Effect of high doses of Natuphos E 5,000 G phytase on growth performance of nursery pigs

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ABSTRACT: A total of 360 pigs (DNA 200 × 400, initially 5.9 ± 0.1 kg) were used in a 42 d trial to determine the effect of high doses of a recently available phytase source (Natuphos E 5000 G, BASF Corporation, Florham Park, NJ) on nursery pig growth and bone ash. Pigs were randomly allotted to pens at weaning by BW and pens were allotted to one of eight corn-soybean meal-based dietary treatments in a randomized complete block design. There were five pigs per pen and nine pens per treatment. Diets were fed in three phases (d 0 to 7, 7 to 21, and 21 to 42) with formulated total calcium:phosphorus (Ca:P) of 1.07, 1.05, and 0.93, respectively. Treatments included a negative control (NC) with 0.40, 0.30, or 0.25% aP from monocalcium P for Phases 1, 2, and 3 respectively; and NC with either 500, 1,000, 2,000, 3,000, or 4,000 FTU/kg phytase. The last two treatments were a positive control (PC) with 0.55, 0.45, or 0.40% aP from monocalcium P for Phases 1, 2, and 3, respectively, or PC with 2,000 FTU/kg phytase. The NC diet with 500 FTU/kg and PC without added phytase were formulated to be equivalent in available Ca and P. On d 42, one pig per pen was euthanized and the right fibula was removed for bone ash analysis. From d 0 to 42, pigs fed increasing phytase in the NC tended to have increased (quadratic, $P = 0.064$) ADG and (linear, $P = 0.082$) ending BW and had improved (quadratic, $P = 0.008$) G:F. Adding 2,000 FTU/kg phytase to the PC did not influence ADG or ADFI, but tended to improve ($P = 0.060$) G:F compared with the PC. In addition, percentage bone ash increased as phytase increased in the NC (linear, $P < 0.001$) or when 2,000 FTU/kg was added to the PC diets ($P < 0.001$). Pigs fed the PC had increased ($P = 0.007$) ADFI and tended to have greater ($P = 0.099$) percentage bone ash than pigs fed NC+500 FTU/kg phytase, but the pigs fed NC+500 FTU/kg phytase had improved ($P = 0.032$) G:F compared to pigs fed the PC. In summary, increasing concentrations of dietary phytase in a P-deficient diet improved growth and bone ash measurements, and was optimized at 1,000 FTU/kg. There were varied improvements when 2,000 FTU/kg phytase was added in P adequate diets.

Key words: bone ash, calcium, growth, nursery pig, phosphorus, phytase

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INTRODUCTION

Phytase enzymes have been commercially available since the early 1990's (Adeola and Cowieson, 2011). Cereal grains and oilseeds can contain 60 to 82% of total phosphorus (P) in the form of phytate-bound P (Ravindran et al., 1994). Because the pig cannot produce enough endogenous phytase for P absorption, a phytase enzyme is commonly added to aid in P absorption. Phosphorus is an important mineral that contributes to bone development and other physiological functions. The ability of a

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phytase enzyme to improve the available P in swine diets has been well documented (Augspurger et al., 2003; Selle and Ravindran, 2008). The addition of phytase also allows for reduced inclusion of inorganic P, and consequently reduces P excretion from the pig (Simons et al., 1990; Jongbloed et al., 1997).

Previous studies have shown improved growth performance in nursery pigs fed high concentrations of phytase at or above 10,000 FTU/kg (Kies et al., 2006; Nyannor et al., 2007; Zeng et al., 2014). The suggested mode of action for high concentrations of phytase comes in the form of non-P related benefits from improved digestibility of energy, AA, and other minerals (Kies et al., 2001). However, it is noted that greater growth performance improvement is seen when digestible P, AA, and other nutrients are at marginal concentrations relative to the dietary predicted requirements (Goncalves et al., 2016).

