgalate and corn grits.

<sup>v</sup> Tylan supplies 110 mg tylosin phosphate per kilogram of diet.

<sup>u</sup> Corn starch, in experiment 1, was replaced by 4 or 8 g of calcium carbonate in diets containing 4.5 or 6.0 g/kg Ca and by 4 or 8 g of calcium carbonate plus 1600 phytase units/kg in diets containing 4.5 or 6.0 g/kg Ca plus phytase. In experiment 2, corn starch was replaced by 1.4 or 2.8 g of calcium carbonate in diets containing 3.0 or 3.5 g/kg Ca and by 1.4 or 2.8 g calcium carbonate plus 800 phytase units/kg in diets containing 3.0 or 3.5 g/kg Ca plus phytase.

<sup>t</sup> Crude protein, total Ca, and total P values in parenthesis are analyzed composition.

Table 2. Growth performance and plasma phosphorus and calcium concentrations of pigs receiving different dietary levels of calcium in low-phosphorus phytase-supplemented diets (experiment 1).

Diet #	Ca (total) %	P (total) %	Phytase units/lb	Initial Weight lb	Final Weight lb <sup>a,b,c</sup>	Weight Gain lb/d <sup>a,b,c</sup>	Feed Intake lb/d <sup>b</sup>	Gain:Feed lb/1000lb <sup>a,b</sup>	Plasma Phosphorus mg/L <sup>a,b,c,d</sup>	Plasma Calcium mg/L <sup>b</sup>
1	.30	.34	0	41.8	84.0	1.51	4.09	377	75.11	90.83
2	.45	.34	0	41.6	76.3	1.25	3.92	321	63.00	86.25
3	.60	.34	0	40.9	74.8	1.21	3.67	338	56.16	95.22
4	.30	.34	720	39.6	86.5	1.61	4.11	394	84.86	96.21
5	.45	.34	720	41.8	84.7	1.53	3.76	407	98.50	94.72
6	.60	.34	720	42.2	85.8	1.56	4.03	392	97.20	96.35
7	.60	.54	0	41.4	90.0	1.75	4.38	405	92.40	98.80
n				8	8	8	8	8	8	8
SD				2.13	5.24	.16	0.62	46.9	10.86	10.93

<sup>a</sup> Effect of phytase (diets 1 to 3 vs. 4 to 6),  $P < .05$ .

<sup>b</sup> Effect of control diet (diets 1 to 3 vs. 7),  $P < .05$ .

<sup>c</sup> Linear effect of calcium for diets 1 to 3,  $P < .05$ .

<sup>d</sup> Linear effect of calcium for diets 4 to 6,  $P < .05$ .

Table 3. Growth performance and plasma phosphorus and calcium concentrations of pigs receiving different dietary levels of calcium in low-phosphorus phytase-supplemented diets (experiment 2).

Diet #	Ca (total) %	P (total) %	Phytase units/kg	Initial Weight lb	Final Weight lb <sup>a,b</sup>	Weight Gain lb/d <sup>a,b</sup>	Feed Intake lb/d <sup>c</sup>	Gain:Feed lb/1000lb <sup>a,b</sup>	Plasma Phosphorus mg/L <sup>a,b</sup>	Plasma Calcium mg/L <sup>a,b</sup>
1	.25	.34	0	41.6	80.9	1.41	3.67	385	68.25	95.99
2	.30	.34	0	41.6	81.2	1.42	4.09	346	61.81	89.53
3	.35	.34	0	42.5	85.6	1.53	4.44	350	65.85	97.41
4	.25	.34	360	41.4	84.7	1.54	3.83	401	89.84	99.86
5	.30	.34	360	41.8	88.2	1.66	4.27	391	102.64	102.99
6	.35	.34	360	41.6	86.7	1.61	3.89	413	91.33	100.40
7	.60	.54	0	42.0	88.2	1.65	3.89	424	100.57	104.79
n				8	8	8	8	8	8	8
SD				1.8	6.2	0.22	0.48	44.7	12.12	6.76

<sup>a</sup> Effect of phytase (diets 1 to 3 vs. 4 to 6),  $P < .05$ .

<sup>b</sup> Effect of control diet (diets 1 to 3 vs 7),  $P < .05$ .

<sup>c</sup> Linear effect of calcium for diets 1 to 3,  $P < .05$ .

Table 4. Growth performance of pigs receiving phytase- and cholecalciferol-supplemented low-calcium and low-phosphorus diets (experiment 3).

Diet #	Ca (total) %	P (total) %	Phytase units/kg	Cholecalciferol (total) ppm	Initial Weight lb	Final Weight lb	Weight Gain lb/d	Feed Intake lb/d	Gain:Feed lb/1000 lb
1	.35	.34	0	.008	41.8	84.9 <sup>B</sup>	1.54 <sup>C</sup>	4.44 <sup>a</sup>	348 <sup>b</sup>
2	.35	.34	360	.008	42.5	88.2 <sup>A</sup>	1.63 <sup>ABC</sup>	3.89 <sup>b</sup>	418 <sup>a</sup>
3	.35	.34	0	.8	42.0	88.0 <sup>A</sup>	1.64 <sup>AB</sup>	3.96 <sup>b</sup>	417 <sup>a</sup>
4	.35	.34	360	.8	42.2	87.8 <sup>A</sup>	1.63 <sup>ABC</sup>	3.89 <sup>b</sup>	419 <sup>a</sup>
5	.60	.54	0	.008	42.2	89.1 <sup>A</sup>	1.67 <sup>A</sup>	4.03 <sup>b</sup>	417 <sup>a</sup>
n					12	12	12	12	12
SD					1.63	3.78	0.12	0.33	28.3

<sup>a,b,c</sup> Means in a column with different superscripts are significantly different at  $P < .05$ .

<sup>A,B,C</sup> Means in a column with different superscripts are significantly different at  $P < .10$ .