# The Effect of Calcium Level on Microbial Phytase Activity and Nutrient Balance in Swine

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**ABSTRACT** : Three barrows weighing 45.0 kg, fitted with simple T-cannulas in both the duodenum and terminal ileum, were assigned to diets in a 3×3 Latin Square design experiment to determine the effects of two calcium levels (0.8% vs 0.4%) on phytase activity and nutrient balance in growing pigs. The control diet contained 0.8% calcium, with no added inorganic phosphorus (0.45% total phosphorus) and no added phytase. The two additional experimental diets contained microbial phytase (750 phytase units/kg) and supplied either 0.8% or 0.4% calcium. With added microbial phytase, ileal and total tract digestibility of total phosphorus were improved by 20.9 and 13.8 percentage units, respectively (p=0.01). The apparent duodenal and ileal digestibility of phytate phosphorus were increased by 51.8 and 49.7 percentage units (p=0.001). Lowering dietary calcium in the presence of microbial phytase increased the digestibility of phytate phosphorus by an additional 10.9 (p=0.001) and 5.7 percentage units for duodenal and ileal digestibility, respectively. Supplementation with microbial phytase significantly reduced fecal excretion of nitrogen and phosphorus and increased the percentage of these nutrients retained by the pig. Lowering dietary calcium further increased the percentage of dietary phosphorus retained. Overall, reducing dietary calcium appeared to increase the effectiveness of added microbial phytase in degrading phytate phosphorus. As a result, care should be taken to avoid high levels of dietary calcium when supplementing swine diets with microbial phytase. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 2 : 197-202*)

**Key Words** : Pigs, Microbial Phytase, Phytate Phosphorus, Calcium

## INTRODUCTION

Most of the phosphorus in a typical corn-soybean meal diet is in the form of phytate (Nelson et al., 1968; Reddy et al., 1982). Phytate phosphorus is largely unavailable to swine, as phytase activity of the gastrointestinal tract of pigs is limited (Pointillart et al., 1984; 1987; Jongbloed et al., 1992). Since dietary phytate is not hydrolyzed efficiently into inorganic phosphate and made available for absorption, large amounts of phosphorus are excreted in feces, leading to phosphorus wastage, and environmental pollution (Cromwell and Coffey, 1991; Kornegay, 1996; Kornegay and Harper, 1997).

Many reports have indicated that the addition of microbial phytase to swine diets can release inorganic phosphate from phytate, thus improving phosphorus availability (Simons et al., 1990; Cromwell et al., 1993; Lei et al., 1993; Pallauf et al., 1994; Cromwell et al., 1995ab; Yi et al., 1996; Radcliffe et al., 1998). Microbial phytase also releases other nutrients bound to phytate phosphorus, thus improving the digestibility and retention of nitrogen (Ketaren et al., 1993; Pallauf et al., 1994), some amino acids (Mroz et al., 1994; Murry et al., 1997), and calcium (Nasi, 1990; Adeola et al., 1995).

High dietary calcium concentrations may have a negative effect on the utilization of phosphorus because of the formation of an insoluble calcium-phytate complex (Wise, 1983; Fisher, 1992). As a consequence, in studies conducted both with starter (Lei et al., 1994; Qian et al., 1996) and grower-finisher pigs (Lantzsch et al., 1995; Skoglund et al., 1997; Lui et al., 1998), as the calcium level of the diet increased, the ability of microbial phytase to improve phytate phosphorus availability declined. The following experiment was conducted to test the effects of two dietary calcium levels on microbial phytase activity in various sections of the gastrointestinal tract and on nutrient balance (nitrogen, calcium, phosphorus) for growing pigs.

## MATERIALS AND METHODS

### Surgical procedures

Three crossbred (Duroc×Landrace×Large White) barrows weighing 45 kg body weight, were fitted with two simple T-cannulas, one in the duodenum (25 cm posterior to the pylorus) and one in the terminal ileum (12 to 15 cm anterior to the ileal-cecal junction). The nylon T-cannula, with a threaded 1.2 cm outside diameter tube and curved T-flange 6 cm long, were prepared at the Beijing Agricultural University Machine Shop from nylon rod stock purchased locally. A detailed description of the procedures used to install the cannulas was published previously (Zhu et al., 1998). Following surgery, the pigs were allowed a 10 day recuperation period.

