Calcium Level Affects the Efficacy of Supplemental Microbial Phytase in Corn-Soybean Meal Diets of Weanling Pigs

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ABSTRACT: A 2 × 2 × 2 factorial experiment was conducted with 64 pigs (4 wk old, 8.04 ± .50 kg BW) to determine the effect of various dietary concentrations of Ca, vitamin D, and microbial phytase (Aspergillus niger) on phytate-P utilization. A low-P, corn-soybean meal diet was supplemented with two levels of phytase (unit/gram), 750 (suboptimal) and 1,200 (optimal); of vitamin D (international unit/kilogram), 660 (normal) and 6,660 (high); and of Ca (percentage), .4 (low) and .8 (normal). Pen feed consumption and individual pig weights, plasma inorganic P and Ca concentrations, and plasma alkaline phosphatase (AP) activity were measured at d 10, 20, and 30. The normal dietary Ca concentration had an adverse effect (P < .05) on all the response measures. The depressive effect of the normal dietary Ca on performance was greater (P < .05) at the normal vitamin D level or at the optimal phytase level than at the other levels of these two factors. The elevation in plasma AP activity in pigs fed the normal dietary Ca was greater (P < .05) at the suboptimal than at the optimal phytase level. The decreases in plasma inorganic P concentration and increases in plasma Ca concentration associated with the normal dietary Ca were substantial. In conclusion, the normal level of Ca in the diet greatly reduced the efficacy of supplemental phytase. Raising vitamin D in the diet partially offset this adverse effect but did not produce further improvement when the Ca level was low.

Key Words: Pigs, Phytase, Vitamin D, Calcium, Phytate

Introduction

Supplementing swine diets with microbial phytase (Aspergillus niger) greatly improves phytate-P bioavailability and decreases P excretion in the manure (Nasi, 1990; Simons et al., 1990; Cromwell, 1991; Jongbloed et al., 1992; Leunissen and Young, 1992). Lei et al. (1993b) demonstrated that supplemental phytase at 1,200 phytase units per gram of a corn-soybean meal diet maximized phytate-P utilization and essentially obviated the need for inorganic P addition for weanling pigs. However, only single dietary levels of Ca and vitamin D, two key nutrients that strongly interact with P (Littledike and Goff, 1987) and affect phytate-P utilization (Wise, 1983), were used in these studies. High levels of Ca in the diets of rats (Taylor and Coleman, 1979; Nahapetian and Young, 1980) and poultry (Edwards and Veltman, 1983; Ballam et al., 1985; Sheideler and Sell, 1987) consistently decreased the availability of phytate-P. Likewise, vitamin D deficiency was found to aggravate the disturbances of P and Ca metabolism resulting from high dietary phytate-P (Pointillart et al., 1985). In contrast, dietary supplements of cholecalciferol improved phytate-P bioavailability to pigs (Fontaine et al., 1985). Recently, Mohammed et al. (1991) reported that the adverse effects of a high-phytate, low-inorganic-P diet on chicks were overcome by lowering Ca and(or) simultaneously raising cholecalciferol in the diets. This experiment was conducted to determine the effect of dietary concentrations of Ca and vitamin D on the ability of microbial phytase to improve phytate-P utilization by weanling pigs.

Materials and Methods

Experimental Design. This study was designed as a 2 × 2 × 2 factorial in randomized complete blocks. Because of repeated measurements over time, the
entire design was a split-plot. The three treatment factors included dietary levels of phytase, Ca, and vitamin D. The two dietary levels (as-fed basis) of phytase (phytase units/gram) were 750 (suboptimal) and 1,200 (optimal), of Ca (percentage) were .4 (low) and .8 (normal), and of vitamin D (international units/kilogram) were 660 (normal) and 6,660 (high).

**Phytase and Diets.** The microbial phytase (A. niger, Alko Ltd., Rajamaki, Finland), the confirmation of the actual activity of the enzyme product, and the process of phytase incorporation into diets were the same as described previously (Lei et al., 1993a). A phytase unit (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. Calcium carbonate (Calcium Products, Swayzee, IN) and vitamin D₃ (Carl Akeley, Lewisburg, OH) were used to produce the desired dietary levels of these two nutrients. Total dietary concentrations of Ca and P were analyzed (Lei et al., 1993b) and of vitamin D calculated (Table 1).

