Determining the available phosphorus release of Natuphos E 5,000 G phytase for nursery pigs

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ABSTRACT: A total of 288 pigs (PIC 327 × 1050; initially 11.1 ± 0.1 kg, and day 40 of age) were used in a 21-d growth trial to determine the available P (aP) release curve for a novel source of 6-phytase (Natuphos E 5,000 G; BASF Corporation, Florham Park, NJ). Natuphos E is a bacterial derived 6-phytase of which the phytase gene is assembled from a hybrid of phytase-producing bacteria and produced through the fermentation of *Aspergillus niger*. Pigs were randomly allotted to pens at weaning. From day 15 to 18 postweaning, a common corn-soybean meal diet containing 0.12% aP was fed to all pigs to acclimate them to a P-deficient diet. On day 0 of the experiment (day 19 after weaning), pens were allotted in a randomized complete block design to one of eight treatments. There were four pigs per pen and nine pens per dietary treatment. Pigs were fed a corn-soybean meal-based diet formulated to 1.25% standardized ileal digestible Lys. Experimental diets were formulated to contain 0.73% Ca and increasing aP supplied by either monocalcium P (0.12%, 0.18%, and 0.24% aP) or from increasing phytase (150, 250, 500, 750, and 1,000 phytase unit [**FTU**]/kg) added to the 0.12% aP diet. Analyzed phytase concentrations were 263, 397, 618, 1,100, and 1,350 FTU/kg, respectively. On day 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 day 0 to 21, increasing P from monocalcium P or phytase improved (linear, $P < 0.01$) ADG and G:F. Bone ash weight and percentage bone ash increased (linear, $P < 0.01$) with increasing monocalcium 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, growth, nursery pig, phosphorus, phytase

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INTRODUCTION

Phosphorus is an important macro mineral in swine nutrition. Along with Ca and vitamin D, it contributes to bone development and is a component of other physiological functions. Most swine diets are formulated with cereal grains and oilseed meals, which contain 60% to 82% of total P in the form of phytate (Ravindran et al., 1994). Monogastrics do not produce enough enzyme endogenously to cleave the phosphates from the phytate for absorption and consequently much of the phytate-bound P is unavailable to the pig. The ability for a phytase enzyme to improve the available P in swine diets has been well documented (Cromwell et al., 1993; Augspurger et al., 2003; Selle and Ravindran, 2008). As a result, a phytase

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enzyme is commonly added to diets to make P more available for swine and other animals. This allows for a reduced dietary inclusion of P from inorganic P sources in swine diets and results in reduced P excretion (Simons et al., 1990; Jongbloed et al., 1997).

There are many manufacturers of phytase, and the site in which phosphorus is cleaved from phytate and origin can vary between phytase sources. Although many existing phytase products have already undergone evaluation to determine their unique release curve (Jones et al., 2010; Kerr et al., 2010), new generation phytases are being developed and have not been thoroughly tested to determine their efficacy.

Therefore, the objective for this trial was to evaluate the effects of a novel 6-phytase (Natuphos E 5,000 G; BASF Corporation, Florham Park, NJ) on nursery pig growth performance and bone ash to develop an available phosphorous (aP) release curve.

MATERIALS AND METHODS

The Kansas State Institutional Animal Care and Use Committee approved the protocol for this study. Ingredients containing Ca or P were analyzed in duplicate prior to manufacturing the diets in order to determine nutrient loading values used for formulation (Table 1). Dietary treatments were corn-soybean meal-based and were formulated to meet or exceed NRC (2012) nutrient requirement estimates with the exception of P and were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. All diets were formulated to contain the same amount of Ca regardless of increasing aP. Available P coefficients were derived from the 10th edition NRC (1998).

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

Ingredient	Ca, %	P, %
Corn	0.04	0.37
Soybean meal	0.41	0.82
Limestone	36.79	0.01
Monocalcium P	16.85	22.22
Vitamin premix	17.51	0.02
Trace mineral premix	18.43	0.06

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

Diet manufacturing started with the production of 10 identical 907 kg batches of basal diet that were packaged in 22.3 kg bags and stored to maintain batch identity (Table 2). For each experimental diet, a subset of bags from each basal diet batch 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.

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 four-hole dry self-feeder and a nipple waterer for ad libitum access to feed and water.

