Efficacy of Different Commercial Phytase Sources and Development of a Phosphorus Release Curve[1]

C. K. Jones, M. D. Tokach, B. W. Ratliff[2], N. L. Horn[3], S. S. Dritz[4], R. D. Goodband, J. M. DeRouchey, and J. L. Nelssen

Summary

Two experiments used 184 pigs (PIC, 22.7 and 21.3 lb BW, respectively) to develop an available P (aP) release curve for commercial phytase products. In Exp. 1 and 2, pigs were fed a basal diet (0.06% aP) and 2 levels of added aP from inorganic P (monocalcium P) to develop a standard curve. In Exp. 1, 100, 175, 250, or 500 phytase units (FTU)/kg OptiPhos (Enzyvia LLC, Sheridan, IN) or 200, 350, 500 or 1,000 FTU/kg Phyzyme XP (Danisco Animal Nutrition, Marlborough, UK) was added to the basal diet. In Exp. 2, 250, 500, 750, or 1,000 FTU/kg OptiPhos; 500, 1,000, or 1,500 FTU/kg Phyzyme XP; or 1,850 or 3,700 phytase units (FYT)/kg Ronozyme P (DSM Nutritional Products, Basel, Switzerland), was added to the basal diet. Manufacturer-guaranteed phytase levels were used in diet formulation. Diets were analyzed for phytase using both the Phytex and AOAC methods. Pigs were blocked by sex and weight and allotted to individual pens with 8 pens per treatment. Pigs were euthanized on d 21, and fibulas were analyzed for bone ash. In Exp. 1, pigs fed increasing monocalcium P had improved (linear; $P = 0.01$) ADG, G/F, and percentage bone ash. Similarly, pigs fed increasing monocalcium P in Exp. 2 tended to have improved (quadratic; $P = 0.09$) ADG in addition to significantly improved (linear; $P \leq 0.001$) G/F and percentage bone ash. In Exp. 1, pigs fed increasing OptiPhos had increased (linear; $P \leq 0.02$) ADG, G/F, and percentage bone ash. Likewise, pigs fed increasing OptiPhos in Exp. 2 had improved (linear; $P \leq 0.001$) ADG and G/F, as well as increased (quadratic; $P \leq 0.001$) percentage bone ash. In Exp. 1, pigs fed increasing Phyzyme XP had increased (linear; $P \leq 0.04$) ADG and G/F and tended to have improved (linear; $P = 0.06$) percentage bone ash. Pigs fed increasing Phyzyme XP in Exp. 2 had increased (quadratic; $P \leq 0.001$) G/F and percentage bone ash. In Exp. 2, pigs fed increasing Ronozyme P had improved (linear; $P \leq 0.001$) ADG in addition to increased (quadratic; $P \leq 0.03$) G/F and percentage bone ash. When AOAC analyzed values and bone ash are used as the response variable, aP release for up to 1,000 FTU/kg of *Escherichia coli*-derived phytases (OptiPhos and Phyzyme XP) can be predicted by the equation ($y = -0.000000125x^2 + 0.000236245x + 0.015482000$), where x is the phytase level in the diet.

Key words: bone strength, phytase, phytase source

[1] Appreciation is expressed to JBS United for providing the pigs and facilities and for partial funding of this trial.

[2] Enzyvia LLC, Sheridan, IN.
[3] JBS United, Sheridan, IN.
[4] Food Animal Health and Management Center, College of Veterinary Medicine, Kansas State University.

Introduction

Phosphorus is one of the most significant minerals in swine nutrition. It is essential for bone development, plays a key role in metabolic processes such as the formation of cellular membranes, and is vital for enzymatic systems involved in fat and carbohydrate metabolism.

In cereal grains and oilseed meals, a large amount of P is in the form of phytic acid (myo-inositol hexaphosphate). The P in phytic acid is largely unavailable to the pig. Thus, a phytase enzyme is added to diets to enhance the pig's ability to use P from phytic acid. Many trials have been conducted to evaluate different sources of the phytase enzyme, including some prominent versions of the enzyme obtained from *Escherichia coli* or *Aspergillus oryzae*.

Because manufacturers have their own individual analytical techniques, it is often confusing to compare phytase sources by a single analytical method. To avoid this confusion, the current study used inclusion rates as directed by the product labels, which gives field-applicable available P (aP) release values. To further clarify comparisons, the current industry standard analysis (AOAC) was also conducted on all phytase samples.

Current data from JBS United demonstrates that 0.12% aP can be replaced in a corn-soybean meal-based diet with 250 phytase units (FTU)/kg OptiPhos (Enzyvia LLC, Sheridan, IN). Recommendations for Phyzyme XP (Danisco Animal Nutrition, Marlborough, UK) and Ronozyme P (DSM Nutritional Products, Basel, Switzerland) are that 500 FTU/kg or 1,850 phytase units (FYT)/kg, respectively, should be used to replace 0.10% aP. Phytase may be added at levels less than that needed to replace the 0.12% or 0.10% P. However, more data is needed to determine a response curve for OptiPhos, Phyzyme XP, and Ronozyme P. The development of dose response curves for *P* release could allow the optimum use of the different sources of the enzyme at all levels.

Our objectives for these trials were to evaluate the effects of three different sources of commercially available phytase on late nursery pig performance and to develop a P release curve.

