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Evaluating the Efficacy of a Novel Phytase Source[1]

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Summary

A total of 350 nursery pigs (PIC 1050 barrows, initially 33.2 lb and 49 d of age) were used in a 21-d study to determine the aP release curve for a novel phytase product (Microtech 5,000, VTR Bio-tech Co., Guangdong, China). Pigs were randomly allotted to pens at arrival to the facility, and on d 0 of the trial pens were allotted to 1 of 7 treatments in a randomized complete block design. There were 5 pigs per pen and 10 pens per treatment. Pigs were fed corn-soybean meal-based diets formulated to 1.25% SID lysine. A single batch of the basal diet (0.12% aP) was manufactured and subsequently divided and used as the major ingredient in experimental diet manufacturing. Experimental diets were formulated to contain increasing available P supplied by either an inorganic source (0.12%, 0.18%, and 0.24% aP from monocalcium P) or from increased phytase (250, 500, 750, 1000 FTU/kg). Diets were analyzed for phytase using the AOAC method, and analyzed concentrations were lower than formulated. Diets formulated to contain 250, 500, 750 and 1000 FTU/kg had analyzed concentrations of 155, 335, 465, and 780 FTU/kg, respectively. On d 21, one pig per pen was euthanized and fibulas were collected to determine bone ash weight and percentage bone ash. From d 0 to 21, increasing P from inorganic P or increasing phytase resulted in increased (linear, $P < 0.01$) ADG, improved (quadratic, $P < 0.02$) F/G, and heavier (linear $P < 0.01$) ending BW. Bone ash weight and percentage bone ash were increased (linear, $P < 0.01$) with increasing inorganic P and increasing phytase. Response criteria, which remained in the linear portion of the quadratic phytase curve (ADG, bone ash weight, and percentage bone ash), were used to calculate aP release curves. When analyzed phytase values and percentage bone ash are used as the response variable, aP release percentage for up to 780 FTU/kg of Microtech 5,000 phytase can be predicted by the equation ($y = 0.000002766761x - 0.000000002225x^2 - 0.000201841391$), where x is the phytase concentration in the diet (FTU/kg).

Key words: nursery pig, phosphorus, phytase

[1] Appreciation is expressed to VTR Bio-Tech Co., Guangdong, China, for partial funding of this project.

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Introduction

Phosphorus is an important mineral in swine nutrition. Most notably it is essential for proper bone metabolism and development, along with calcium and vitamin D. Additionally, P is heavily involved in metabolic processes, such as energy transport, enzymatic activity, and osmotic balance, and it is a structural component in nucleic acids, phospholipids, and proteins.

In most swine diets, the largest ingredient constituents include cereal grains and oilseed meals. These ingredients have moderate concentrations of P in the form of phytic acid (myo-inositol hexaphosphate). Monogastric species lack the enzyme needed to cleave the bound phosphate found in phytic acid. As a result, a phytase enzyme is regularly added to swine diets to improve the pig's ability to use P from phytic acid. The use of phytase has allowed the swine industry to rely less on inorganic forms of P in diets, and inherently reduce P excretion of the pig.

Many studies have evaluated different sources of the phytase enzyme, and each source has its own unique aP release curve. These release curves are used to determine its value as a provider of aP in the diet.

Our objective for this trial was to evaluate the effects of a novel phytase (Microtech 5,000; VTR Bio-tech Co.; Guangdong, China) source on nursery pig performance and bone ash values to develop an aP release curve.

Procedures

The Kansas State University Institutional Animal Care and Use Committee approved the protocol for this study. The study was conducted at the K-State Segregated Early Weaning Facility in Manhattan, KS.

A total of 350 nursery pigs (PIC 1050 barrows, initially 33.2 lb and 49 d of age) were used in a 21-d growth trial. When pigs arrived at the facilities after weaning, they were randomly allotted to pens within 2 identical barns. Pens of pigs were blocked by barn (35 pens per barn) and were randomly allotted to 1 of 7 dietary treatments. There were 5 pigs per pen and 10 pens per dietary treatment (5 pens/barn). Barns were environmentally controlled and each pen (5 × 5 ft) had tri-bar flooring and contained a 4-hole dry self-feeder and one cup waterer to provide ad libitum access to feed and water.