A total of 288 nursery pigs (PIC 327×1050; initially 11.1 ± 0.1 kg and day 40 of age) were used in a 21-d growth trial. Pigs were initially weaned and randomly allotted to pens and fed common starter diets. On day 15 postweaning, pens of pigs were blocked by BW and randomly allotted to one of eight dietary treatments with four pigs per pen (two barrows and two gilts) and nine replications (pens) per treatment. From day 15 to 18 postweaning, a common corn-soybean meal diet containing 0.12% aP was fed to all pigs to acclimate them to a P-deficient diet. Starting on day 19 postweaning and continuing for 21 d, pens were fed their respective treatment diets which consisted of three diets containing increasing (0.12%, 0.18%, or 0.24%) levels of aP from inorganic P, provided by monocalcium P, or the 0.12% aP inorganic P diet with one of five concentrations of added phytase (150, 250, 500, 750, or 1,000 phytase unit [**FTU**]/kg; Natuphos E 5,000 G; BASF Corporation, Florham Park, NJ). The analyzed phytase activity (5,320,000 FTU/kg) was used for determining the amount of phytase to include in each diet.

During the experiment, pigs and feeders were weighed every 7 d to determine ADG, ADFI, and G:F. On day 21 of the study, the median weight gilt in each pen was euthanized via captive bolt. The right fibula was removed from euthanized pigs to determine percentage bone ash criteria. Once collected, all fibulas were stored at −20°C. 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 105°C for 7 d. Then dried fibulas were ashed in a muffle furnace at 600°C for 24 h to determine total ash weight and calculate percentage bone ash (Flohr et al., 2016).

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

Ingredient	%
Corn	63.67
Soybean meal, 48% CP	33.85
Monocalcium P, 22% 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[3]	0.15
Vitamin premix[4]	0.25
	100
Calculated analysis	
Standardized ileal digestibility (SID) amino acids, %	
Lys	1.25
Ile:Lys	63
Leu:Lys	129
Met:Lys	33
Met & Cys:Lys	57
Thr:Lys	63
Trp:Lys	18.7
Val:Lys	69
Total Lys, %	1.40
CP, %	21.8
ME, kcal/g	3,353
NE, kcal/g	2,464
SID Lys:ME, g/Mcal	3.78
Ca, %	0.64
P, %	0.54
Available P[5], %	0.12
STTD P, %	0.24

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

[2]Analyzed Ca and P values were used in formulation.

[3]Provided per kilogram of premix: 26.5 g Mn from manganese oxide, 110 g Fe from iron sulfate, 110 g Zn from zinc sulphate, 11 g Cu from copper sulfate, 198 mg I from calcium iodate, and 198 mg Se from sodium selenite.

[4]Provided per kg premix: 4,409,171 IU vitamin A; 551,150 IU vitamin D3; 17,637 IU vitamin E; 15 mg vitamin B12; 1,764 mg menadione; 3,307 mg riboflavin; 11,023 mg pantothenic acid, 19,841 mg niacin.

[5]Coefficients for formulation were derived from NRC (1998).

Chemical Analysis

One sample per dietary treatment from the pooled feed samples was sent to a commercial laboratory (Ward Laboratories, Kearney, NE) for CP (AOAC 990.03, 2006), Ca (AOAC 965.14/985.01, 2006), and P (AOAC 965.17/985.01, 2006) analysis. In addition, ingredients containing Ca and P were analyzed (Ward Laboratories, Kearney, NE) in duplicate prior to manufacturing diets to determine nutrient loading values (Table 1). One sample was sent to another commercial laboratory (Eurofins Scientific Inc., Des Moines, IA) and analyzed in duplicate for complete dietary phytase (AOAC 2000.12, 2006).

Statistical 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 7 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 GLIMMIX 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 concentrations. Contrast coefficients for phytase concentrations were adjusted to account for the unequal treatment spacing on phytase inclusion.

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 basal 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 treatments based on the observed value for each response criteria. This value was then converted to a marginal aP% using the pen ADFI. Available P release curves were developed for bone ash weight and percentage bone ash.

Mixed model analysis of variance with weight block as a random effect was then performed to evaluate aP release as a function of the phytase concentration using linear and quadratic contrasts. Next, mixed model regression was performed to predict aP release as a function of phytase concentration assuming no aP release for the diet containing 0.12% aP and no phytase.

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

RESULTS

Chemical Analysis

Analyzed CP and P of the experimental diets were similar to those expected from diet

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 batch	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[1]	---	---	---	0.003	0.005	0.009	0.014	0.019
	100	100	100	100	100	100	100	100
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	1,000
Ca:P ratio	1.35	1.22	1.11	1.35	1.35	1.35	1.35	1.35
Analyzed composition[3]								
CP, %	21.5	19.8	22.0	21.4	22.2	22.9	22.1	23.1
Ca, %	0.75	0.79	0.87	0.77	0.82	0.89	0.80	0.86
P, %	0.50	0.57	0.64	0.49	0.50	0.48	0.50	0.51
Phytase, FTU/kg	95	<60	<60	263	397	618	1,100	1,350
Ca:P ratio	1.50	1.39	1.35	1.57	1.64	1.85	1.60	1.68

[1]Natuphos E 5,000 G (BASF Corporation, Florham Park, NJ) was analyzed for phytase concentration, and it contained 5,320,000 phytase units (FTU)/kg.