Procedures

In Exp. 1, a total of 88 barrows (initially 22.7 lb) were used in a 21-d growth trial. Pigs were blocked by weight and allotted to 1 of 11 dietary treatments. In Exp. 2, a total of 104 pigs (initially 21.3 lb) were used in a 21-d growth trial. Pigs were blocked by sex and weight and allotted to 1 of 13 dietary treatments. In both experiments, there was 1 pig per pen and 8 pens per treatment. Each pen (31.6 × 39 in.) contained a 2-hole, dry self-feeder and a nipple water to provide ad libitum access to feed and water. The study was conducted in 4 adjacent rooms in the Discovery Nursery at JBS United's Burton Russell Research Farm in Frankfurt, IN. Samples of phytase and inorganic phosphorus premixes and complete feed were taken at the time of diet preparation and analyzed for phytase.

A common starter diet (meal form) containing 0.06% aP was fed to pigs for 6 d prior to the experiment while pigs were being acclimated to the barn. In Exp. 1 and 2, pigs were fed a basal diet (0.06% aP) and 2 levels of added aP monocalcium P (0.075 and 0.15 for Exp. 1 and 0.07 and 0.14 for Exp. 2) to develop a standard curve. In Exp. 1, 100, 175, 250, or 500 FTU/kg OptiPhos or 200, 350, 500, or 1,000 FTU/kg Phyzyme XP was added to the basal diet. In Exp. 2, 250, 500, 750, or 1,000 FTU/kg OptiPhos; 500, 1,000, or 1,500 FTU/kg Phyzyme XP; or 1,850 or 3,700 FYT/kg Ronozyme P was added to the basal diet.

In Exp. 1, all treatment diets were constructed from a single basal diet (Table 1) made in two batches at the Kansas State University (K-State) Animal Science Feed Mill. Each bag was marked with batch and bagging order. The first 3 and last 2 bags of each batch were not used in diet preparation. Individual treatments were mixed from the basal diet at the K-State Poultry Feed Mill. A total of 197.5 lb of each batch of the basal diet were used to create 395 lb of each treatment diet. Each of the 2 batches contributed 98.75 lb (a total of 197.5 lb) and was mixed for 2 min. Five pounds (2 lb phytase premix and 3 lb P premix) of premix was added to the mixer while the mixer hands were on the upside, and the diet was mixed for an additional 2 min. The additional 98.75 lb of each batch of the basal diet was added, and the diet was mixed for an additional 2 min. Approximately 30 lb of feed was removed from the mixer discharge and deposited back into the top of the mixer. The treatment was mixed for an additional 6 min, for a total treatment addition mixing time of 12 min. Treatments were bagged into 30-lb bags and tagged with labels including the K-State and JBS United protocol number and correlating treatment letter.

In Exp. 2, premixes were manufactured at K-State and shipped to Sheridan, IN, where they were added to a single basal diet (Table 1), which was made in 3 batches at the Burton Russell Research Farm Feed Mill in Frankfort, IN. Each bag was marked with batch and bagging order. The first and last 2 bags of each batch were not used in diet preparation trial. A total of 92, 152, and 150 lb of batches 1, 2, and 3 of the basal diet, respectively, were used to create 394 lb of each treatment diet. Half of each batch (a total of 197 lb) was added to the mixer and mixed for 2 min. Six pounds (2 lb phytase premix and 4 lb inorganic P premix) of premix was added to the mixer while the mixer hands were on the upside, and the diet was mixed for an additional 2 min. The remainder each batch of the basal diet was added, and the diet was mixed for an additional 2 min. Approximately 30 lb of feed was removed from the mixer discharge and deposited back into the top of the mixer. The treatment was mixed for an additional 2 min for a total treatment addition mixing time of 8 min. Treatments were bagged into 30-lb bags and tagged with labels including the K-State and JBS United protocol number and correlating treatment letter.

In both experiments, treatment premixes were made at the K-State Swine Research Laboratory. The phytase premixes consisted of a phytase source (OptiPhos, Phyzyme XP, or Ronozyme P) and/or cornstarch. The same lot of each OptiPhos and Phyzyme XP were used to make both Exp. 1 and 2 premixes. Phytase was stored in a freezer for approximately 3 mo between experiments. The negative control and diets with monocalcium P were made with no phytase and 2 lb of cornstarch. In Exp. 1, a single batch of the 500 FTU/kg OptiPhos premix and the 1,000 FTU/kg Phyzyme XP premix was manufactured and analyzed for lysine, Ca, P, and phytase content (Table 2). Micro-

ingredients were also analyzed for Ca (Table 3). In Exp. 2, a single batch of the 1,000 OptiPhos premix, 1,500 FTU/kg Phyzyme XP premix, and 3,700 FYT/kg Ronozyme P premix was made and analyzed for Ca, P, and phytase content. Cornstarch was added in increasing levels to the base mixes to dilute them to the various phytase levels used in the trials. In both experiments, the P premixes consisted of monocalcium phosphate (21% P) and/or sand of similar particle size. The negative control and diets containing phytase were made with no monocalcium P and 3 (Exp. 1) or 4 (Exp. 2) lb of sand. Premixes were analyzed for Ca and P, and phytase analysis was conducted according to the AOAC and Phytex methods (Table 4).

Treatment diets were fed in meal form for 21 d. Average daily gain, ADFI, and G/F were determined by weighing pigs and measuring feed disappearance on d 0 and 21 of the trial. Animals were euthanized via lethal injection with Euthanasia-III Solution (Exp. 1; Med-Pharmex) or Beuthanasia-D Special (Exp. 2; Schering-Plough) according to the K-State Institutional Animal Care and Use Committee standards. The right fibula was removed without cartilage caps from each animal, autoclaved, and boiled for 45 to 60 min. Fibulas were cleaned of adhering tissue, dried at 105°C for 24 h, and ashed in a muffle furnace at 600°C for 24 h. Total ash weight and percentage ash were measured.