Dietary treatments were manufactured at the O.H. Kruse Feedmill in Manhattan, KS. Ingredients containing Ca and/or P were analyzed (CP, Ca, P) prior to manufacturing diets in order to determine nutrient loading values (Table 1). Phytase premix was also analyzed to determine inclusion rate in the experimental diets and contained 265,129 FTU/lb. The dietary treatments were 0.12, 0.18, or 0.24% aP from inorganic P, provided by increasing monocalcium P, or increased phytase levels of 250, 500, 750, or 1000 FTU/kg.

All experimental dietary treatments were derived from a single basal batch (Table 2). After manufacturing the basal batch, it was stored in an overhead bin, and for each experimental diet a subset amount of the basal diet (Table 3) was weighed into the major ingredient scale and treatment-specific ingredients were hand added to achieve the final dietary treatments. During bagging of experimental diets, feed samples were collected from the 5th, 15th, 25th, and 35th bags, and these samples were pooled and used for phytase and nutrient analysis.

In total, 4 subset samples from the pooled feed samples were sent to a commercial laboratory (Midwest Laboratories, Omaha, NE) for proximate nutrient analysis, including CP, P, and Ca. Additionally, 2 subset samples were sent to another commercial feed laboratory (New Jersey Feed Laboratories, Ewing, NJ) for complete diet phytase analysis. The laboratory used standard analysis (AOAC; method 2000.12) to determine complete diet phytase concentrations.

Prior to initiation of the study, all pigs were fed the negative control (0.12% aP) for a 4-d pre-test period. During the experiment, pigs and feeders were weighed every 7 d to determine ADG, ADFI, and F/G. On d 21 of the study, 1 pig per pen was euthanized with an intravenous overdose of sodium pentobarbital (Fatal Plus, Vortech Pharmaceuticals, Dearborn, MI) in accordance with the K-State Institutional Animal Care and Use Committee standards. Pigs selected for euthanasia were the median weight pig within each pen. Fibulas were sampled from euthanized pigs to determine a percent bone ash value. Once collected, all fibulas were stored at -4°F.

The right fibulas were used to determine bone ash concentrations. To do so, cartilage caps were removed, and bones were boiled for 60 min. Adhering tissue was removed and bones were dried at 221°F for 7 d. Then dried fibulas were ashed in a muffle furnace at 1,112°F for 24 h with total ash weight and percentage ash determined.

Data Analysis

All values at least three SD outside the mean of each response criterion were considered outliers and removed from analysis. No outliers were observed for the growth data. Two pigs with outliers for bone ash weight and percentage bone ash were removed from the ash results but were used for calculation of growth data.

Data were analyzed as a randomized complete block design with pen as the experimental unit and barn as the blocking criterion. 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 all treatments that included inorganic P showed that all treatments remained in the linear portion of the quadratic curve of phytase release for ADG, ash weight, and percentage ash; however, for F/G the treatment supplementing an additional 0.12% aP from inorganic P (0.24% total aP) was in the quadratic portion of the phytase curve. Because aP release curves must be generated from the data that are only in the linear portion of the curve, a release curve for F/G was not determined based on the current data set.

Intake of aP from each diet was calculated, and this value was termed the dosage of aP administered to each pig through its diet. Using the calculated aP dosages, simple regression using PROC REG of SAS was used to determine the aP release from the novel 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 the phytase (Y) was calculated by adding the slope to the product of the response criteria (x) multiplied by the intercept, and then subtracting the aP intake of each diet, and then dividing by total feed intake multiplied by 100.

Results

Crude protein, Ca, and P analysis of the experimental diets resulted in concentrations similar to those used in diet formulation (Table 3). According to the AOAC, analysis of the phytase concentration in the complete diet was lower than expected for all phytase treatments. Diets formulated to contain 250, 500, 750, and 1000 FTU/kg had analyzed concentrations of 155, 335, 465, and 780 FTU/kg, respectively. As a result, the analyzed AOAC phytase levels are used to describe the phytase treatments throughout the results from the study.

