# THE EFFECT OF DIETARY PHYTASE LEVEL ON PHOSPHOROUS AND CALCIUM BALANCE IN NURSERY PIGS.

**T. C. TSAI, C. R. DOVE, and M. J. AZAIN**

## ABSTRACT

The objective of this study was to evaluate the effect of an E. coli phytase on growth performance, and phosphorous (P) and Calcium (Ca) balance in nursery pigs. A 2 x 5 factorial arrangement of treatments was used, with main effects of available P ( 0.13% or 0.35%) and phytase (0, 250, 500, 2500, 12500 U phytase /kg diet). Barrows (initial wt. 18 kg) were housed individually in metabolism cages in an environmentally controlled room and fed test diets for 14 d. There was a 10-d adaptation period and a 4 d collection period. Body weight gain increased as the phytase level increased during the adaptation period and the overall period in pigs fed both low-P and adequate-P diet ($P<0.01$). There was also a significant effect of dietary P level, with pigs fed the 0.35% P diet gaining more than those fed 0.13% ( $P < 0.001$). Fecal P excretion was increased ($P < 0.0001$) in pigs fed the 0.35% P diet and reduced as the phytase level increased ($P<0.0001$). Urinary P excretion was less than 50 mg per day in pigs fed the 0.13% P diet and was unaffected by phytase. Urinary P was greater in pigs fed the 0.35% P diet and increased with phytase addition ($P < 0.01$) from approximately 3% of total excretion with no added phytase to 30% of the total P excretion with 12,500 U phytase. There was no effect of phytase on fecal Ca loss, but urinary Ca was reduced by phytase ($P < 0.001$) in both diets. Overall, Ca retention (%) was improved ($P < 0.001$) by the addition of phytase to both LP and adequate-P diets. These results demonstrate that addition of phytase to either low or adequate P diets, decreases fecal P excretion. On the adequate P diets, phytase addition results in P absorption that exceeds the animal's requirement, resulting in greater urinary loss.

## INTRODUCTION

The benefits of exogenous, fungal or E. coli phytase has been well established. E. coli-derived phytase (6-position) has been shown to improve growth performance at a level of 500 U/kg phytase in the diet (Augspurger et al. 2003, Veum et al. 2006). Improved thermostability (Rodrique et al. 1999) of the E. Coli. phytase is an advantage, particularly in pelleted diets fed to monogastric animals. E. Coli-derived phytase, expressed in pichia pastoris, was reported to have a greater benefits on nutrient digestibility and reducing P excretion than a fungal phytase in broiler chicks fed a P deficient diet (Silversides et al. 2004) and in pigs (Augspurger et al. 2003). A greater understanding of P utilization and balance with phytase supplementation in the diet can maximize the phytase benefit of growth performance and the reduction of environmental contamination. The objective of this study was to determine the effect of E. Coli. phytase supplementation on P and Ca balance in young pigs.

## MATERIALS AND METHODS

The experimental protocols used in this study were approved by the Animal Care and Use Committee of the University of Georgia. A total of 40 weanling barrows (7-week age, average weight 18.50 $\pm$ 1 kg, PIC42 X PIC280) were randomly selected from the University of Georgia Animal & Dairy Science Department Swine Unit. Pigs used for this research were weaned at approximately 21 days of age and fed a common diet for four weeks before the experiment started. There were 20 pigs per replicate and 2 replicates of the 14-d trial. Pigs were housed in an environmentally regulated room in the LARU (Large Animal Research Unit). Room temperature was set at 23 $^{0}$C, and a 12-hr light/dark (0700/1900) cycle was set. The pigs were trained to meal feeding twice each day at approximately 0800 and 1600hr at the rate of 4-5% of body weight per day. Feeding time was 45 minutes. Water was available ad libitum. Feed consumption was recorded at each meal, and feed refusals and spillage were collected. Pigs were weighed at day 0, and placed into individual stainless steel metabolism cages (0.71m x 0.81m), equipped with a nipple waterer, feeding bowl holder, and plastic-coated expanded metal floors. On day 10, pigs were weighed, and cages were cleaned and set up for a 4-day collection trial. During the 4-day collection period, the total fecal output was collected twice daily from each pig. Urine was collected twice daily into containers with 25ml 3N HCL. The individual pig urine total volume was recorded, and 10% of the total was reserved in a 1L bottle. Fecal and urine samples were stored at -20 $°C$ until further analysis. The screens and trays were washed after every collection. At the end of the trial, the pigs were returned to the farm.