Data Analysis

All values that were at least three SD away from the mean of each response criteria were considered outliers. In Exp. 1, 4 pigs with outliers for growth data (ADG, ADFI, or G/F) were removed from both the growth and bone (ash weight and percentage ash) results. Two pigs with outliers for percentage ash were removed from the ash weight and percentage ash results but were used for the calculation of growth data. One pig with an outlier for ash weight was removed from the ash weight results but was used in the calculation of percentage ash and growth data. Three fibulas were broken during analysis, preventing ash weight and percentage ash for these fibulas from being calculated. Growth data from these pigs were used. In Exp. 2, 1 pig was deemed an outlier for G/F and was removed from all data. One pig was considered an outlier for percentage ash and was removed from the ash weight and percentage ash results but was used for the calculation of growth data.

Data were analyzed as a randomized complete block design with pig as the experimental unit. Treatment was fixed, whereas pigs and room were randomly assigned. Analysis of variance was performed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Results were considered to be significant if their *P*-values were ≤ 0.05 and were considered to be a trend if their *P*-values were ≤ 0.10. Main effects from Exp. 1 showed that all treatments that included inorganic P remained in the linear portion of the quadratic curve of phytase release, and so all treatments were used for analysis. Conversely, main effects from Exp. 2 showed that the treatment supplemented with an additional 0.21% aP from inorganic P (0.27% total aP) was in the quadratic portion of the phytase curve. Because aP release curves must be generated from data that are only in the linear portion of this curve, the treatment was removed from all data analysis. For reference, adding 0.21% aP from monocalcium P (0.27% total aP) resulted in pigs with an ADG of 1.10 lb/d, an ADFI of 1.57 lb/d, a G/F of 0.70, a bone ash weight value of 775 mg, and a bone ash percentage of 41.9.

A regression equation was calculated for ADG, G/F, ash weight, and percentage ash to predict the percentage aP released from the *E. coli*-derived phytases, given each response criteria. First, the total intake of aP from the diet was calculated and termed to be the dosage of aP administered to each pig through its diet. Dosage for pigs fed the negative control, OptiPhos, Phyzyme XP, and Ronozyme P diets was the product of 0.06 and individual grams of feed intake. In Exp. 1, dosage for pigs fed the negative control diet plus 0.075% aP from the monocalcium P diet was the product of 0.135 and individual grams of feed intake. Dosage for pigs fed the negative control plus 0.15% aP from the monocalcium P diet was the product of 0.21 and individual grams of feed intake. In Exp. 2, dosage for pigs fed the negative control diet plus 0.07% a*P* from the monocalcium P diet was the product of 0.13 and individual grams of feed intake. Dosage for pigs fed the negative control plus 0.14 a*P* from the monocalcium P diet was the product of 0.20 and individual grams of feed intake.

Using these aP dosages, regression was used to determine the aP release from each phytase source for a given aP dosage (intercept) and the aP release from each response variable for a given aP dosage (slope). The percentage aP released from each phytase source (Y) was then calculated by adding the value of aP release from each phytase source for a given aP dosage to the product of the value of aP release from each response variable for a given aP dosage and the value of the response variable (X).

Results

In Exp. 1, lysine and P analysis of the diets resulted in concentrations similar to those used in diet formulation (Table 2). However, Ca levels were higher than expected because of higher than anticipated Ca levels in the microingredients. The high Ca levels resulted in high Ca to total P ratios (2.04 to 2.20) for the negative control and all phytase diets. As previous research suggests, these ratios likely decreased ADG and G/F. However, these ratios did not appear to affect percentage bone ash or the aP release levels calculated from percentage bone ash. Lower Ca:P ratios were used in Exp. 2, in which analysis of the diets resulted in concentrations similar to those used in diet formulation.

According to the AOAC analysis, the phytase concentration in OptiPhos was nearly 3.1 and 2.5 times the concentration listed on the label by the manufacturer for Exp. 1 and 2, respectively (Tables 5 and 6). The phytase level in Phyzyme XP was at the concentration listed on the label by the manufacturer in Exp. 1 and 0.7 times the listed concentration in Exp. 2. Ronozyme P was used and analyzed only in Exp. 2, in which the analyzed values were similar to levels reported on the label by the manufacturer.

Results of the AOAC analysis in both experiments indicated that, as expected, phytase levels increased linearly as more phytase premix was added to the diet. Phytase analysis with the Phytex assay found much lower phytase levels for all premixes and diets. Results from the Phytex analysis assay were not as consistent with added dietary levels as the AOAC assays; however, the Phytex assay was conducted only by one laboratory, whereas the AOAC assay was an average of results from three (Exp. 1) or two (Exp. 2) laboratories. Within laboratory, the Phytex assay was less consistent with our calculated values than any single AOAC assay.

Experiment 1

Pigs fed increasing monocalcium P had improved (linear; $P = 0.01$) ADG, ADFI, G/F, bone ash weight, and percentage ash (Tables 7 and 8). Pigs fed increasing OptiPhos had improved (linear; $P \leq 0.02$) ADG, G/F, and bone percentage ash, as well as increased (quadratic; $P = 0.05$) bone ash weight. Pigs fed increasing Phyzyme XP had improved (linear; $P \leq 0.04$) ADG and G/F, as well as a tendency for increased (linear; $P = 0.06$) percentage bone ash.