### Experimental design

The pigs were assigned to diets in a 3×3 Latin Square design experiment. The control diet (table 1) provided 0.8% calcium and was formulated to NRC (1988) specifications using corn, soybean meal, rapeseed meal and cottonseed meal. However, the diet contained

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no added inorganic phosphorus (0.45% total phosphorus) and no added phytase. The two experimental diets were similar to the control diet but were supplemented with microbial phytase (750 phytase units/kg), and supplied either 0.8% (microbial phytase diet) or 0.4% (calcium+ phytase diet) calcium. Chromic oxide (0.25%) was used as a digestibility marker.

**Table 1.** Composition of diets fed to determine the effects of dietary calcium levels on the efficacy of microbial phytase

| | Control | Phytase | ½ Ca +Phytase |
|---|---|---|---|
| Ingredients (% as fed) | | | |
| Corn | 74.29 | 74.29 | 75.87 |
| Soybean meal | 10.65 | 10.65 | 10.50 |
| Rapeseed meal | 4.00 | 4.00 | 4.00 |
| Cottonseed meal | 7.00 | 7.00 | 7.00 |
| Soybean oil | 0.55 | 0.55 | 0.20 |
| Limestone | 1.76 | 1.76 | 0.77 |
| Salt | 0.30 | 0.30 | 0.30 |
| L-Lysine HCl | 0.20 | 0.20 | 0.20 |
| Vit-Min premix[1] | 1.00 | 1.00 | 1.00 |
| Chromic oxide | 0.25 | 0.25 | 0.25 |
| Microbial phytase[2] | - | + | + |
| Nutrient level (% dry matter unless stated) | | | |
| Crude protein | 17.24 | 17.24 | 17.24 |
| Calcium | 0.80 | 0.80 | 0.40 |
| Total phosphorus | 0.45 | 0.45 | 0.45 |
| DE (kcal/kg)[3] | 3,630 | 3,630 | 3,630 |
| Lysine[3] | 0.86 | 0.86 | 0.86 |
| Met.+Cys.[3] | 0.55 | 0.55 | 0.55 |

[1] Supplied the following amounts per kilogram of diet: 50 ppm Fe; 250 ppm Cu; 50 ppm Mn; 60 ppm Zn; 0.3 ppm Se; 0.3 ppm I; 50 ppm olaquindox; 5512 IU vitamin A; 551 IU vitamin $D_3$; 66.1 IU vitamin E; 2.0 mg vitamin K; 0.2 mg folic acid; 40 mg choline; 0.8 mg thiamine; 60 mg riboflavin; 100 mg pantothenic acid; 120 mg niacin; 12 $\mu$g vitamin $B_{12}$.

[2] 0.3 g Phytase Novo (750 phytase units) was added per kg of diet.

[3] Calculated according to the China Feed Database (1995). All remaining values were determined by chemical analysis.

The source of the microbial phytase was Phytase Novo (Novo Nordisk Company, Bagsvaerd, Denmark) produced by the organism *Aspergillus oryzae*, using a gene recombination technique. The product was in the form of a coated granule (2500 phytase units/g), and was included at a rate of 0.3 kg/tonne. One phytase unit is defined as the quantity of enzyme that liberates 1 $\mu$mol of inorganic phosphorus per minute from 5.1 mmol of sodium phytate at 37℃ and pH 5.5 (manufacturer's specifications). Contaminating levels of proteases and carbohydrases were also present in the enzyme cocktail.

**Animal housing**

During the experimental period, the barrows were housed in an environmentally controlled room with the temperature of the room maintained between 25-27℃. Ventilation was provided by a 600 mm diameter fan while lighting was continuous throughout the experiment. The barrows were housed in 0.5×1.5 m cast iron metabolic crates equipped with a 0.25 $m^3$ round bottom feeder located at the front of the crate. The pigs were fed a wet feed (approximately 1200 g) twice daily (8:00, 20:00 h) and were provided with water in the feeder bowl at all times except during feeding.