All pigs were fed a standard weaning diet for 4 wk before the trial. During the trial, a corn-soybean basal diet was used, and each treatment met or exceeded the NRC recommendation, except for the P content. The diet contained 1.15% of lysine and 3400 kcal/kg metabolism energy, in both LP and adequate-P. The ratio of calcium and total phosphorous was 1.3:1 in adequate-P and 2:1 in LP. Both diets were supplemented with 0, 250, 500, 2500, 12500 U/kg phytase at the expense of corn. The phytase used in this study was the Quantum, E. coli product from Syngenta Animal Nutrtition. The low P diet had no added inorganic P. The diet composition is shown in Table 1.

## STATISTICAL ANALYSIS

All data were analyzed using the PROC GLM procedure in SAS as a 2 x 5 design with main effects of level of phytase (0, 250, 500, 2500, 12500) and level of phosphorous (0.13 and 0.35%) as well as the diet P and phytase interactions. Results are presented as least square means for the phytase x P level effects. Each pig was used as the experimental unit in ANOVA.

## RESULTS

Growth Performance:

Pigs fed the adequate-P diet had greater growth rates than those fed the LP diet (P<0.005). Addition of phytase to both the LP (0.13% aP) and adequate-P (0.35% aP) diets resulted in improved body weight gain for day 0-10 and overall (P< 0.01, Table 2). On the LP diet, addition of 250, 500, 2500 and 12500 U/kg phytase/kg improved gain by 37, 33, 61 and 49% over the non-supplemented control. In the adequate-P diet, addition of phytase improved pigs' growth rate by 3, 8, 12 and 17% over the no supplemented control in this group. There was no significant phytase x dietary phosphorous interaction on growth rate. Pigs fed the low-P diet, with the addition 2500 and 12500 U/kg phytase had growth rates that were similar to those of pigs fed the adequate-P no phytase treatment, indicating a normalization of growth.

There was no significant phytase effect on feed intake. Feed efficiency was better in pigs fed adequate-P diets than in those were fed LP for day 0-10 (P<0.01) or day 0-14 (P<0.001), and there was no difference during the collection period. As with growth rate, there were no significant diet x phytase interactions on G : F ratio for day 0-10 or day 0-14. Efficiency in pigs fed the LP diets with 2500 or 12500 U phytase/kg was not different from that in pigs fed the adequate-P no phytase diet.

Phosphorous Excretion and Digestibility:

As expected, phosphorus intake was significantly higher in the adequate P group (P<0.0001, Table 3). Pigs fed the adequate-P diet had greater average fecal P excretion than the pigs fed the LP diet (P<0.001). Supplementing 250, 500, 2500, and 12500 U/kg phytase in the low-P (LP) diet reduced fecal P excretion by 1.7%, 18%, 49% and 60%, while adding the same levels of phytase in the adequate-P group resulted in 12%, 22%, 31%, and 35% less P output in the feces. Since feed intake was not significantly different and fecal P was reduced with phytase, the apparent total tract digestibility (ATTD) of P increased significantly with the addition of phytase (P<0.001, Table 5). The ATTD of P was increased 23%, 30%, 58%, and 67% or 12%, 15%, 29% and 25% as 250-12500 U/kg phytase was added to the LP or adequate-P groups, respectively, as compared to pigs fed non-phytase diet. There was a phytase x dietary P interaction for ATTD of P that was accounted for by the greater response to P in pigs fed the LP diet, as compared to those fed the adequate-P (P<0.01).

Urinary P in pigs fed the low P diet was less than 100 mg/d and was unaffected by dietary phytase addition. In contrast, feeding phytase to pigs fed the adequate P diet resulted in a significant increase in urine P (P<0.0001). The phytase level and phosphorous level interaction was significant and this due to the lack of change in urine P with phytase addition to the LP as compared to adequate-P (P<0.0001). Total P output was reduced significantly (P<0.01) by increasing the phytase level, similar to fecal P output. However, the total P excretion was increased in pigs fed 12500 U/kg phytase in adequate-P because urine P excretion dramatically affected the result of the total P output. The percentage of P retention increased by 23%, 30%, 58%, and 68% as 0-12500 U/kg phytase was supplemented in the LP group, and by 13%, 13%, 23%, and 7% in adequate-P.