[2]Sand was used to equalize inclusion rate of the basal batch with experimental ingredients.

[3]Seven samples per dietary treatment were pooled and used to create a composite sample. One composite sample was sent to a commercial laboratory (Ward Laboratories, Kearney, NE) for CP, Ca and P analysis. Another composite sample was sent to a commercial laboratory (Eurofins Scientific Inc., Des Moines, IA) and analyzed in duplicate for complete dietary phytase.

formulation. There was some variation in Ca analysis, which increased the Ca:P ratios; however this was unexpected due to the analysis of all major Ca containing ingredients prior to diet formulation. The level of phytase analyzed slightly greater than expected across all diets (Table 3). This was unexpected due to the use of the analyzed phytase level for dietary formulation and careful sequencing of diets. Nevertheless, the phytase levels increased in a stepwise fashion with increasing phytase.

Growth Performance

From day 0 to 21, pigs fed increasing aP from inorganic P had improved (linear, $P < 0.001$, Table 4) ADG, ending BW, ADFI, and G:F. In addition, pigs fed increasing phytase had improved (linear, $P < 0.001$) ADG, ending BW, ADFI, and G:F.

For bone composition, bone ash weights were increased for pigs fed either increasing inorganic P (linear, $P = 0.003$) or phytase (linear, $P < 0.001$). As a result, percentage bone ash values increased for pigs fed inorganic P (linear, $P = 0.005$) or phytase (linear, $P < 0.001$).

Percentage aP released from this phytase source varied depending on the response criteria (Table 5). As phytase concentrations increased, calculated aP increased linearly ($P < 0.001$) to the highest phytase concentration for all response criteria. However, the rate of increase from the prediction equation varied by response variable with a release of 0.159% aP for bone ash weight and 0.227% aP for percentage bone ash at 1,000 FTU/kg. Based on the linear response for aP release associated with percentage bone ash, a prediction equation (aP release = 0.000212 × FTU/kg) was developed that predicts the aP release at different dietary phytase concentrations (Table 6).

DISCUSSION

Phosphorous is a key mineral in animal diets for bone development and other physiological functions. However, the majority of P in cereal grains and oilseeds commonly fed to swine is bound in the form of phytate and not available for absorption (Ravindran et al., 1994). Swine are unable to cleave P from phytate because they produce insufficient amounts of endogenous phytase in their

small intestine (Jongbloed et al., 1992; Humer et al., 2015). While mircoflora activity in the large intestine produces larger amounts of endogenous phytase, absorption of P takes place in the small intestine, thus P released in the large intestine will be excreted (Smith et al., 1955; Bohlke et al., 2005, Rutherfurd et al., 2014).

Commercially produced microbially-derived phytase is one of the most significant enzyme discoveries used in swine diets (Cromwell, 2009). The phytase enzyme (*myo*-inositol hexaphosphate phosphohydrolase) catalyzes the hydrolysis of phytate to inorganic phosphate (PO_4) and *myo*-inositol (Humer et al., 2015). While intermediate products are synthesized in the stepwise dephosphorylation reaction, only *o*-phosphate ions (PO_4) can pass through the gastrointestinal wall and be utilized by the animal (Jongbloed et al., 1992). Phytase inclusion in swine diets allows more dietary P to be absorbed in the proximal end of the small intestine and results in less excretion of P from the pigs (Gonzalez-Vega and Stein, 2014).

Phytase activity is measured in the form of phytase units (FTU). One FTU is defined as the quantity of phytase enzyme required to liberate 1 micromol of inorganic P per minute, at pH 5.5, from an excess of 15 µmol/L of sodium phytate at 37°C (AOAC, 2006). A common method to evaluate the efficacy of a phytase source is to determine the phytase activity needed to reach a specific aP release value in the diet (Goncalves et al., 2016). Several microbial phytase sources are available for swine producers, yet each source can have a different aP release value (Jones et al., 2010). Consequently, it is important to determine aP release for each specific phytase source and compare to other sources on an equal FTU inclusion basis.

The previous Natuphos product (Natuphos) was a 3-phytase and was derived from fermentation of Aspergillus *niger*. The new generation, Natuphos E, is a bacterial derived 6-phytase of which the phytase gene is assembled from a hybrid of phytase-producing bacteria and produced through the fermentation of *A. niger*. Currently, literature is limited regarding the use of Natuphos E in swine diets. Torrallardona and Ader (2016) conducted a 42-d study to determine growth performance, bone ash values, and apparent total tract digestibility (**ATTD**) for P in nursery pigs fed 125 to 1,000 FTU/kg Natuphos E in P-deficient diets. Over the entire 42-d study, increasing Natuphos E improved (linear, $P < 0.03$) ADG, ADFI, G:F, and bone characteristics compared to pigs receiving a P-deficient diet with no phytase. These findings are in agreement with the current study, where ADG, ADFI, and bone ash values increased linearly in P-deficient diets when phytase inclusion increased from 150 to 1,000 FTU/kg. Torallardona and Ader (2016) further observed that increasing phytase improved (linear and quadratic, $P \leq 0.026$) ATTD for P, Ca, and ash with the greatest improvement occurring up to 250 FTU/kg.