**Collection of feces, digesta and urine**

Each test period lasted 16 days, consisting of a five day period during which the pigs were fed the control diet to consume intrinsic phosphorus, followed by a five day adaptation period and then a three day collection of feces and urine. Beginning on the first day of collection, feces were collected as soon as they were excreted, then placed into a sealed container. The amount of feces excreted each day was weighed and one-fifth of the fresh feces was conserved and mixed with feces from the other two day collection. Approximately 25% of the total fecal collection was mixed with 10% tartaric acid and dried at 65℃ for 70 hours. The total 24 h urinary excretion of each pig was weighed and placed into a bottle containing 50 ml of 20% HCl and 10 ml methyl benzene. One tenth of the total urinary excretion was then frozen at 20℃ until needed for analysis.

Digesta was collected for a three day period following the fecal collection. Starting 1 h after feeding, approximately 50 ml of duodenal digesta was collected every two hours, six times daily. Ileal digesta was collected continuously (150 ml each day). At the end of the trial, samples of ileal and duodenal digesta were freeze dried and frozen at -20℃ until analysis. The dried samples were ground to pass through a 1 mm sieve.

**Sample analyses**

Samples of feeds, feces and urine were analyzed for their nitrogen, calcium and total phosphorus content using the methods of the AOAC (1990). Nitrogen was analyzed using the Kjeldahl method (AOAC method 988.05), calcium by titration with 0.1 N $KMnO_4$ (AOAC method 927.02) and total phosphorus was determined colorimetrically using a molybodovanadate reagent (AOAC method 965.17). The phytate phosphorus content of feed and digesta was analyzed according to the procedures described by Wheeler and Ferrel (1971). Microbial phytase activity was measured using the procedure of Simons et al. (1990). Chromic oxide analysis was conducted according to the description provided by Christian and Coup (1954).

Total tract digestibilities of phosphorus, calcium, nitrogen and dry matter were calculated using the total fecal collection technique. Digestibilities of phosphorus and phytate in the duodenum and terminal ileum were calculated according to the external marker method.

**Statistical analysis**

The data were analyzed according to a Latin Square Variance Analysis. When the data were significant, Tukey's multicomparison was used to compare significant differences between average means (SAS, 1989).

## RESULTS

There was no difference between treatments (p>0.05) in the total phosphorus content of feed or duodenal digesta (table 2). The total phosphorus content of ileal digesta and feces were significantly (p=0.01) lower in pigs supplemented with microbial phytase. Reducing the calcium level of the diet further reduced (p=0.01) the total phosphorus concentration of ileal digesta but not of feces.

**Table 2.** Effect of microbial phytase and dietary calcium levels on total phosphorus and phytate phosphorus concentrations as well as phytase activity in feed, digesta and feces of growing (45 kg) pigs[1,2]

| | Control | Phytase | ½ Ca +Phytase | SEM[3] | p value[4] |
|---|---|---|---|---|---|
| Total phosphorus (%) | | | | | |
| Feed | 0.45 | 0.45 | 0.45 | - | - |
| Duodenal digesta | 0.33 | 0.33 | 0.34 | 0.01 | 0.99 |
| Ileal digesta | 0.66[a] | 0.52[b] | 0.34[c] | 0.02 | 0.01 |
| Feces | 1.99[a] | 1.57[b] | 1.44[b] | 0.08 | 0.01 |
| Phytate phosphorus (%) | | | | | |
| Feed | 0.28 | 0.28 | 0.28 | - | - |
| Duodenal digesta | 0.18[a] | 0.01[b] | 0.01[b] | 0.06 | 0.04 |
| Ileal digesta | 0.56[a] | 0.12[b] | 0.09[b] | 0.06 | 0.03 |
| Phytase activity (umol/min/kg) | | | | | |
| Feed | <50 | 786 | 786 | - | - |
| Duodenal digesta | <50[a] | 655[b] | 710[b] | 61.0 | 0.09 |
| Ileal digesta | <50 | <50 | <50 | 6.4 | 0.96 |

[1] Data are based on dry matter.
[2] Data within rows with the same or no superscript are not significantly different (p<0.05).
[3] Standard error of the mean.
[4] Probability of difference being significant.