Calcium Excretion and Digestibility:

Calcium intake was not different between treatmentd. Phytase addition did not change fecal Ca excretion significantly in either LP or adequate-P group. Phytase addition in the diet increased ATTD of Ca (P<0.05, Table 4). Pigs fed the LP diet tended to show a greater response to phytase than pigs fed adequate-P diet (P< 0.06). Urinary Ca excretion was reduced in both LP and adequate-P after phytase was supplied in the diet (P<0.005). Total Ca output (urine + feces) was significantly reduced in both LP and adequate-P (P< 0.02) as phytase was added. Calcium retention increased with phytase (P< 0.001) and was greater in the adequate-P diet (P=0.001). Phytase improved calcium retention more in pigs fed the 0.13% aP diet than in those fed 0.35% aP diets (P< 0.05).

## DISCUSSION

Historically, inorganic P (iP) has been used in diets to provide sufficient available P (Haper et al. 1997). In the present study, supplementation of dicalcium phosphate increased BW gain (P<0.001), and G:F ratio (P<0.001) as compared to that with the unsupplemented, LP (0.13% aP) diet. However, addition of inorganic phosphorous resulted in significantly greater P excretion in the feces, which would have a negative impact on the environment. Phytase addition is an alternative to the use of dicalcium phosphate and numerous studies demonstrate improvements in growth performance, P and Ca digestibility, and bone characteristics (Augspurger et al. 2004, Kies et al, 2006). In our work, phytase addition reduced total fecal P % to 49% and 60% (2500, 12500 U/kg), compared to no phytase in pigs fed the LP diet, and 31% and 35%, compared to no phytase in adequate-P diets (0.35% aP). The addition of 2500 U/kg phytase in the diet can totally replace inorganic phosphate treatment.

One of the potential concerns with the use of phytase is that while it clearly reduces fecal P excretion, it may result in increased urinary P output. The results of this research demonstrate that until total P absorbed reaches a threshold, there is no increase in urinary output. In the present study, none of the pigs fed the low P diet supplemented with phytase reached this threshold. Pigs fed the adequate P diet, with 0 or 250 U phytase/kg diet had urinary P that was greater than that in any of the pigs fed the low P diets, but remained less than 100 mg/d. Urinary P did not begin to increase significantly until the pigs were fed the adequate-P diet supplemented with 500 U phytase /kg diet or greater. This indicates that once the P needs of the animal are met, any additional P will be excreted in the urine. In this study, the pig’s capacity to utilize P is approximately 3.2 g/d.

The addition of 500 U/kg E. coli phytase in our study increased BW gain by 33% when compared to no phytase treatment (Table 2) in LP. This is similar or higher than what has been observed with the same level of other phytases (Augspurger et al. 2004, Veum et al. 2006). Moreover, there was a full recovery of P and Ca digestibility with 2500, or 12500 U/kg phytase supplementation of LP in our study, compared to adequate-P without phytase. Kies et al. (2006) demonstrated that use of 15000 FTU/kg phytase in a deficient P diet improved Ca and P digestibility up to 75.8% and 83.8%. Addition of 12500U/ Kg phytase of LP in our study increased Ca and P digestibility up to 79.33% and 82.39%, respectively. Addition of phytase to an adequate-P diet in our study resulted in improved BW gain, and G:F ratio over the unsupplemented control. The result of our study agreed with Veum et al.(2006), who also demonstrated benefits of phytase in diets with adequate-P levels.

The influence of the ratio of calcium and total phosphorous in the diets and the effectiveness of phytase has been discussed. Maintaining the Ca: tP ratio at 1.2:1 or 1.6:1 with phytase improved growth performance, bone characteristics, and P and Ca digestibility by comparison of to 2:1 (Qian et al., 1996). The studies examining the relationship of Ca and tP level to the response to phytase in pigs have been done with fungal phytase (Liu et al. 2000). There is a lack of research defining the effect of Ca: tP ratio with E. Coli. phytase in the pig. In this study, the ratio of calcium and total P for LP was 2.08:1 and 1.29:1 in the adequate-P diet. It is possible that some of the results could have been influenced by the ratio. The effect of Ca and P ratio with this enzyme still needs to be determined.

Urinary Ca excretion was decreased in pigs fed either the LP or adequate-P diets that were supplemented with phytase. The reason for this is not clear, but this may represent a means to reduce formation of calcium stones in species where this is a problem. Serum P levels were not determined, but clearly phytase increased P digestibility which may have resulted in greater Ca reabsorption from the kidney (Laiken, 1985).

