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Cover Page Footnote

Appreciation is expressed to BASF Corporation, Florham Park, NJ, for partial funding of this project.

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Determining the Phosphorus Release for Natuphos E 5,000 G Phytase for Nursery Pigs[1]

K.M. Gourley, J.C. Woodworth, J.M. DeRouchey, M.D. Tokach, S.S. Dritz,[2] and R.D. Goodband

Summary

A total of 286 nursery pigs (PIC 327 × 1050; initially 24.3 lb and d 42 of age) were used in a 21-d growth trial to determine the available P (aP) release curve for a novel phytase source (Natuphos E 5,000 G, BASF Corporation, Florham Park, NJ). Pigs were randomly allotted to pens at weaning. On d 0 of the experiment (d 18 after weaning), pens were allotted in a randomized complete block design to 1 of 8 treatments. There were 4 pigs per pen and 9 pens per treatment. Pigs were fed a corn-soybean meal-based diet formulated to 1.25% standardized ileal digestible (SID) lysine. Ten 1-ton batches of basal feed (0.12% aP) were manufactured and subsequently divided to be the major portion of experimental diet manufacturing. Experimental diets were formulated to contain increasing aP supplied by either an inorganic source (0.12, 0.18, and 0.24% aP from monocalcium P) or from increased phytase (150, 250, 500, 750, and 1,000 FTU/kg). Diets were analyzed for phytase using the AOAC method and actual analyzed concentrations were 263, 397, 618, 1,100, and 1,350 FTU/kg, respectively. On d 21 of the study, one pig per pen was euthanized and the right fibula was collected for bone ash and percentage bone ash calculations. From d 0 to 21, increasing P from inorganic P or increasing phytase resulted in improved (linear, $P < 0.01$) ADG, F/G and ending BW. Bone ash weight and percentage bone ash increased (linear, $P < 0.01$) with increasing inorganic P or phytase. When formulated phytase values and percentage bone ash are used as the response variables, aP release for up to 1,000 FTU/kg of Natuphos E 5,000 G phytase can be predicted by the equation: aP release = 0.000212 × FTU/kg phytase.

Key words: bone ash, nursery pigs, phosphorus, phytase

Introduction

Phosphorus is an important macro mineral in swine nutrition. Along with calcium and vitamin D, it contributes to bone development. Most swine diets are formulated with cereal grains and oilseed, which contain P in the form of phytic acid. Monogastrics do not produce the enzyme needed to cleave the phosphates from the phytic acid for

[1] Appreciation is expressed to BASF Corporation, Florham Park, NJ, for partial funding of this project.
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absorption. As a result, a phytase enzyme is commonly added to swine diets to make P more available for animal use. This allows for reduced dietary inclusion of P from inorganic P sources, and results in reduced P excretion by the pig.

There are several phytase sources commercially available for swine producers to utilize, and as new generations of these products become available, updated P release values need to be determined. While some phytase products have already undergone evaluation to determine their unique release curve, other newer products have not been thoroughly tested.

Therefore, the objective for this trial was to evaluate the effects of a second generation phytase (Natuphos E 5,000 G, BASF Corporation, Florham Park, NJ) source on nursery pig performance and bone ash to develop an aP release curve.

Procedures

The Kansas State Institutional Animal Care and Use Committee approved the protocol for this study. The study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. The nursery barn was environmentally controlled and each pen contained a 4-hole dry self-feeder and a nipple waterer for ad libitum access to feed and water.

A total of 286 nursery pigs (PIC 327 × 1050; initially 24.3 lb and d 42 of age) were used in a 21-d growth trial. Pigs were initially weaned and randomly allotted to pens and fed a common diet. After 14 d post-weaning, pens of pigs were blocked by BW and randomly allotted to 1 of 8 dietary treatments with 4 pigs per pen (2 barrows and 2 gilts) and 9 replications (pens) per dietary treatment. The dietary treatments consisted of 3 treatments (0.12, 0.18, or 0.24% aP) of increasing inorganic P, provided by monocalcium P, or 5 treatments consisting of added phytase (150, 250, 500, 750, or 1,000 FTU/kg) with the phytase added to the 0.12% aP inorganic P diet. Prior to the beginning of the 21-d study (d 15 to 18 post-weaning), all pigs were fed the negative control diet (0.12% aP) for a 4-d pre-test period.