From d 0 to 21, pigs fed increasing aP from inorganic P had increased (linear; $P < 0.01$, Table 4) ADG resulting in heavier (linear; $P < 0.01$) ending BW and improved (quadratic; $P < 0.01$) F/G. Pigs fed increasing phytase had increased (linear; $P < 0.01$) ADG resulting in heavier ending BW (linear, $P < 0.04$) and improved (quadratic, $P < 0.02$) F/G.

Bone ash weights were increased for pigs fed increasing inorganic P (linear, $P < 0.01$), or increasing phytase (linear, $P < 0.01$). Additionally, percentage bone ash values were increased for pigs fed increased inorganic P (linear, $P < 0.01$) and increasing phytase (linear, $P < 0.01$).

Percentage aP released from this phytase source varied depending on the response criteria (Table 5). As phytase concentrations increased, calculated aP increased in a quadratic fashion to the highest phytase dose. The greatest aP release was calculated with percentage bone ash as the response criteria. Additionally, analyzed phytase explained the greatest amount of variation (96%; Figure 1) in percentage bone ash than in other response variables (0.80 and 0.84 for ADG and bone ash weight, respectively). However, based on the shape of the quadratic response (Figure 2) for aP release associated with percentage bone ash, it appears that the associated prediction equation (Table 6) may underestimate the response from 155 to 335 FTU/kg, and slightly overestimate the response from 465 to 780 FTU/kg.

Overall, this study has provided an aP release curve that can be used to value Microtech 5,000 phytase as a source of aP in nursery swine diets when included at levels between 155 and 780 FTU/kg. Available P release percentage for up to 780 FTU/kg of Microtech 5,000 phytase can be predicted by the equation ($y = 0.000002766761x - 0.000000002225x^2 - 0.000201841391$), where x is the phytase concentration in the diet (FTU/kg).

Table 1. Analyzed ingredient composition[1]

	Analyzed value,%		
Ingredient	CP	Ca	P
Corn	7.92	0.05	0.24
Soybean meal	48.77	0.37	0.70
Limestone	---	40.90	---
Monocalcium P	---	19.00	19.57
Vitamin premix	---	23.77	---
Trace mineral premix	---	18.80	---

[1] Ingredient samples were pooled, and analysis was performed in triplicate by a commercial laboratory (Midwest Laboratories; Kearney, NE).

Table 2. Composition of basal batch (as-fed basis)[1]

Ingredient, %	
Corn	63.33
Soybean meal	33.89
Monocalcium P	0.20
Limestone	0.85
Sodium chloride	0.35
L-lysine HCL	0.30
DL-methionine	0.12
L-threonine	0.12
Trace mineral premix	0.15
Vitamin premix	0.25
Titanium dioxide	0.40
Sand[2]	0.04
Total	100.00
Calculated analysis	
SID[3] lys, %	1.25
Total lys, %	1.40
SID amino acid ratios	
Ise:lys	63
Leu:lys	129
Met:lys	33
Met & cys:lys	57
Thr:lys	63
Trp:lys	18.7
Val:lys	69
CP, %	21.8
NE, kcal/lb	1,097
SID Lys:ME, g/Mcal	5.17
Ca, %	0.61
P, %	0.44
Available P, %	0.12

[1] The basal batch was used as the major ingredient within each experimental diet.
[2] Sand was used to displace corn in experimental diets when ingredient inclusion rates varied to achieve treatment aP levels, as a result experimental diets were isocaloric.
[3] Standardized ileal digestible.