**Animals.** Sixty-four weanling crossbred pigs (Landrace-Yorkshire-Hampshire, 4 wk old) were grouped into heavy (8.91 ± .61 kg BW) and light (7.17 ± .36 kg BW) blocks based on body weight. The 32 pigs within each block were allotted into eight pens of four pigs each and balanced by BW, litter, and sex. The experiment was conducted for 30 d. Housing, management, and P-depletion procedures were the same as in previous feeding trials (Lei et al., 1993a,b).

**Vitamin D-SeC**

<table>
<thead>
<tr>
<th>Ingredient, g/kg</th>
<th>Vitamin D, IU/kg:</th>
<th>Phytase, PU/a/g:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Item</td>
<td>Calcium, g/kg:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Corn</td>
<td>773.6</td>
<td>762.9</td>
</tr>
<tr>
<td>Soybean meal (44% CP)</td>
<td>200.0</td>
<td>200.0</td>
</tr>
<tr>
<td>L-lysine-HCl</td>
<td>2.6</td>
<td>2.5</td>
</tr>
<tr>
<td>Salt (NaCl)</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Premixb</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Vitamin E-SeCf</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Antibioticd</td>
<td>.5</td>
<td>.5</td>
</tr>
<tr>
<td>Phytasea</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>8.3</td>
<td>19.0</td>
</tr>
<tr>
<td>Vitamin D²</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Nutritive values</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P, g/kgb</td>
<td>3.2</td>
<td>2.9</td>
</tr>
<tr>
<td>Ca, g/kgb</td>
<td>4.8</td>
<td>8.8</td>
</tr>
<tr>
<td>Vitamin D, IU/kgc</td>
<td>660</td>
<td>660</td>
</tr>
</tbody>
</table>

*aPhytase unit: 1 nmol of inorganic P released from sodium phytate per minute at 37°C.  
bSupplied the following amounts per kilogram of diet: vitamin A, 3,307 IU; vitamin D₃, 660 IU; menadione, 5.5 mg; riboflavin, 3.3 mg; niacin, 18.4 mg; d-pantothenic acid, 13.4 mg; choline, 110 mg; vitamin B₁₂, 20 µg; Zn, 75 mg; Fe, 58 mg; Mn, 33 mg; Cu, 10 mg; I, 20 mg.  
cSupplied 17 IU of vitamin E and .3 mg of Se/kg of diet.  
dSupplied 55 mg of chlortetracycline/kg of diet.  
eAspergillus niger phytase (Alko Ltd., Rajamaki, Finland), 500,000 phytase units/g of product.  
fContained 38% Ca.  
gContains 3,000 IU of vitamin D/µg of product.  
hCalculated values.  
iAnalyzed values.
vitamin D and their four combinations, this decrease in ADG was significant \((P < .02)\) only at the normal vitamin D level and/or at the combined optimal level of phytase and normal level of vitamin D. Similarly, ADFI was suppressed by the normal dietary Ca level at d 20 (742 vs 861 g, \(P < .1\)) and 30 (918 vs 1,116 g, \(P < .01\)). This decrease in ADFI was largest at the combined levels of optimal phytase and normal vitamin D at d 30 (\(P < .05\)). Poor feed efficiency \((P < .01)\) resulted from feeding the pigs the diet with normal Ca through the entire study. The overall gain:feed (grams/kilogram) at d 10, 20, and 30 were 377, 486, and 582 for pigs fed normal dietary Ca and 431, 530, and 648 for pigs fed low dietary Ca, respectively. The lower gain:feed associated with normal dietary Ca was significant \((P < .05)\) only at the normal vitamin D level and was more pronounced at the optimal level of phytase \((P < .06)\) than at the suboptimal level of phytase \((P < .1)\). Compared to the suboptimal phytase level, optimal phytase level did not improve ADG \((P < .68)\), ADFI \((P < .95)\), or gain:feed \((P < .31)\).