Dietary treatments were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. Ingredients containing Ca or P were analyzed (Ca, P) prior to manufacturing the diets in order to determine nutrient loading values used for formulation (Table 1). Phytase premix was also analyzed to determine inclusion rate in the experimental diets and contained 5,111,000 FTU/kg.

All dietary treatments were derived from 10, 1-ton basal batches (Table 2). After manufacturing, each basal batch was bagged off into 10 separate tons. For each experimental diet, a subset of bags (50 lb each) from the basal diet was added to the mixer along with treatment-specific ingredients to achieve the final dietary treatments (Table 3). During bagging of experimental diets, feed samples were collected from the 5th, 10th, 15th, 20th, 25th, 30th, and 35th bags, and these samples were pooled and used for phytase and nutrient analysis.

One sample per dietary treatment from the pooled feed samples was sent to a commercial laboratory (Ward Laboratories, Kearney, NE) for proximate analysis, including

CP, Ca, and P. In addition, one sample was sent to another commercial feed laboratory (Eurofins Scientific Inc., Des Moines, IA) for complete diet phytase analysis (AOAC; method 2000.12).

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 via captive bolt. Pigs selected were the median weight gilt in each pen. The right fibula was removed from euthanized pigs to determine percentage ash bone criteria. Once collected, all fibulas were stored at -4°F. For processing of fibulas for bone ash, 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 to determine total ash weight and percentage bone ash.

Data Analysis

Studentized residuals were evaluated for pen means or individual bone ash measurements to ensure data met the assumption of normal distribution. One pig had a bone ash weight and percentage bone ash greater than 3 SD from the mean and was removed from bone ash analysis, but the pen data were retained for the evaluation of growth data.

Data were analyzed as a randomized complete block design with pen as the experimental unit. An initial base model was evaluated using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Treatment was considered the fixed effect and linear and quadratic contrasts were evaluated within increasing inorganic P or phytase doses. Contrast coefficients for phytase doses were adjusted to account for the unequal spacing.

For pens fed inorganic phosphorus diets, the marginal intake of aP per day was calculated for each pen. The calculation was: dietary aP% minus 0.12% (the aP in the base diet) multiplied by ADFI. Subsequently, a standard curve was developed for each response criteria using marginal aP release as the predictor variable. The equation for the standard curve was then used to calculate aP release for each pen fed the different phytase dosages based on the observed value for each response criteria. This value was then converted to a marginal aP% using the pen ADFI.

Mixed model ANOVA with weight block as a random effect was then performed to evaluate aP release as a function of the phytase dosage using linear and quadratic contrasts. Next, mixed model regression was performed to predict aP release as a function of phytase dosage assuming an intercept of no aP release for the control diet without phytase.

Results were considered to be significant with P-values ≤ 0.05 and were considered marginally significant with P-values ≤ 0.10.

Results and Discussion

Crude protein and P of the experimental diets were similar to those expected from diet formulation. There was some variation in Ca analysis, which is similar to what we have observed in analysis of other experimental diets. The level of phytase analyzed slightly higher than expected across all diets (Table 3). This was unexpected due to the use of

the analyzed phytase level for dietary formulation. Analysis of phytase in final diets is much more difficult and variable than analysis of phytase in concentrated products, which may contribute to the higher analyzed values. Nevertheless, the phytase levels increase in a stepwise fashion as would be expected.

From d 0 to 21, pigs fed increasing aP from inorganic P had improved (linear, $P < 0.01$, Table 4) ADG, ending BW and F/G. Additionally, pigs fed increasing phytase had improved (linear, $P < 0.01$) ADG, ending BW, and F/G.

For bone composition, bone ash weights were increased (linear, $P < 0.01$) for pigs fed either increasing inorganic P or phytase. As a result, percentage bone ash values increased (linear, $P < 0.01$) for pigs fed inorganic P or phytase.

Percentage aP released from this phytase source varied depending on the response criteria (Table 5). As phytase concentrations increased, calculated aP increased in a linear ($P < 0.01$) fashion to the highest phytase dose. The greatest aP release was calculated with percentage bone ash as the response criteria. Based on the linear response (Figure 2) for aP release associated with percentage bone ash, it appears the associated prediction equation (Table 6) will very closely predict the aP release. Release values for performance criteria (ADG and F/G) were lower than the release values for percentage bone ash. This might be a result of the Ca level used in the basal diets. Recent research indicates that high Ca to P ratios increase percentage bone ash, but may impair feed intake and growth rate.