Table 3. Ingredient composition of experimental diets (as-fed basis)[1]

	Experimental diet						
	Inorganic P			Phytase [2]			
Ingredient, %	0.12% aP	0.18% aP	0.24% aP	250	500	750	1,000
Basal mix	99.35	99.35	99.35	99.35	99.35	99.35	99.35
Limestone	0.31	0.16	---	0.31	0.31	0.31	0.31
Monocalcium P	---	0.325	0.65	---	---	---	---
Sand[3]	0.34	0.165	---	0.30	0.26	0.21	0.17
Phytase	---	---	---	0.04	0.09	0.13	0.17
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis							
CP, %	21.70	21.70	21.70	21.70	21.70	21.70	21.70
Ca, %	0.73	0.73	0.73	0.73	0.73	0.73	0.73
P, %	0.44	0.50	0.57	0.44	0.44	0.44	0.44
Phytase, FTU/kg	---	---	---	250	500	750	1,000
Ca:P ratio	1.66	1.46	1.28	1.66	1.66	1.66	1.66
Analyzed composition							
CP, %	23.2	23.9	23.3	23.7	23.4	23.7	22.6
Ca, %	0.67	0.64	0.72	0.62	0.69	0.62	0.54
P, %	0.48	0.56	0.61	0.47	0.48	0.49	0.49
Phytase, FTU/kg	< 50	< 50	< 50	155	335	465	780
Ca:P ratio	1.40	1.14	1.18	1.32	1.44	1.27	1.10

[1] A total of 350 pigs (PIC 1050 barrows, initially 33.2 lb) were used in a 21-d study examining the influence of increasing available P from inorganic P or from a novel phytase source.
[2] Microtech 5,000, FTU/kg[2] (VTR Bio-tech Co.; Guangdong, China).
[3] Sand was used to displace corn in the diet as experimental ingredient inclusion rates varied; as a result, experimental diets were isocaloric.
[4] Phytase premix was analyzed for phytase level, and it contained 265,129 FTU/lb.

Table 4. Effects of increased aP from inorganic P or a novel phytase source on nursery pig growth performance and bone ash values[1]

	Inorganic P[2], % aP			Phytase					*Probability, P <*			
									Inorganic P		Phytase[3]	
Item	0.12	0.18	0.24	155	335	465	780	SEM	Linear	Quadratic	Linear	Quadratic
d 0 to 21												
ADG, lb	1.22	1.47	1.60	1.31	1.33	1.30	1.38	0.051	0.01	0.26	0.01	0.58
ADFI, lb	2.46	2.47	2.64	2.48	2.41	2.41	2.50	0.119	0.12	0.44	0.86	0.43
F/G	2.02	1.69	1.65	1.90	1.82	1.85	1.82	0.038	0.01	0.01	0.01	0.02
Weight, lb												
d 0	33.2	33.2	33.2	33.2	33.2	33.2	33.2	---	---	---	---	---
d 21	58.3	64.1	66.8	60.2	61.1	61.0	62.4	1.66	0.01	0.35	0.04	0.59
Bone ash weight, g[4]	1.17	1.40	1.66	1.14	1.27	1.22	1.38	0.083	0.01	0.83	0.01	0.49
Bone ash, %[4]	33.50	36.20	39.23	32.81	35.02	35.20	36.04	1.892	0.01	0.86	0.01	0.87

[1] A total of 350 nursery pigs (initially 33.2 lb) were used to evaluate the influence of increasing aP from inorganic P or from a novel phytase on pig growth performance and bone ash values. Pigs were fed the negative control diet formulated to contain 0.12% aP for a 4-d pre-test period and then were fed experimental diets for 21d. There were 5 pigs per pen and 10 pens per treatment.

[2] Inorganic P was added to the diet by increasing monocalcium P.

[3] Microtech 5,000, (VTR Bio-tech Co.; Guangdong, China).

[4] One pig per pen was euthanized and fibulas were used to determine bone ash weight and percentage bone ash.

Table 5. Calculated aP release values based on different response criteria

	Phytase, FTU/kg[1]				
	155	335	465	780	SEM
ADG	0.034	0.043	0.037	0.051	0.007
Bone ash weight	0.019	0.045	0.035	0.058	0.009
Percentage bone ash	0.016	0.054	0.055	0.061	0.024

[1] Microtech 5,000 (VTR Bio-tech Co., Guangdong, China).