**Phytase, PU/kg:**

<table>
<thead>
<tr>
<th>Phyrase, PU/kg:</th>
<th>750</th>
<th>1,200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D, IU/kg:</td>
<td>660</td>
<td>6,660</td>
</tr>
<tr>
<td>Calcium, g/kg:</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td><strong>Daily gain, g</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days 0 to 10</td>
<td>252</td>
<td>166</td>
</tr>
<tr>
<td>Days 11 to 20</td>
<td>374</td>
<td>303</td>
</tr>
<tr>
<td>Days 21 to 30</td>
<td>497</td>
<td>307</td>
</tr>
<tr>
<td><strong>Feed intake, g/d (as-fed)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days 0 to 10</td>
<td>505</td>
<td>397</td>
</tr>
<tr>
<td>Days 11 to 20</td>
<td>434</td>
<td>416</td>
</tr>
<tr>
<td>Days 21 to 30</td>
<td>467</td>
<td>348</td>
</tr>
</tbody>
</table>

**Discussion**

Dietary Ca and vitamin D have been shown to affect the utilization of phytate-P metabolism in animals (Wise, 1983; Pointillart et al., 1985). However, recent studies on the effect of microbial phytase...
on utilization of phytate-P by pigs during starting
(Lei et al., 1993a;b; Leuniessen and Young, 1992) and
growing-finishing phases (Nasi, 1990; Simons et al.,
1990; Cromwell, 1991; Jongbloed et al., 1992) were
almost exclusively conducted with a single level of Ca
or vitamin D. In most cases, dietary Ca levels were
relatively low. In the present study, the ability of
phytase to improve phytate-P availability was greatly
reduced at a normal level of dietary Ca compared to
that at a low level of dietary Ca. When all pigs were
fed a corn-soybean meal diet without added inorganic
Ca. In contrast, the same amount of phytase activity
itself, is largely unavailable for absorption (Wise,
1983). Consequently, phytate-P, as well as Ca
status (Lei et al., 1993b) only in pigs receiving low
Ca. In contrast, the same amount of phytase activity
in the same diets but with normal Ca concentration
(.8%) resulted in appreciably lower ADG, ADFI,
and gain:feed and unfavorable changes in plasma
inorganic P and Ca concentrations and AP activity.
Calcium, the major diveral cation in the diets for
many species, can progressively precipitate all the
phytate by forming extremely insoluble Ca-phytate
complexes in the intestine (Wise, 1983; Nelson and
Kirby, 1987). Consequently, phytate-P, as well as Ca
itself, is largely unavailable for absorption (Wise,
1983). It has been conclusively demonstrated that
high levels of Ca in the diets of rats (Taylor and
Coleman, 1979; Nahapetian and Young, 1980) and of
poultry (Edwards and Veltman, 1983; Ballam et al.,
1985; Sheideler and Sell, 1987) decreased the availa-
bility of phytate-P considerably. Lowering dietary Ca
from 1 to .5% enhanced phytate-P digestibility by 15%
reported that absorption of P from diets without
supplemental inorganic P was inversely related to
dietary Ca up to .65%. Pointillart et al. (1989) found
that elevating dietary Ca from .9 to 1.4% in the diets
containing .5% P (all from plants) intensified the P
deficiency secondary to high phytate feeding. But they
failed to observe a detrimental effect of high dietary
Ca on phytate-P absorption or retention and suggested
that the failure to find a decreased P absorption might
have resulted from use of an appropriate dietary
vitamin D level (1,000 IU/kg).

The effect of dietary Ca on performance and plasma
P status varied considerably with dietary vitamin D
level, although vitamin D alone had no significant
effect on any of the response measures. The high level
of vitamin D in the diets seemed to reduce the adverse
effects of normal dietary Ca on ADG, ADFI, gain:feed,
and plasma inorganic P concentrations. This interac-
tion between vitamin D and Ca was in agreement with
that observed by Mohammed et al. (1991). They
found that inclusion of a high level of cholecalciferol
(10 times normal) in chick diets led to a marked
increase in circulating levels of 1,25-(OH)2D3. As a
result, availability of Ca and phytate were signifi-
cantly increased, and the hypophosphatemia asso-
ciated with the low P intake was alleviated.