The phytate phosphorus content (table 2) in the duodenal (p=0.04) and ileal digesta (p=0.03) of pigs fed microbial phytase was significantly lower than the control group. Calcium level had no further effect on either duodenal or ileal phytate phosphorus concentrations. Analysis of the duodenal and ileal digesta demonstrated high microbial phytase activity in the duodenal digesta and very low microbial phytase activity in the ileal digesta.

With added microbial phytase, the digestibility of total phosphorus measured at the terminal ileum (27.5 vs. 48.4%; p=0.01) and over the entire digestive tract (35.1 vs. 48.9%; p=0.03) was significantly improved (table 3). Reducing the dietary calcium level had no further effect on total phosphorus digestibility measured either at the terminal ileum (48.4 vs. 58.2%) or over the entire digestive tract (48.9 vs. 56.7%).

**Table 3.** The effect of microbial phytase and dietary calcium levels on the digestibility of total phosphorus and phytate phosphorus in different parts of the digestive tract in growing pigs[1,2]

| | Control | Phytase | ½ Ca +Phytase | SEM[3] | p value[4] |
|---|---|---|---|---|---|
| Total phosphorus (%) | | | | | |
| Terminal ileum | 27.5[a] | 48.4[b] | 58.2[b] | 3.50 | 0.01 |
| Total tract | 35.1[a] | 48.9[b] | 56.7[b] | 3.41 | 0.03. |
| Phytate phosphorus (%) | | | | | |
| Duodenum | 16.5[a] | 68.3[b] | 79.2[c] | 3.72 | 0.001 |
| Terminal ileum | 8.9[a] | 58.6[b] | 64.3[b] | 5.43 | 0.001 |

[1] Within rows, means followed by same or no letter are not significantly different (p<0.05).
[2] Total phosphorus digestibility in the total tract was calculated according to the total fecal collection technique; the remaining values were calculated according to the external marker method.
[3] Standard error of the mean.
[4] Probability of difference being significant.

The digestibility of phytate phosphorus measured at the duodenum (16.5 vs. 68.3%) and at the terminal ileum (8.9 vs. 58.6%) was significantly (p=0.001) improved by the addition of microbial phytase (table 3). Reducing the calcium level of the diet produced further increases (p=0.001) in phytate phosphorus digestibility measured at the duodenum (68.3 vs. 79.2%) but not at the terminal ileum (58.6 vs. 64.3%).

Dry matter digestibility was unaffected by microbial phytase addition regardless of dietary calcium level (table 4). With added microbial phytase, fecal nitrogen excretion was significantly reduced (p=0.03), while urinary nitrogen excretion was reduced significantly (p=0.03) only when the dietary calcium level was lowered. A significant (p=0.05) increase in nitrogen digestibility was observed with added microbial phytase but no further increase was obtained with the reduced calcium level. The percentage of both nitrogen intake (p=0.03) and absorbed nitrogen (p=0.04) that were retained was significantly increased by microbial phytase but lower dietary calcium produced no further effect.

Fecal phosphorus excretion was significantly reduced (p=0.01) by the addition of microbial phytase and further reduced (p=0.01) when both microbial phytase and a lower dietary calcium level were fed (table 5). As a consequence, the amount of dietary phosphorus both absorbed (p=0.01) and retained (p=0.01) was increased by microbial phytase and further increased (p=0.01) by the reduction in dietary calcium.

Calcium intake (p=0.03) as well as fecal (p=0.01) and urinary calcium (p=0.01) excretion were significantly lower for pigs fed the low calcium diet than for pigs fed either the control or the microbial phytase supplemented diet (table 5).

**Table 4.** The effect of added microbial phytase and dietary calcium levels on nitrogen and dry matter balance in growing pigs[1]