Overall, this study has provided an aP release curve that can be used to value Natuphos E 5,000 phytase as a source of aP in nursery diets when included at levels between 150 and 1,000 FTU/kg. Available P release of percentage bone ash for up to 1,000 FTU/kg of Natuphos E 5,000 can be predicted by the equation: aP release = 0.000212 × FTU/kg phytase.

Table 1. Analyzed ingredient composition (as-fed basis)[1]

	Analyzed value, %	
Ingredient	Ca	P
Corn	0.03	0.29
Soybean meal	0.36	0.71
Limestone	29.25	0.14
Monocalcium P	17.37	20.85
Vitamin premix	17.15	0.04
Trace mineral premix	27.65	0.02

[1]Ingredient samples were pooled and analysis was performed by two commercial laboratories (Ward Laboratories, Kearney, NE, and Cumberland Valley Analytical Services, Hagerstown, MD).

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

Item	
Ingredient, %	
Corn	63.67
Soybean meal	33.85
Monocalcium P	0.20
Limestone	1.04
Sodium chloride	0.35
L-Lys-HCL	0.30
DL-Met	0.12
L-Thr	0.12
Trace mineral premix	0.15
Vitamin premix	0.25
Calculated analysis	
SID[2] Lys, %	1.25
Total Lys, %	1.40
SID amino acid ratios	
Ile:Lys	63
Leu:Lys	129
Met:Lys	33
Met and Cys:Lys	57
Thr:Lys	63
Trp:Lys	18.7
Val:Lys	69
CP, %	21.8
NE, kcal/lb	1,100
SID Lys:ME, g/Mcal	3.78
Ca, %	0.64
P, %	0.54
Available P, %	0.12
STTD P, %	0.36

[1]The basal batch was used as the major ingredient within each experimental diet.
[2]Standardized ileal digestible.

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

	Experimental diet							
	Inorganic P			Phytase[1]				
Ingredient, %	0.12%	0.18%	0.24%	150	250	500	750	1,000
Basal mix	99.01	99.01	99.01	99.01	99.01	99.01	99.01	99.01
Limestone	0.25	0.13	---	0.25	0.25	0.25	0.25	0.25
Monocalcium P	---	0.27	0.54	---	---	---	---	---
Titanium dioxide	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Sand[2]	0.34	0.20	0.05	0.34	0.34	0.33	0.33	0.32
Phytase	---	---	---	0.003	0.005	0.009	0.014	0.019
Calculated analysis								
CP, %	21.7	21.7	21.7	21.7	21.7	21.7	21.7	21.7
Ca, %	0.73	0.73	0.73	0.73	0.73	0.73	0.73	0.73
P, %	0.54	0.60	0.66	0.54	0.54	0.54	0.54	0.54
Phytase, FTU/kg	---	---	---	150	250	500	750	1000
Ca:P ratio	1.35	1.22	1.11	1.35	1.35	1.35	1.35	1.35
Analyzed composition								
CP, %	21.5	19.8	22.0	21.4	22.2	22.9	22.1	23.1
Ca, %	0.89	0.81	0.68	0.86	0.89	0.73	0.78	0.58
P, %	0.49	0.55	0.63	0.48	0.48	0.47	0.45	0.47
Phytase, FTU/kg	95	< 60	< 60	263	397	618	1100	1350
Ca:P ratio	1.81	1.48	1.08	1.81	1.85	1.55	1.72	1.23

[1]Natuphos E 5,000 G (BASF Corporation, Florham Park, NJ). Phytase premix was analyzed for phytase level, and it contained 5,111,000 FTU/kg.
[2]Sand was used equalize inclusion rates of experimental ingredients.
[3] Phytase premix was analyzed for phytase level, and it contained 5,111,000 FTU/kg.