Table 3. Plasma concentrations of inorganic P and Ca and plasma alkaline phosphatase activity of pigs
receiving different levels of supplemental microbial phytase, vitamin D, and calcium in the diets

<table>
<thead>
<tr>
<th>Phytase, PU/g</th>
<th>Vitamin D, IU/kg</th>
<th>Calcium, g/kg</th>
<th>Plasma inorganic P, mg/dL</th>
<th>Plasma CA, mg/dL</th>
<th>Plasma alkaline phosphatase, U/dL</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>600</td>
<td>800</td>
<td>6,660</td>
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<td></td>
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<td>Day 0</td>
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<td>Day 20</td>
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<td>11.9</td>
<td>12.0</td>
<td>12.5</td>
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<td>11.2</td>
<td>12.3</td>
<td>12.8</td>
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<td></td>
<td></td>
<td></td>
<td>9.4</td>
<td>12.9</td>
<td>12.9</td>
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<td></td>
<td></td>
<td></td>
<td>Day 10</td>
<td>Day 20</td>
<td>Day 30</td>
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<td></td>
<td>11.9</td>
<td>12.3</td>
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<td>9.4</td>
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<td>12.9</td>
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<td>Day 20</td>
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<td></td>
<td>11.2</td>
<td>12.8</td>
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<td>11.1</td>
<td>12.5</td>
<td>12.9</td>
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<td>12.9</td>
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<td>Day 30</td>
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<td></td>
<td></td>
<td></td>
<td>11.9</td>
<td>12.5</td>
<td>9.4</td>
</tr>
</tbody>
</table>

aPhytase unit: 1 nmol of inorganic P released from sodium phytate per minute at 37°C.
bProbabilities of significance (P < .05): phytase, vitamin D, Ca, .0002; phytase × vitamin D, .41; phytase × Ca, .53; vitamin D × Ca, .35; and phytase × vitamin D × Ca, .57. Standard errors of mean differences (milligrams/deciliter): main effect, .22; two-way interactions, .31; and three-way interactions, .44. Approximate df of error, 13.
cProbabilities of significance (P < .05): phytase, .06; vitamin D, .17; Ca, .0001; phytase × vitamin D, .79; phytase × Ca, .29; vitamin D × Ca, .30; and phytase × vitamin D × Ca, .12. Standard errors of mean differences (milligrams/deciliter): main effect, .23; two-way interactions, .32; and three-way interactions, .45. Approximate df of error, 16.
dProbabilities of significance (P < .05): phytase, .56; vitamin D, .43; Ca, .06; phytase × vitamin D, .77; phytase × Ca, .25; vitamin D × Ca, .74; and phytase × vitamin D × Ca, .24. Standard errors of mean differences (units/deciliter): main effect, .92; two-way interactions, 1.30; and three-way interactions, 1.84. Approximate df of error, 14.
Likewise, absorption or retention of P in a diet for pigs with .6% P (80% phytate-P) and .6% Ca was nearly doubled when the diet was supplemented with cholecalciferol at 1,000 IU/kg (Fontaine et al., 1985). However, the formation of unavailable Ca-phytate, suggested by a simultaneous increase in fecal Ca and P excretion (r > .92, P < .05) with time, took place in vitamin D-depleted pigs (Pointillart et al., 1985).

Vitamin D supplementation was shown to have no effect on phytase or AP activity in the intestinal mucosa of pigs (Fontaine et al., 1985), so it may oppose the depressive effect of Ca on phytate-P availability by promoting Ca absorption and thus preventing, or at least reducing, Ca-phytate formation (Pointillart et al., 1989).

Phytase level also influenced the action of dietary Ca. An optimal level of dietary phytase seemed to magnify the differences in growth performance measures between the two levels of dietary Ca and minimized the differences in plasma AP activity, and to a lesser extent in plasma inorganic P and Ca concentrations. However, the differences in most measures resulting from the two dietary levels of phytase were relatively smaller than those observed previously (Lei et al., 1993b). Interactions between phytase and Ca or vitamin D may have somewhat confounded the effect of phytase.

Among the eight combinations of the three dietary treatments, optimal phytase combined with normal vitamin D and low Ca produced the best overall response. Simultaneous lowering of Ca and elevation of vitamin D in the diets did not cause additive benefits compared with a singular reduction in dietary Ca. In contrast, decreasing Ca and increasing vitamin D in a low-P diet for chicks additively improved phytate-P utilization (Mohammed et al., 1991). However, when microbial phytase was incorporated into the diets in this study, dietary Ca rather than vitamin D became more crucial. Moreover, pigs may be different from chicks in response to these interactions.

Implications

Supplemental microbial phytase in corn-soybean meal diets improves phytate phosphorus utilization more efficiently at moderately low levels of dietary calcium than at normally recommended levels. Introducing high levels of vitamin D in diets with normal calcium concentration may partially offset this adverse effect of calcium but does not enhance phytase efficacy in low-calcium diets.

Literature Cited