## Conclusion

The benefit of E. Coli. phytase on BW gain, P and Ca retention and digestibility was clearly shown. In this study, 2500 U/kg phytase supplementation to a low phosphorous diet (0.13% aP) seems to maximize the phytase efficiency, and most importantly reduced the environmental contamination from excess P excretion. Phytase addition to the diet reduced fecal P output and there was no increase in urinary P excretion unless both high levels of P were fed and phytase was added.

## Literature Cited


Augspurger, N. R., D. M. Webel, X. G. Lei, and D. H. Baker. 2003. Efficacy of *E. coli* phytases expressed in yeast for releasing phy-tate-bound phosphorus in young chicks and pigs. J. Anim. Sci. 81:474–483.

Augspurger, N. R., D. H. Baker. 2004. High dietary phytase levels maximize phytate-phosphorus utilization but do not affect protein utilization in chicks fed phosphorusor amino acid-deficient diets. J. Anim. Sci. 82:1100-1107.

Harper, A. F., E. T. Kornegay, and T. C. Schell. 1997. Phytase Supplementation of Low-Phosphorus Growing-Finishing Pig Diets Improves Performance, Phosphorus Digestibility, and Bone Mineralization and Reduces Phosphorus Excretion. J. Anim. Sci. 75:3174–3186.

Kies, A. K., P. A. Kemme., L. B. J. Sebek., J, Th. M. van Diepen., and A. W. Jongbloed., 2006, Effect of graded doses and a high dose of microbial phytase on digestibility of various minerals in weaner pigs. J. Anim. Sci. 84: 1169-1175.

Laiken, N. D., Fanestil D. D. 1985. Regulation of calcium, magnesium, and phosphorous excretion. Pages 537-540 in Physiological Basis of Medical Practice. Eleven edition. John B. West, MD, PHD. Williams & Wilkins Press.

Liu, J., D. W. Bollinger, D. R. Ledoux, and T. L. Veum. 2000. Effects of dietary calcium:phosphorus ratios on apparent absorption of calcium and phosphorus in the small intestine, cecum, and colon of pigs. J. Anim. Sci. 78:106–109.

NRC, 1998. Nutrient Requirements of Swine, 10th ed. National Academy Press, Washington, DC.

Qian, H., E. T. Kornegay, and D. E. Conner, Jr. 1996. Adverse effects of wide calcium:phosphorus ratios on supplemental phytase efficacy for weanling pigs fed two dietary phosphorus levels. J. Anim. Sci. 74:1288-1297.

Rodriguez, E., Y. Han, and X. G. Lei. 1999. Cloning, Sequencing, and Expression of an *Escherichia coli* Acid Phosphatase/Phytase Gene (*appA2*) Isolated from Pig Colon. Bio. and Biophy. Res. Com. 257:117–123.

Silversides, F. G., T. A. Scott, and M. R. Bedford. 2004. The Effect of Phytase Enzyme and Level on Nutrient Extraction by Broilers. Poultry Science 83:985–989.

Veum, T. L., D. W. Bollinger, C. E. Buff, and M. R. Bedford. 2006. A genetically engineered *Escherichia coli* phytase improves nutrient utilization, growth performance, and bone strength of young swine fed diets deficient in available phosphorus. J. Anim. Sci. 2006. 84:1147–1158.

Table 1. Diet Composition.

| | Low P diet | Adequate P diet |
|---|---|---|
| Corn | 64.75 | 64.15 |
| Soybean meal | 29.53 | 29.62 |
| Fat | 3.0 | 3.0 |
| Salt | 0.35 | 0.35 |
| Limestone | 1.73 | 1.08 |
| Dicalcium Phosphate | 0.0 | 1.15 |
| Vitamin Premix[a] | 0.25 | 0.25 |
| Mineral Premix[b] | 0.15 | 0.15 |
| Lysine | 0.14 | 0.14 |
| Calculated Analysis: | | |
| ME[d], kcal/kg | 3420 | 3400 |
| Crude protein, % | 20.16 | 20.15 |
| Lysine, % | 1.15 | 1.15 |
| Calcium, % | 0.75 | 0.75 |
| Total P, % | 0.36 | 0.58 |
| Available P, % | 0.14 | 0.35 |

Low P treatment simply removed inorganic P.