Percentage aP released from each phytase source varied depending on the response criteria used to calculate the value (Table 9). The lowest aP release value for both phytase sources was calculated with ADG as the response criteria. The aP release values calculated with G/F as the response criteria were nearly identical for all levels of OptiPhos, whereas levels generally increased with increasing Phyzyme XP to an overall release value that was similar for both phytase sources. The aP release values calculated from bone ash weight were similar for all levels of Phyzyme XP, with the exception of 500 FTU/kg. However, the calculated aP release values were not as consistent for OptiPhos, as evidenced by the second lowest phytase dose releasing the highest percentage aP. The clearest response to percentage aP release was calculated with percentage bone ash as the response criteria. As both OptiPhos and Phyzyme XP levels increased, calculated a*P* increased in a quadratic fashion to the highest phytase dose.

Experiment 2

Pigs fed increasing monocalcium P had improved (linear; $P < 0.001$) G/F and percentage bone ash, improved (quadratic; $P = 0.01$) ADFI, and a tendency for improved (linear; $P = 0.07$, quadratic; $P = 0.09$) ADG (Tables 10 and 11). Pigs fed increasing OptiPhos had improved (linear; $P \leq 0.01$) ADG, G/F, and bone ash weight, increased (quadratic; $P < 0.001$) percentage bone ash, and tended to have increased (linear; $P = 0.07$) ADFI. Pigs fed increasing Phyzyme XP had improved (linear; $P < 0.001$) percentage bone ash, improved (quadratic; $P = 0.05$) G/F, and tended to have increased (linear; $P = 0.09$) bone ash weight. Pigs fed increasing Ronozyme P had improved (linear; $P \leq 0.004$) ADG, ADFI, and bone ash weight, as well as improved (quadratic; $P \leq 0.03$) G/F and percentage bone ash.

Percentage aP released from each phytase source and level again varied depending on the response criteria used to calculate the value (Table 12). The lowest aP release value for 250 FTU/kg of OptiPhos was calculated from ADG. The lowest aP release values for 500, 750, and 1,000 FTU/kg of OptiPhos was calculated from bone ash weight. In contrast, the highest aP release level for all OptiPhos levels was calculated from bone ash percentage. The lowest aP release level for 500 FTU/kg of Phyzyme XP was calculated from bone ash percentage, whereas the lowest levels for 1,000 and 1,500 FTU/kg of Phyzyme XP were calculated from ADG. The highest aP release level for 500 FTU/kg of Phyzyme XP was calculated from G/F, whereas the highest levels for 1,000 and 1,500 FTU/kg of Phyzyme XP were calculated from bone ash percentage. Finally, the lowest aP release level for 1,850 and 3,700 FTU/kg of Ronozyme P was calculated from bone ash weight and G/F, respectively. The highest aP release level for both Ronozyme *P* levels was calculated from bone ash percentage.

Experiments 1 and 2

By using the average values of the AOAC phytase assays from both *E. coli* phytase sources, the response to various criteria were plotted against the analyzed phytase level. Approximately 77% of the variation in response in percentage bone ash was explained by the analyzed phytase level in the diet (Figure 1). Similarly, by plotting the aP released for each phytase level against the analyzed AOAC phytase level, a P release curve was calculated. With percentage bone ash as the response criteria, approximately 73% of the variation in aP release was explained by the analyzed phytase level in the diet (Figure 2). When AOAC analyzed values and bone ash are used as the response variable, aP release for up to 1,000 FTU/kg of *E. coli*-derived phytases (OptiPhos and Phyzyme XP) can be predicted by the equation ($y = -0.000000125x^2 + 0.000236245x + 0.015482000$), where x is the phytase level in the diet.

Previous K-State recommendations, based on Kornegay (1996) P release curves[5], agree well with the phytase release suggested by the aP curve developed from percentage bone ash (Figure 3). The curve previously used by K-State was valid only to 700 FTU/kg, whereas the new curve suggested by this research is valid to 1,000 FTU/kg.

Discussion

Higher phytase concentrations in the AOAC analysis compared with the Phytex analysis were expected because of the key differences between the Phytex assay used by the manufacturer of OptiPhos and the AOAC method. The Phytex assay extracts P with a 0.2M sodium citrate buffer, whereas the AOAC assay uses a 0.2M sodium acetate buffer, Tween 20, and bovine serum albumin. The Phytex assay incubation time is 15 min; the AOAC assay incubation time is 60 min. Additionally, the color reagent used to measure the P released from phytic acid has a wavelength of 820 nm in the Phytex assay and 415 nm in the AOAC assay. Finally, the Phytex assay diafiltrates feed samples to remove high background P levels from monocalcium or dicalcium P before they are assayed; the AOAC assay does not.

The influence of *E. coli*-derived phytase source on level of percentage bone ash follows the typical quadratic response for aP release that has been shown in previous research. The 77% of variation in percentage bone ash that was explained by analyzed phytase value was the highest of any of the measured variables (63, 36, and 39 for ADG, GF, and bone ash weight, respectively). This reinforces that percentage bone ash was the best variable to use to predict aP release. The predicted aP release values from trials in which analyzed AOAC values were used agree largely with Kornegay's summary for *E. coli*-derived phytase levels, suggesting that we can predict aP release levels from *E. coli*-derived phytases when their AOAC assayed value is less than 1,000 FTU/kg. More research needs to be conducted to further evaluate release values for higher phytase levels.

In summary, when percentage bone ash was used as the response criteria, the aP release for these phytase sources was similar to the manufacturers' recommendations when the products were used according to label phytase levels (0.12% for 250 FTU/kg of

[5] Kornegay, E. T., 1996. Nutritional, environmental and economical consideration for using phytase in pig and poultry diets. Pages 277-302 in Nutrient Management of Food Animals to Enhance and Protect the Environment. E. T. Kornegay, ed. CRC Press, Boca Raton, FL.