| | Control | Phytase | ½ Ca+ Phytase | SEM[2] | P value[3] |
|---|---|---|---|---|---|
| Dry Matter (g/d) | | | | | |
| DM intake | 2020.6 | 2192.3 | 2162.3 | 74.87 | 0.23 |
| Fecal DM | 297.2 | 319.5 | 292.6 | 11.75 | 0.09 |
| DM digestibility, % | 85.3 | 85.4 | 86.5 | 0.54 | 0.31 |
| Nitrogen Balance (g/d) | | | | | |
| N intake | 55.4 | 56.6 | 54.2 | 1.35 | 0.23 |
| Fecal N | 11.5[a] | 10.2[b] | 9.6[b] | 0.39 | 0.03 |
| Urinary N | 10.7[a] | 7.2[ab] | 5.5[b] | 1.12 | 0.03 |
| N absorption | 43.9 | 46.4 | 44.6 | 1.46 | 0.34 |
| N digestibility, % | 79.2[a] | 81.9[b] | 82.3[b] | 0.67 | 0.05 |
| N retention | 33.2 | 39.2 | 39.1 | 3.44 | 0.12 |
| Retention/ intake, % | 59.9[a] | 69.3[b] | 72.1[b] | 2.69 | 0.03 |
| Retention/ absorption, % | 75.6[a] | 84.5[b] | 87.6[b] | 2.78 | 0.04 |

[1] Within rows, means followed by same or no letter do not differ ($p<0.05$).
[2] Standard error of the mean.
[3] Probability of difference being significant.

**Table 5.** The effect of added microbial phytase and calcium levels on phosphorus and calcium balance in growing pigs[1]

| | Control | Phytase | ½ Ca +Phytase | SEM[2] | P Value[3] |
|---|---|---|---|---|---|
| Phosphorus Balance (g/d) | | | | | |
| P intake | 9.0 | 9.8 | 9.7 | 1.40 | 0.21 |
| Fecal P | 5.9[a] | 5.0[b] | 4.2[c] | 0.22 | 0.01 |
| Urinary P | 0.02 | 0.03 | 0.04 | 0.01 | 0.34 |
| P absorption | 3.2[a] | 4.8[b] | 5.5[c] | 0.18 | 0.01 |
| P digestibility, % | 35.1[a] | 48.9[b] | 56.7[b] | 3.41 | 0.03 |
| P retention | 3.2[a] | 4.8[b] | 5.5[c] | 0.32 | 0.01 |
| Retention/ intake, % | 35.1[a] | 48.9[b] | 56.7[c] | 2.05 | 0.01 |
| Retention/ absorption, % | 99.0 | 99.4 | 99.4 | 0.15 | 0.22 |
| Calcium Balance (g/d) | | | | | |
| Ca intake | 16.2[a] | 17.5[a] | 9.3[b] | 3.52 | 0.03 |
| Fecal Ca | 9.0[a] | 7.2[a] | 1.5[b] | 1.26 | 0.01 |
| Urinary Ca | 0.66[a] | 0.62[a] | 0.32[b] | 0.05 | 0.01 |
| Ca absorption | 7.2 | 10.3 | 7.8 | 1.59 | 0.15 |
| Ca digestibility, % | 44.2[a] | 59.0[ab] | 83.8[b] | 5.84 | 0.01 |
| Ca retention | 6.6 | 10.0 | 7.5 | 1.85 | 0.14 |
| Retention/ intake, % | 40.4[a] | 58.0[b] | 79.9[c] | 3.92 | 0.01 |
| Retention/ absorption, % | 90.9 | 94.2 | 95.9 | 0.84 | 0.08 |

[1] Within rows, means followed by same or no letter do not differ ($p<0.05$).
[2] Standard error of the mean.
[3] Probability of difference being significant.

Calcium digestibility was increased ($p=0.01$) by microbial phytase addition and further increased ($p=0.01$) by lowering dietary calcium content in the presence of microbial phytase. The percentage of dietary calcium that was retained was significantly ($p=0.01$) increased by microbial phytase addition and further increased ($p=0.01$) by lowering the calcium content of the diet.

## DISCUSSION

A positive effect of added microbial phytase on phytate hydrolysis was demonstrated by measuring the digestibility of both total phosphorus and phytate phosphorus. With added phytase, 68.3% of phytate phosphorus was hydrolyzed prior to the duodenum. In the absence of microbial phytase, only 16.5% of phytate phosphorus was hydrolyzed. The results of the present study are therefore consistent with earlier reports showing improved phytate phosphorus utilization as a result of microbial phytase supplementation of diets fed to growing pigs (Simons et al., 1990; Jongbloed et al., 1992; Han et al., 1997; Murry et al., 1997; Harper et al., 1997).