Table 6. aP release equations for Microtech 5,000[1] phytase based on various response criteria

Response	aP release equation	Coefficient of determination, r^2
ADG	$Y = 0.000000018319x^2 + 0.00000780861x + 0.033528707672$	0.9483
Bone ash weight	$Y = 0.000000009439x^2 + 0.000340238685x + 1.102654003555$	0.8024
Percentage bone ash	$Y = -0.000000002225x^2 + 0.000002766761x - 0.000201841391$	0.8395

[1] Microtech 5000 (VTR Bio-tech Co.; Guangdong, China).

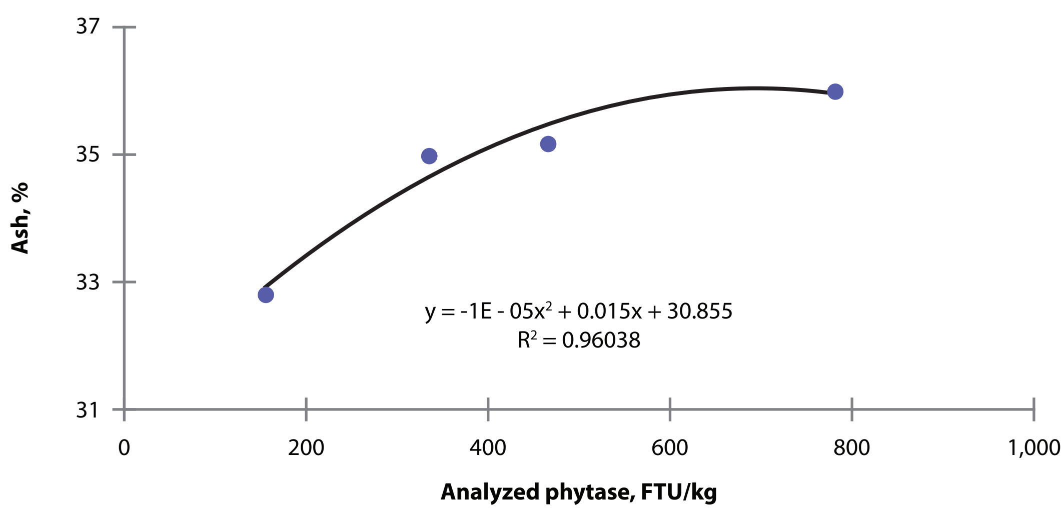

Figure 1. Influence of Microtech 5,000 phytase source and level on percentage bone ash.

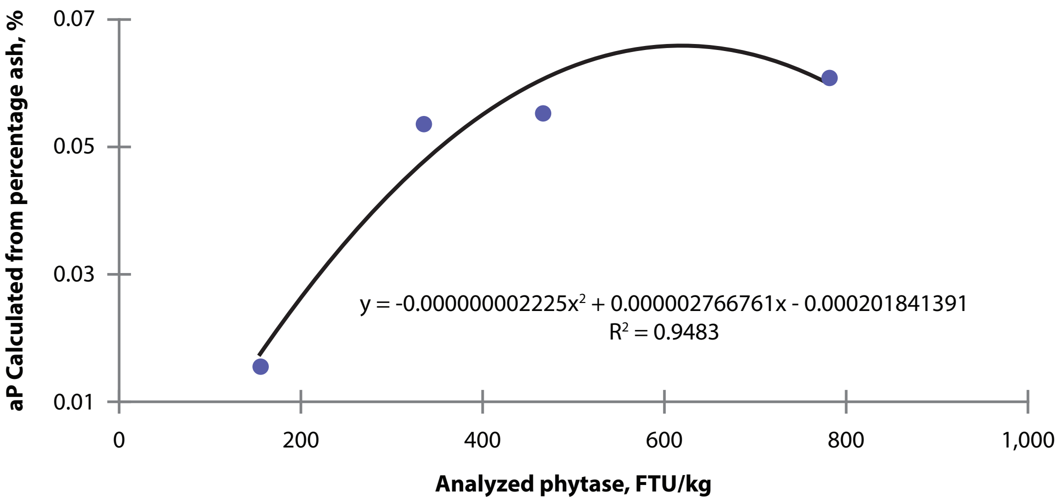

Figure 2. Influence of Microtech 5,000 phytase source and level on predicted available P (aP) release, calculated from percentage bone ash.