OptiPhos, 0.10% for 500 FTU/kg of Phyzyme XP, and 0.10% for 1,850 FTU/kg of Ronozyme P). When analyzed on an AOAC basis, the a*P* release curves for the *E. coli* phytases had similar release curves, at least up to 1,000 FTU/kg.

Table 1. Composition of experimental control diets (as-fed basis)[1]

Ingredient, %	Exp. 1	Exp. 2
Corn	57.98	58.11
Soybean meal, 46.5% CP	34.98	35.01
Additive premixes[2]	0.50	0.60
Soybean oil	3.00	3.00
Limestone	1.50	0.25
Salt	0.35	0.35
Vitamin premix	0.25	0.25
Trace mineral premix	0.15	0.15
Lysine-HCl	0.17	0.17
DL-methionine	0.07	0.07
L-threonine	0.05	0.05
Mecadox	1.00	1.00
Total	100.00	100.00
Calculated analysis		
SID[3] lysine, %	1.20	1.20
Total lysine, %	1.34	1.34
SID amino acid ratios		
Isoleucine:lysine ratio	68	68
Leucine:lysine ratio	138	139
Methionine:lysine ratio	39	30
Met & Cys:lysine ratio	58	57
Threonine:lysine ratio	64	62
Tryptophan:lysine ratio	20	19
Valine:lysine ratio	76	74
Crude protein, %	21.4	21.5
ME, kcal/lb	1,565	1,569
SID lysine:ME ratio, g/Mcal	3.51	3.48
Ca, %	0.71	0.49
P, %	0.40	0.39
Available P, %	0.06	0.06

[1] Pigs were fed experimental diets from d 0 to 21 of the trial.
[2] Premixes were added by hand for each treatment and consisted of 3 or 4 lb P premix.
[3] Standardized ileal digestible.

Table 2. Analyzed nutrient composition of ingredients (Exp. 1)

	Lysine, %		Calcium, %		Phosphorus, %		Ca:P
Item	Forumlated[1]	Analyzed[2]	Forumlated[1]	Analyzed[3]	Forumlated[1]	Analyzed[3]	Analyzed[3]
OptiPhos 2000[4]		0.11		16.35		0.07	233.57
Phyzyme XP 1200[5]		0.14		0.05		0.26	0.19
OptiPhos base premix[6]		0.03		2.82		0.04	70.50
Phyzyme XP base premix[6]		0.02					
Negative control	1.34	1.27	0.71	0.92	0.40	0.41	2.24
0.075% aP[7] from monocalcium P	1.34	1.30	0.77	1.00	0.48	0.49	2.04
0.15% aP from monocalcium P	1.34	1.25	0.84	0.90	0.55	0.58	1.55
100 FTU OptiPhos	1.34	1.32	0.71	0.90	0.40	0.41	2.20
175 FTU OptiPhos	1.34	1.34	0.71	0.98	0.40	0.41	2.39
250 FTU OptiPhos	1.34	1.30	0.71	0.90	0.40	0.43	2.09
500 FTU OptiPhos	1.34	1.37	0.71	0.95	0.40	0.43	2.21
200 FTU Phyzyme XP	1.34	1.32	0.71	0.93	0.40	0.43	2.16
350 FTU Phyzyme XP	1.34	1.36	0.71	1.00	0.40	0.42	2.38
500 FTU Phyzyme XP	1.34	1.31	0.71	0.92	0.40	0.43	2.14
1,000 FTU Phyzyme XP	1.34	1.30	0.71	0.97	0.40	0.43	2.26

[1] Nutrient values provided by the manufacturer.
[2] Mean value of 2 samples analyzed in duplicate.
[3] Mean value of 4 samples analyzed in duplicate.
[4] Enzyvia LLC, Sheridan, IN.
[5] Danisco A/S Corporation, Marlborough, UK.
[6] Created from the pure product and cornstarch.
[7] Available P.

Table 3. Calcium concentration of microingredients (Exp. 1)

Ingredient	Analyzed[1]
Antibiotic	18.18
Trace mineral premix	10.44
Vitamin premix	16.93

[1] Mean value of 2 samples analyzed in duplicate.

Table 4. Analyzed nutrient composition of ingredients (Exp. 2)

	Calcium, %		Phosphorus, %		Ca:P
Item	Forumlated[1]	Analyzed	Forumlated[1]	Analyzed	Analyzed
Negative control	0.49	0.48	0.39	0.36	1.33
0.07% aP[2] from monocalcium P	0.55	0.53	0.46	0.43	1.23
0.14% aP from monocalcium P	0.61	0.58	0.53	0.48	1.21
250 FTU OptiPhos[3]	0.49	0.53	0.39	0.36	1.47
500 FTU OptiPhos[3]	0.49	0.47	0.39	0.36	1.31
750 FTU OptiPhos[3]	0.49	0.48	0.39	0.36	1.33
1,000 FTU OptiPhos[3]	0.49	0.49	0.39	0.36	1.36
500 FTU Phyzyme XP[4]	0.49	0.53	0.39	0.37	1.43
1,000 FTU Phyzyme XP[4]	0.49	0.50	0.39	0.37	1.35
1,500 FTU Phyzyme XP[4]	0.49	0.47	0.39	0.37	1.27
1,850 FYT Ronozyme P[5]	0.49	0.49	0.39	0.36	1.36
3,700 FYT Ronozyme P[5]	0.49	0.47	0.39	0.36	1.31

[1] Nutrient values provided by the manufacturer.
[2] Available P.
[3] Enzyvia LLC, Sheridan, IN.
[4] Danisco A/S Corporation, Marlborough, UK.
[5] DSM Nutritional Products, Basel, Switzerland.