Both low P diet and adequate P diet supplemented with 0, 250, 500, 2500, and 12500 U/kg phytase, formed ten treatments.

Higher limestone in low P diet was to compliment Ca.

a Supplied per kg of premix: vitamin A 4400 IU; vitamin D 660000 IU; vitamin E 17600 IU; vitamin K 1760 IU; riboflavin 3960 mg; niacin 22000 mg; vitamin B12 17600 $\mu g$ .

b Supplied per kg of premix: iron 110000 mg; copper 11000 mg; manganese 26400 mg; zinc 110000 mg; iodine 198 mg; selenium 198 mg.

c Titanium dioxide used as an indigestible marker.

d Metabolizable energy

Table 2. The Effect of Different Levels Phytases in LP (0.13% aP) and HP (0.35% aP) on Growth Performance.

| Diet | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | P Value | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | LP (13%) | | | | | HP (35%) | | | | | SEM | Plevel | Phytase | Plevel x Phytase |
| Phytase | 0U | 250U | 500U | 2500U | 12500U | 0U | 250U | 500U | 2500U | 12500U | | | | |
| Gain, kg | | | | | | | | | | | | | | |
| Day 0-10 | 2.964 | 4.197 | 4.050 | 5.374 | 5.050 | 4.546 | 4.950 | 5.422 | 5.122 | 5.857 | 0.439 | 0.0046 | 0.0055 | 0.2863 |
| Day 10-14 | 1.689 | 2.193 | 2.122 | 2.122 | 2.287 | 2.481 | 2.272 | 2.202 | 2.769 | 2.490 | 0.266 | 0.0415 | 0.6248 | 0.5131 |
| Day 0-14 | 4.653 | 6.390 | 6.172 | 7.497 | 6.930 | 7.027 | 7.222 | 7.624 | 7.891 | 8.230 | 0.543 | 0.001 | 0.0144 | 0.4265 |
| Intake, g/d | | | | | | | | | | | | | | |
| Day 0-10 | 836 | 875 | 845 | 936 | 909 | 883 | 909 | 945 | 904 | 904 | 43.12 | 0.2965 | 0.7054 | 0.5964 |
| Day 10-14 | 822 | 990 | 938 | 1036 | 939 | 985 | 979 | 993 | 1013 | 1049 | 68.12 | 0.1821 | 0.4861 | 0.6166 |
| Day 0-14 | 832 | 908 | 871 | 965 | 918 | 912 | 929 | 959 | 935 | 945 | 45.19 | 0.1993 | 0.5267 | 0.6897 |
| Gain:Feed | | | | | | | | | | | | | | |
| Day 0-10 | 0.347 | 0.471 | 0.466 | 0.569 | 0.549 | 0.507 | 0.541 | 0.564 | 0.563 | 0.645 | 0.044 | 0.0052 | 0.0067 | 0.4552 |
| Day 10-14 | 0.505 | 0.525 | 0.577 | 0.508 | 0.690 | 0.604 | 0.579 | 0.556 | 0.668 | 0.588 | 0.059 | 0.3219 | 0.6431 | 0.2645 |
| Day 0-14 | 0.392 | 0.489 | 0.496 | 0.550 | 0.534 | 0.536 | 0.551 | 0.561 | 0.592 | 0.621 | 0.033 | 0.0006 | 0.013 | 0.5611 |

Results are LS Means for 8 pigs each treatment, the interaction between two phosphorous levels (0.13% and 0.35% aP) and five ohytase levels (0, 250, 500, 2500, and 12500 U/kg). Significance was set at $P<0.05$.

Table 3. The Effect of Different Levels Phytases in LP (0.13% aP) and HP (0.35% aP) on P Balance.