Table 4. Effects of increasing aP from inorganic P or Natuphos E 5,000 G on nursery pig growth performance and bone ash values[1]

	Inorganic P, % aP[2]				Phytase, FTU/kg[3]					Inorganic P		Phytase	
Item	0.12	0.18	0.24	150	250	500	750	1,000	SEM	Linear	Quadratic	Linear	Quadratic
BW, lb													
d 0	24.7	24.6	24.6	24.0	24.0	24.3	24.5	24.7	0.43	0.724	0.975	0.126	0.133
d 21	44.65	48.86	51.65	46.98	47.66	47.51	49.51	51.28	0.84	<0.001	0.478	<0.001	0.906
d 0 to 21													
ADG, lb	0.96	1.18	1.29	1.08	1.08	1.10	1.19	1.27	0.03	<0.001	0.111	<0.001	0.666
ADFI, lb	1.89	2.06	2.16	2.02	1.99	1.97	2.13	2.14	0.05	<0.001	0.517	<0.001	0.959
F/G	1.98	1.75	1.68	1.89	1.81	1.79	1.79	1.70	0.03	<0.001	0.050	<0.001	0.352
Bone ash weight, g[4]	0.678	0.850	0.856	0.713	0.666	0.769	0.819	0.936	0.041	0.003	0.103	0.001	0.194
Bone ash, %[4]	38.11	41.23	42.05	38.67	39.65	41.36	43.21	45.59	1.010	0.005	0.332	0.001	0.614

[1]A total of 286 nursery pigs (PIC 327 × 1050; initially 24.3 lb and d 42 of age) were used in a 21-d growth study evaluating the effects of increasing available P from inorganic P or from a novel phytase source.

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

[3]Natuphos E 5,000 G (BASF Corporation, Florham Park, NJ).

[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]						Probability, $P <$	
Item	150	250	500	750	1000	SEM	Linear	Quadratic
ADG	0.036	0.042	0.050	0.079	0.103	0.009	0.001	0.325
F/G	0.025	0.046	0.072	0.064	0.109	0.014	0.001	0.226
Bone ash weight	-0.003	-0.036	0.042	0.073	0.159	0.008	0.001	0.206
Percent bone ash	0.000	0.034	0.093	0.144	0.227	0.032	0.001	0.737

[1]Natuphos E 5,000 G FTU/kg (BASF Corporation, Florham Park, NJ).

Table 6. Available P release equations for Natuphos E 5,000 phytase based on various response criteria

Response	aP release equation
Bone ash weight	aP release = 0.000116 × FTU/kg
Percentage bone ash	aP release = 0.000212 × FTU/kg