Table 5. Analyzed phytase content of diets (Exp. 1)

	Additional aP[1] from monocalcium P			OptiPhos[2], FTU/kg				Phyzyme XP[3], FTU/kg			
Analyzed[4]	None[5]	0.075%	0.15%	100	175	250	500	200	350	500	1,000
AOAC assay, FTU/kg											
Laboratory A	50	70	55	335	635	740	1,635	180	465	450	1,225
Laboratory B	33	87	57	344	530	719	1,528	241	385	415	1,100
Laboratory C	88	202	119	354	516	729	1,363	219	370	423	789
Average AOAC assay	57	119	77	344	560	729	1,509	213	407	429	1,038
Phytex assay, FTU/kg	52	86	71	275	270	300	605	225	285	280	385
Average AOAC ratio[6]				3.5	2.9	2.9	2.7	1.1	1.1	0.8	0.8
Phytex ratio[7]				2.8	1.5	1.2	1.2	1.1	0.8	0.6	0.4

[1] Available P.
[2] Enzyvia LLC, Sheridan, IN.
[3] Danisco A/S Corporation, Marlborough, UK
[4] Average of samples taken at the beginning and end of the experiment.
[5] Contained 0.06% aP.
[6] Ratio of AOAC analysis to formulated values.
[7] Ratio of Phytex analysis to formulated values.

Table 6. Analyzed phytase content of diets (Exp. 2)

	Added aP[1] from monocalcium P			OptiPhos[2], FTU/kg				Phyzyme XP[3], FTU/kg			Ronozyme P[4], FYT/kg	
Analyzed	None[5]	0.07%	0.14%	250	500	750	1,000	500	1,000	1,500	1,850	3,700
AOAC assay, FTU/kg												
Laboratory A	50	50	40	710	1,330	2,000	2,600	290	760	1,140	1,790	3,920
Laboratory B	65	105	63	637	1,123	1,697	2,357	447	656	1,042	1,597	3,635
Avg. AOAC assay	58	78	52	674	1,227	1,849	2,479	369	708	1,091	1,694	3,778
Phytex assay, FTU/kg	70	84	160	360	670	800	900	180	240	550	930	1,900
Avg. AOAC ratio[6]				2.69	2.45	2.46	2.48	0.74	0.71	0.73	0.92	1.02
Phytex ratio[7]				1.44	1.34	1.07	0.90	0.36	0.24	0.37	0.50	0.51

[1] Available P.
[2] Enzyvia LLC, Sheridan, IN.
[3] Danisco A/S Corporation, Marlborough, UK.
[4] DSM Nutritional Products, Basel, Switzerland.
[5] Contained 0.06% aP.
[6] Ratio of average AOAC analyses to formulated values.
[7] Ratio of Phytex analyses to formulated values.

Table 7. Effects of different sources of *E. coli*-derived phytase on nursery pig performance (Exp. 1)[1]

	Additional aP[2] from monocalcium P			OptiPhos[3], FTU/kg				Phyzyme XP[4], FTU/kg			
Item	None[5]	0.075%	0.15%	100	175	250	500	200	350	500	1,000
d 0 to 21											
ADG, lb	0.81	1.12	1.32	0.86	0.86	0.92	1.01	0.81	0.87	0.92	0.92
ADFI, lb	1.64	1.96	1.95	1.53	1.58	1.62	1.74	1.56	1.68	1.62	1.61
G/F	0.51	0.57	0.67	0.56	0.56	0.57	0.58	0.52	0.52	0.57	0.58
Bone ash weight, mg	473	579	777	504	650	616	594	586	610	546	593
Bone ash, %	35.6	39.4	41.8	36.2	38.2	39.6	41.1	37.0	39.0	37.9	40.0

[1] A total of 88 pigs (1 pig per pen and 8 pens per treatment) with an initial BW of 22.7 lb. Pigs were fed the control diet (0.06% aP) during a 6-d pretest period and then fed experimental diets for 21 d.
[2] Available P.
[3] Enzyvia LLC, Sheridan, IN.
[4] Danisco A/S Corporation, Marlborough, UK.
[5] Contained 0.06% aP.

Table 8. Probability table of different sources of *E. coli*-derived phytase on nursery pig performance (Exp. 1)[1]

	Monocalcium P		OptiPhos[2]		Phyzyme XP[3]		
Item	Linear	Quadratic	Linear	Quadratic	Linear	Quadratic	SE
d 0 to 21							
ADG, lb	0.01	0.26	0.01	0.88	0.04	0.50	0.046
ADFI, lb	0.01	0.07	0.11	0.22	0.92	0.88	0.075
G/F	0.01	0.54	0.02	0.14	0.01	0.64	0.023
Bone ash weight, mg	0.01	0.47	0.07	0.05	0.27	0.30	55.0
Bone ash, %	0.01	0.69	0.01	0.56	0.06	0.59	1.65

[1] A total of 88 pigs (1 pig per pen and 8 pens per treatment) with an initial BW of 22.7 lb. Pigs were fed the control diet (0.06% available P) during a 6-d pretest period and then fed experimental diets for 21 d.
[2] Enzyvia LLC, Sheridan, IN.
[3] Danisco A/S Corporation, Marlborough, UK.