The finding of a high degree of phytate degradation in the anterior portion of the gastrointestinal tract agrees with the work of Gueguen et al. (1968) who reported that phytate was mainly hydrolyzed in the stomach by wheat bran phytase. Jongbloed et al. (1992) measured the phytase content of different parts of the gastrointestinal tract in growing pigs fitted with two cannulas in the duodenum and terminal ileum and found the phytase activity of duodenum digesta was almost equal to the concentration of the diet with added microbial phytase. However, the phytase activity of ileal digesta was less than 4% of the phytase activity of the original diet.

An explanation for the higher activity in the anterior portion of the gastrointestinal tract can be obtained by examining the pH in the various sections of the tract. Microbial phytase has been shown to be most active either at pH 2.5 or pH 5.5 (Simons et al., 1990). Jongbloed et al. (1992) observed that the pH in the duodenum of pigs fed corn-soybean meal based diets ranged from pH 5 to 6 during the first hour following a meal. In contrast, the pH of ileal digesta remained over 7.5 for 9 h. Therefore, the pH in the stomach and duodenum are favorable for high microbial phytase activity whereas the pH in the lower small intestine is not.

Lowering the calcium level of the diet in the presence of dietary microbial phytase increased phytate phosphorus digestibility. The digestibility of phytate phosphorus measured at the duodenum was 13.7% higher in the diet with the reduced calcium level compared with the diet supplemented with microbial phytase alone. Therefore, our findings are consistent with the earlier studies of Lei et al. (1994) and Qian et al. (1996) with starter pigs and Lantzsch et al. (1995), Skoglund et al. (1997) and Lui et al. (1998) with grower-finisher pigs.

There are at least three potential mechanisms which could explain the detrimental effect of high dietary

calcium on the efficacy of microbial phytase (Lui et al., 1998). Wise (1983) suggested that calcium forms an insoluble complex with phytate that is simply not accessible for hydrolysis by phytase. The second potential mechanism involves the effects of calcium on intestinal pH. High dietary calcium has been shown to increase intestinal pH (Bolduan et al., 1988) which may lower microbial phytase activity by providing an unfavorable intestinal environment (Sandberg et al., 1993). Lastly, the extra calcium could suppress phytase activity directly by competing for the active sites of the enzyme (Qian et al., 1996).

Phytic acid is known to form complexes with protein in the small intestine and any hydrolysis of the phytate-protein complex would be expected to increase nitrogen digestibility (Pallauf et al., 1994). Ketaren et al. (1993), Murry et al. (1997) and Han et al. (1997) have produced results similar to the present experiment, showing a reduction in fecal nitrogen excretion and increases in nitrogen absorption and retention as a result of supplementation with microbial phytase. It is also possible that cleavage of phytic acid by microbial phytase diminishes its inhibitory influence on tyrpsin activity leading to an increase in nitrogen digestibility (Caldwell, 1992).

In agreement with the previous studies of Murry et al. (1997) and Han et al. (1997), the fecal excretion of both phosphorus and calcium were reduced by the addition of microbial phytase. Our finding of a greater absorption and retention of both these nutrients as a result of microbial phytase supplementation is also in agreement with these earlier studies.

The fecal excretion of both calcium and phosphorus were further lowered for pigs fed the diet with the reduced calcium level compared with the diet supplemented with microbial phytase alone. This resulted in more phosphorus but not calcium being absorbed and retained in the body. As such, these findings agree with the earlier reports of Lantzsch et al. (1995) and Liu et al. (1997).

## IMPLICATIONS

The overall results of this experiment indicate that the addition of microbial phytase to the diet of growing pigs was beneficial in improving the utilization of phytate phosphorus. Reducing dietary calcium appeared to increase the effectiveness of microbial phytase in degrading phytate phosphorus. The phosphorus contained in swine manure is considered to be an environmental pollutant, which may cause eutrophication of surface water (NRC, 1998). It is therefore desirable to reduce phosphorus excretion as much as possible. Increasing the efficacy of microbial phytase, through a reduction in dietary calcium levels, may help to meet this goal. However, care must be taken to ensure that animal performance is not compromised. Further research is needed to determine the optimum calcium to phosphorus ratio in diets supplemented with microbial phytase that will both optimize animal performance and, at the same time, minimize nutrient excretion.

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