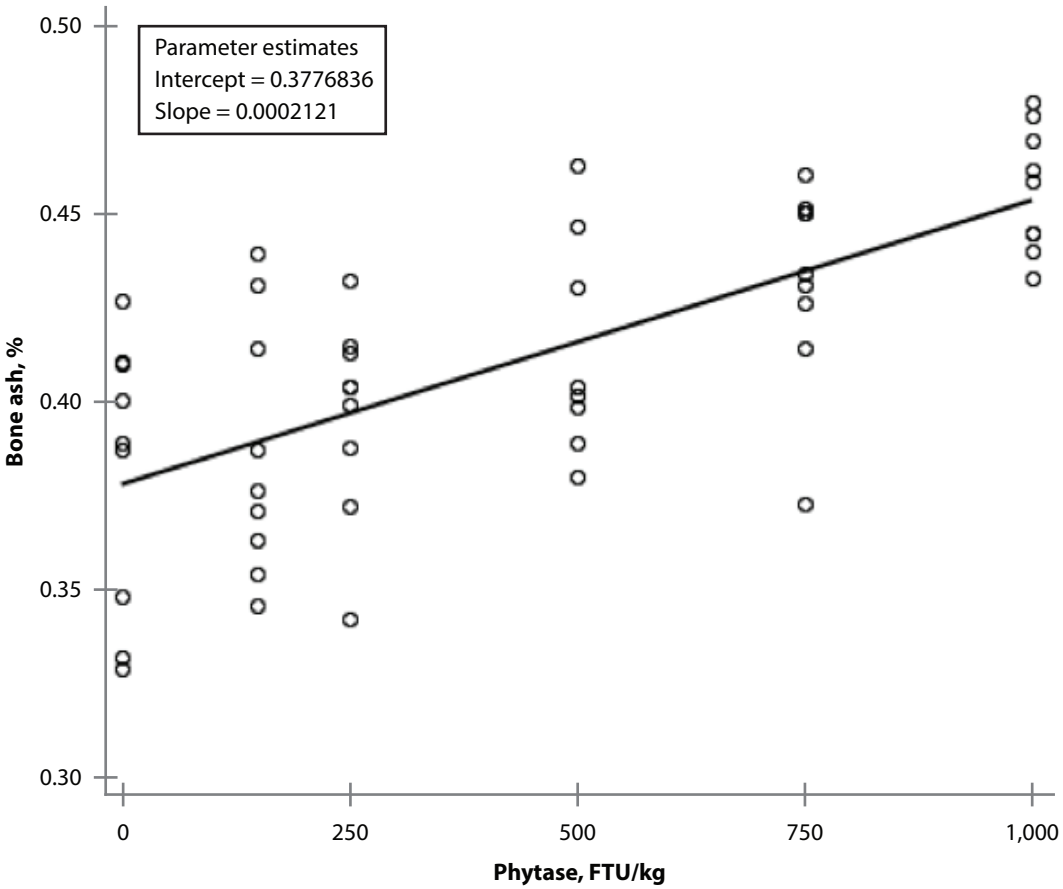

Figure 1. Influence of Natuphos E 5,000 phytase level on percentage bone ash.

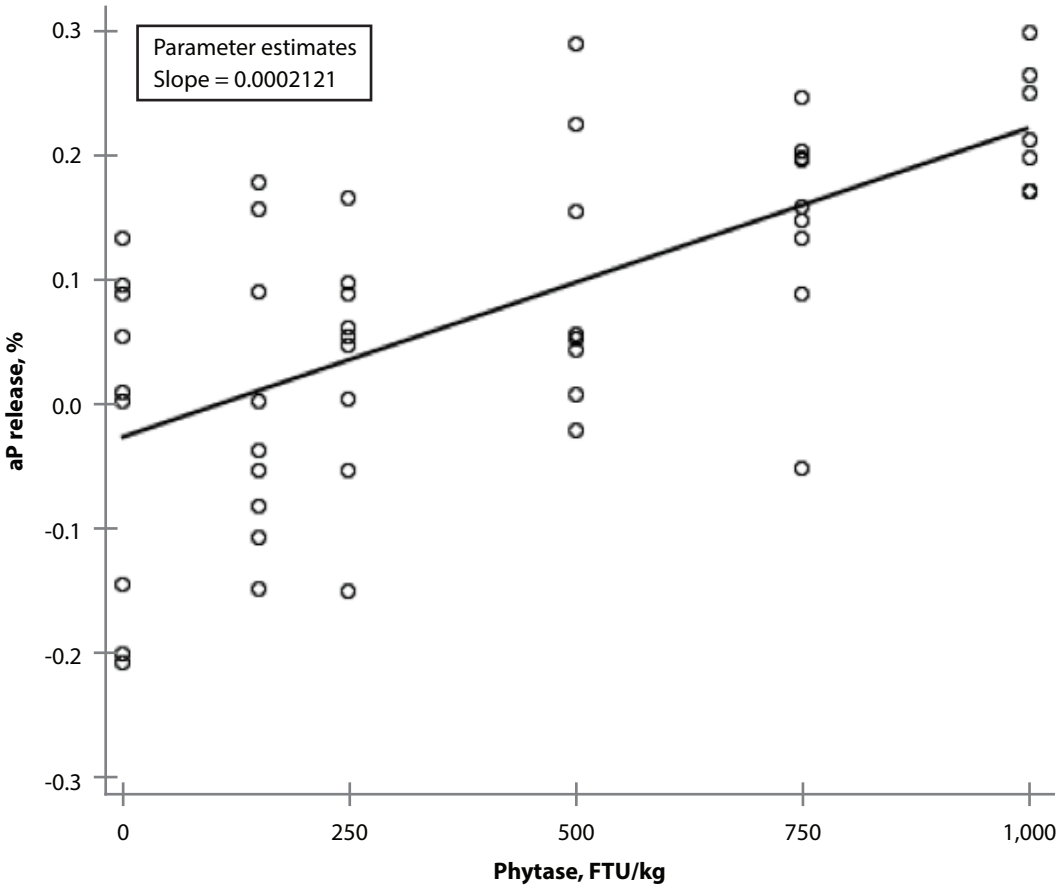

Figure 2. Influence of Natuphos E 5000 phytase level on available P (aP) release, calculated from percentage bone ash.