Table 9. Calculated available P release values based on different response criteria (Exp. 1)

	OptiPhos[1], FTU/kg				Phyzyme XP[2], FTU/kg				
Item	100	175	250	500	200	350	500	1,000	SE
Response criteria									
ADG, lb	0.029	0.029	0.046	0.063	0.013	0.022	0.042	0.044	0.012
G/F	0.099	0.096	0.097	0.089	0.068	0.056	0.093	0.102	0.018
Bone ash weight, mg	0.055	0.127	0.105	0.084	0.092	0.094	0.070	0.094	0.028
Bone ash, %	0.059	0.086	0.117	0.121	0.069	0.094	0.082	0.120	0.028

[1] Enzyvia LLC, Sheridan, IN.
[2] Danisco A/S Corporation, Marlborough, UK.

Table 10. Effects of different sources of *E. coli*-derived phytase on nursery pig performance (Exp. 2)[1]

	Additional aP[2] from monocalcium P			OptiPhos[3], FTU/kg				Phyzyme XP[4], FTU/kg			Ronozyme P[5], FTU/kg	
Item	None[6]	0.07%	0.14%	250	500	750	1,000	500	1,000	1,500	1,850	3,700
d 0 to 21												
ADG, lb	0.89	1.07	1.03	1.05	1.11	1.15	1.15	1.02	1.00	0.98	1.06	1.27
ADFI, lb	1.43	1.69	1.49	1.58	1.62	1.65	1.62	1.49	1.48	1.43	1.53	1.83
G/F	0.63	0.64	0.69	0.66	0.68	0.69	0.71	0.69	0.67	0.68	0.70	0.70
Bone ash weight, mg	626	601	696	731	734	744	799	625	773	681	691	799
Bone ash, %	34.2	39.6	41.2	41.6	41.9	42.7	43.6	37.1	41.9	42.0	41.1	42.3

[1] A total of 128 pigs (1 pig per pen and 8 pens per treatment) with an initial BW of 21.3 lb. Pigs were fed the control diet (0.06% aP) during a 6-d pretest period and then fed experimental diets for 21 d.
[2] Available P.
[3] Enzyvia LLC, Sheridan, IN.
[4] Danisco A/S Corporation.
[5] DSM Nutritional Products, Basel, Switzerland.
[6] Contained 0.06% aP.

Table 11. Main effects of different sources of *E. coli*-derived phytase on nursery pig performance (Exp. 2)[1]

	Probabilities, P <								
	Monocalcium P		OptiPhos[2]		Phyzyme XP[3]		Ronozyme P[4]		
Item	Linear	Quadratic	Linear	Quadratic	Linear	Quadratic	Linear	Quadratic	SE
d 0 to 21									
ADG, lb	0.07	0.09	0.001	0.11	0.33	0.18	0.001	0.76	0.079
ADFI, lb	0.54	0.01	0.07	0.21	0.96	0.43	0.001	0.28	0.112
G/F	0.001	0.19	0.001	0.24	0.01	0.05	0.001	0.03	0.019
Bone ash weight, mg	0.23	0.26	0.01	0.56	0.09	0.28	0.004	0.67	60.2
Bone ash, %	0.001	0.07	0.001	0.001	0.001	0.10	0.001	0.01	1.21

[1] A total of 128 pigs (1 pig per pen and 8 pens per treatment) with an initial BW of 21.3 lb. Pigs were fed the control diet (0.06% available P) during a 6-d pretest period and then fed experimental diets for 21 d.
[2] Enzyvia LLC, Sheridan, IN.
[3] Danisco A/S Corporation.
[4] DSM Nutritional Products, Basel, Switzerland.

Table 12. Effects of different sources of *E. coli*-derived phytase on nursery pig available P (aP) release (Exp. 2)[1]

	OptiPhos[2], FTU/kg				Phyzyme XP[3], FTU/kg			Ronozyme P[4], FTU/kg		
Item	250	500	750	1,000	500	1,000	1,500	1,850	3,700	SE
Predicted aP, %										
ADG	0.075	0.084	0.090	0.093	0.079	0.072	0.073	0.084	0.098	0.008
G/F	0.079	0.082	0.082	0.092	0.098	0.095	0.099	0.097	0.070	0.015
Bone ash weight	0.088	0.079	0.079	0.091	0.072	0.104	0.090	0.081	0.074	0.012
Bone ash, %	0.127	0.115	0.125	0.142	0.056	0.137	0.146	0.117	0.103	0.021

[1] A total of 128 pigs (1 pig per pen and 8 pens per treatment) with an initial BW of 21.3 lb. Pigs were fed the control diet (0.06% aP) during a 6-d pretest period and then fed experimental diets for 21 d.
[2] Enzyvia LLC, Sheridan, IN.
[3] Danisco A/S Corporation.
[4] DSM Nutritional Products, Basel, Switzerland.