Linear improvements in growth performance and bone characteristics were observed when P-deficient diets (0.12% aP) were supplemented

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, kg													
Day 0	11.1	11.2	11.2	10.9	10.9	11.0	11.1	11.2	0.19	0.724	0.975	0.126	0.133
Day 21	20.3	22.2	23.4	21.3	21.6	21.6	22.5	23.3	0.38	<0.001	0.478	<0.001	0.906
Day 0 to 21													
ADG, g	434	535	584	488	495	501	541	575	13.7	<0.001	0.111	<0.001	0.666
ADFI, g	858	936	981	916	901	896	966	970	21.0	<0.001	0.517	<0.001	0.959
G:F, g/kg	505	572	596	532	555	561	561	590	10.1	<0.001	0.084	<0.001	0.204
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]	38.1	41.2	42.1	38.7	39.7	41.4	43.2	45.6	1.01	0.005	0.332	<0.001	0.614

[1]A total of 288 nursery pigs (PIC 327×1050; initially 11.1 kg and d 40 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	1,000	SEM	Linear	Quadratic
ADG	0.036	0.042	0.050	0.079	0.103	0.009	0.001	0.325
G:F	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).

with increasing aP provided by monocalcium phosphate. Previous research has shown P-deficient diets reduced feed efficiency and bone ash values in weanling pigs (Mahan, 1982). Furthermore, Augspurger et al. (2003) demonstrated that feed efficiency and bone ash linearly improved as inorganic P (KH_2PO_4) was added to a basal diet formulated to be low in aP (0.075% aP), which supports our findings of increased feed efficiency and percentage bone ash with increasing monocalcium phosphate in a P-deficient diet.

Kornegay and Qian (1996) evaluated the addition of phytase to P-deficient diets to determine the aP release value of a phytase product. They determined that ADG, apparent P digestibility, and ash content of bone were the more sensitive indicators to develop aP release values. In the current study, aP release values for performance criteria (ADG and G:F) were lower than the release values for percentage bone ash, which might be a result of the elevated analyzed Ca concentrations. The NRC (2012) cites the total Ca requirement estimate for an 11 to 25-kg growing pig to be 0.70%, and our diets were formulated to contain 0.73% total Ca and analyzed to approximately 0.75% to 0.89% Ca. A recent study by Gonzalez-Vega et al. (2016) demonstrated that as STTD Ca was increased from 0.32% to 0.72% in nursery pig diets, growth criteria (ADG and G:F) worsened (linear, $P < 0.05$). Conversely, percentage bone ash increased (quadratic, $P < 0.05$) as dietary Ca increased in the diet. This is in agreement with the growth performance from the current study, where aP release values were lower for ADG and G:F. However, bone ash weight and percentage bone ash did not seem to be effected by the total Ca values as Gonzalez-Vega et al. (2016) would suggest. As a result, bone ash weight and percentage bone ash were used to predict aP release values.

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

In the present study, when using percentage bone ash to predict an aP release curve, the aP release per FTU/kg in the diet is less than suggested by the manufacturer. This could be due to differences in type of bone (fibula vs. metatarsals) used for bone analysis in which an aP release curve was developed from. Fibulas are easier to remove intact and are easier to clean consistently compared to metatarsals, which allows for greater bone ash values (Biehl and Baker, 1996). Another consideration is type of feedstuffs included in the diet in which the aP release curve was developed. Depending on the type of cereal grain used in formulation and the amount of phytate bound P or phytase already in the grain, the phosphorus release could vary. It is suggested that the magnitude of response to phytase is correlated with the level of dietary phytate (Selle and Ravindran, 2008).

In summary, this study has provided an aP release curve that can be used for Natuphos E 5,000 G phytase as a source of aP in nursery diets when included at concentrations between 150 and 1,000 FTU/kg. Using percentage bone ash as the response criteria, aP release for up to 1,000 FTU/kg of Natuphos E 5,000 G can be predicted by the equation: aP release = 0.000212 × FTU/kg phytase. Further research needs to be conducted to determine aP release of Natuphos E when included in grower and finisher diets and in diets containing levels of phytase above 1,000 FTU/kg.

Conflict of interest statement. None declared.

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