| Diet | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | P Value | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | LP(13%) | | | | | HP(35%) | | | | | SEM | Plevel | Phytase | Plevel x Phytase |
| Phytase | 0U | 250U | 500U | 2500U | 12500U | 0U | 250U | 500U | 2500U | 12500U | | | | |
| P intake, g/d | 3.57 | 4.56 | 4.20 | 4.23 | 4.04 | 5.17 | 5.54 | 5.09 | 5.90 | 5.35 | 0.32 | <.0001 | 0.1876 | 0.6686 |
| Fecal P, g/d | 1.81 | 1.78 | 1.47 | 0.92 | 0.72 | 2.17 | 1.91 | 1.70 | 1.49 | 1.40 | 0.16 | 0.0004 | <.0001 | 0.3928 |
| Urine P, g/d | 0.03 | 0.04 | 0.05 | 0.04 | 0.03 | 0.09 | 0.08 | 0.16 | 0.35 | 0.71 | 0.06 | <.0001 | <.0001 | <.0001 |
| Total P, g/d | 1.84 | 1.82 | 1.52 | 0.96 | 0.76 | 2.26 | 1.99 | 1.86 | 1.84 | 2.11 | 0.16 | <.0001 | 0.0011 | 0.0074 |
| P retention, g/d | 1.74 | 2.74 | 2.68 | 3.27 | 3.28 | 2.91 | 3.54 | 3.23 | 4.06 | 3.24 | 0.26 | 0.0004 | 0.0003 | 0.218 |
| P retention, % | 48.60 | 59.91 | 62.99 | 76.99 | 81.57 | 56.15 | 63.35 | 63.46 | 69.24 | 59.95 | 2.35 | 0.0224 | <.0001 | <.0001 |
| Ca intake, g/d | 5.72 | 6.88 | 5.22 | 6.75 | 6.89 | 4.91 | 6.95 | 7.27 | 6.89 | 7.31 | 0.63 | 0.3561 | 0.0524 | 0.2654 |
| Fecal Ca, g/d | 2.06 | 2.39 | 1.96 | 1.47 | 1.48 | 2.02 | 1.80 | 1.75 | 1.70 | 2.11 | 0.23 | 0.9773 | 0.211 | 0.1187 |
| Urine Ca, g/d | 1.77 | 1.88 | 1.67 | 1.40 | 1.08 | 1.03 | 0.32 | 0.43 | 0.24 | 0.20 | 0.17 | <.0001 | 0.0013 | 0.1525 |
| Total Ca, g/d | 3.83 | 4.27 | 3.63 | 2.88 | 2.56 | 3.04 | 2.12 | 2.17 | 1.95 | 2.31 | 0.34 | <.0001 | 0.0156 | 0.0818 |
| Ca retention, g/d | 1.89 | 2.61 | 1.59 | 3.87 | 4.33 | 1.86 | 4.83 | 5.09 | 4.95 | 4.99 | 0.53 | 0.0002 | 0.0002 | 0.0379 |
| Ca retention, % | 32.17 | 36.43 | 26.58 | 57.22 | 64.04 | 37.56 | 65.84 | 70.20 | 71.79 | 66.57 | 4.53 | <.0001 | <.0001 | 0.0004 |

Results are LS Means for 8 pigs each treatment, the interaction between two phosphorous levels (0.13% and 0.35% aP) and five phytase levels (0, 250, 500, 2500, and 12500 U/kg). Significance was set at P<0.05.

Table 4. The Effect of Different Levels Phytases in LP (0.13% aP) and HP (0.35% aP) on Apparent Digestibility.

| Diet | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | P Value | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | LP (13%) | | | | | HP (35%) | | | | | SEM | Plevel | Phytase | Plevel x Phytase |
| Phytase | 0U | 250U | 500U | 2500U | 12500U | 0U | 250U | 500U | 2500U | 12500U | | | | |
| Apparent Digestibility, % | | | | | | | | | | | | | | |
| Energy | 91.63 | 90.54 | 89.64 | 92.07 | 91.787 | 89.27 | 89.39 | 89.43 | 90.63 | 90.305 | 0.524 | 0.0011 | 0.0053 | 0.3611 |
| CP | 90.9 | 90.08 | 88.18 | 91.89 | 90.826 | 88.88 | 87.81 | 88.77 | 89.32 | 89.824 | 0.688 | 0.0016 | 0.0150 | 0.1708 |
| P | 62.69 | 69.86 | 65.24 | 79.41 | 81.726 | 64.28 | 73.3 | 73.58 | 78.15 | 74.801 | 2.643 | 0.5407 | <.0001 | 0.0746 |
| Ca | 73.98 | 73.68 | 68.74 | 81.09 | 82.339 | 64.9 | 68.4 | 75.91 | 77.7 | 70.689 | 3.237 | 0.0585 | 0.0200 | 0.0561 |

Results are LS Means for 8 pigs each treatment, the interaction between two phosphorous levels (0.13% and 0.35% aP) and five phytase levels (0, 250, 500, 2500, and 12500 U/kg). Significance was set at $P<0.05$.