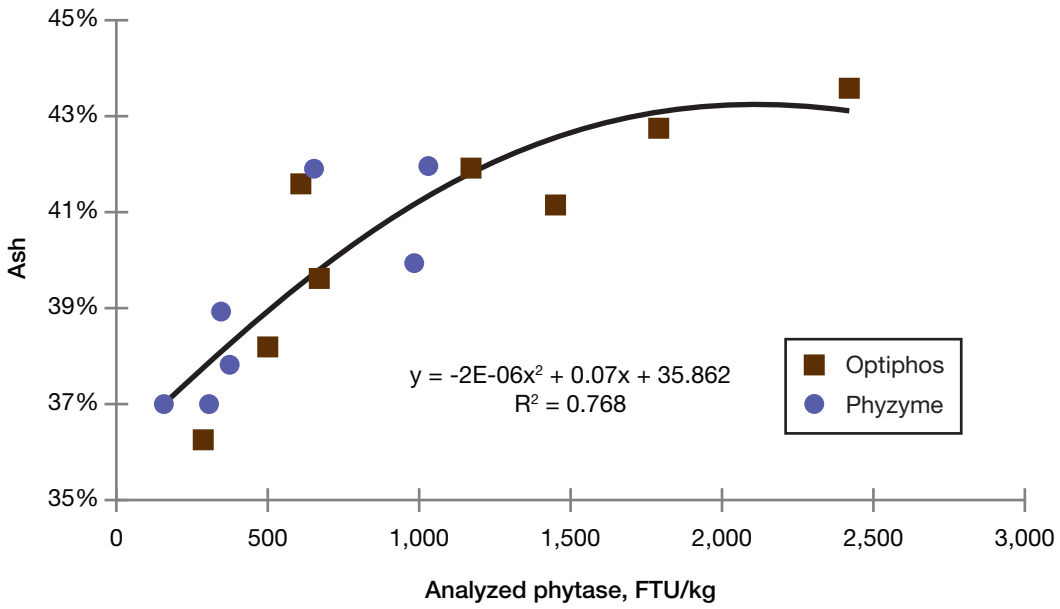

Figure 1. Influence of *E. coli*-derived phytase source and level on percentage bone ash.

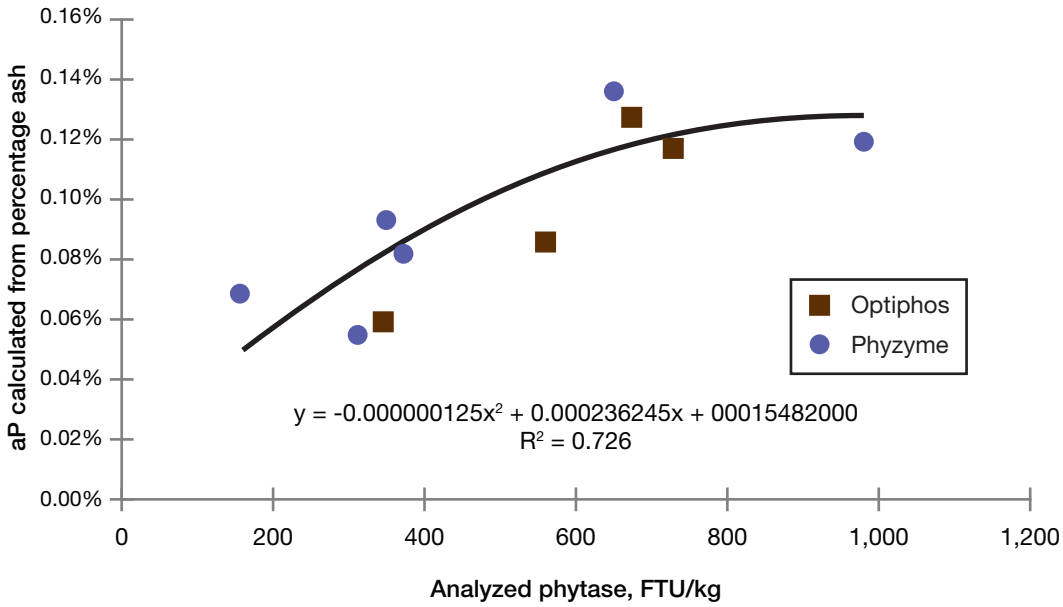

Figure 2. Influence of *E. coli*-derived phytase source and level on predicted available P (aP) release calculated from percentage bone ash.

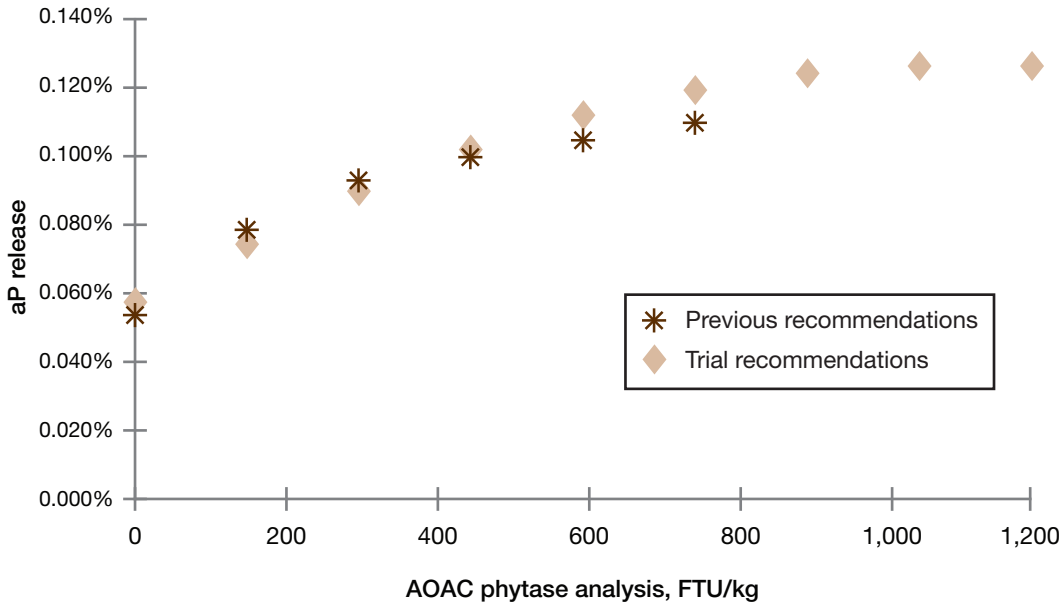

Figure 3. Differences between available P (aP) release values from this trial and previous Kansas State University recommendations.