Natuphos E 5,000 G (BASF Corporation, Florham Park, NJ) is a relatively new source of phytase available to the U.S. swine industry. Natuphos E, is a bacterial derived 6-phytase of which the phytase gene is assembled from sequences of various phytase producing bacteria of the species *Hafnia*, *Yersinia*, and *Buttauxiella*. Production occurred through the fermentation of *Aspergillus niger*. In a recent study (Gourley et al., 2017), Natuphos E 5,000 G improved (linear, $P < 0.01$) ADG, ADFI, G:F, and percentage bone ash as phytase increased from 0 to 1,000 FTU/kg. However, current literature is not available to determine the impact of feeding concentrations above 1,000 FTU/kg of this new phytase source.

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

	Analyzed value, %	
Ingredient	P	Ca
Corn	0.31	0.03
Soybean meal	0.72	0.43
Limestone	0.23	37.73
Monocalcium P	20.54	16.38
Fish meal	3.07	5.59
Dried whey	0.80	0.58
Blood plasma	1.00	0.19
Enzymatically treated soybean meal[b]	0.74	0.38
Corn DDGS, > 6 and < 9% oil	0.98	0.06
Trace mineral premix	0.03	18.28
Vitamin premix	0.04	18.17

[a]Duplicate ingredient samples were pooled and analysis was performed at a commercial laboratory (Ward Laboratory; Kearney, NE).

[b]HP 300, Hamlet Protein Inc. (Findlay, OH).

Therefore, the objective of this study was to evaluate the effect of high doses of Natuphos E 5,000 G on the growth performance and bone ash in nursery pigs.

MATERIALS AND METHODS

The Kansas State University Institutional Animal Care and Use Committee approved the protocol for this study. The study was conducted at the Kansas State University Segregated Early Wean Facility in Manhattan, KS. Two identical barns were environmentally controlled and each pen contained a 4-hole dry self-feeder and a nipple waterer for ad libitum access to feed and water.

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 requirements with the exception of P and were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. The analyzed phytase activity (5,111,000 FTU/kg) was used for determining the amount of Natuphos E 5,000 G to include in each diet.

Dietary treatments included a negative control with 0.40, 0.30, or 0.25% aP (0.44, 0.36, 0.32 % standardized total tract digestible [STTD] P) from inorganic P, provided by monocalcium P, for Phases 1, 2, and 3, respectively. Additional dietary treatments included the negative control plus increasing phytase at 500, 1,000, 2,000, 3,000, or 4,000 FTU/kg (Natuphos E 5,000 G, BASF Corporation, Florham Park, NJ) in each phase; a positive control with 0.55, 0.45, or 0.40% aP (0.57, 0.49, 0.46 % STTD P) from inorganic P for Phases 1, 2, and 3, respectively, or the positive control with 2,000 FTU/kg of phytase in each phase. The positive control was formulated with Ca and P similar to current industry levels, which resulted in Ca being close to NRC (2012) requirement estimates, but P was formulated above the NRC (2012) estimated requirement for the weight range corresponding to each phase. The NC was formulated to be the PC minus 0.15% P and 0.14% Ca, which was the amount the manufacturer suggested would be released by 500 FTU/kg Natuphos E 5,000 G. Available P coefficients were derived from the 10th edition NRC (1998). Using STTD P values, the NC was also below the NRC (2012) requirement estimates, while the PC was formulated well above the STTD P estimates.

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

Ingredient, %	Phase 1	Phase 2	Phase 3
Corn	36.80	52.09	62.98
Soybean meal, 48% CP	20.80	27.46	32.93
Dairylac 80[b]	15.14	5.05	—
Dried whey	8.08	5.05	—
Enzymatically treated soybean meal[c]	5.05	5.05	—
Corn DDGS	5.05	—	—
Blood plasma	4.04	—	—
Fish meal	1.26	1.26	—
Choice white grease	1.01	1.01	1.01
Monocalcium P	0.28	0.56	0.86
Limestone	1.19	0.98	0.83
Sodium chloride	0.30	0.30	0.35
L-Lys-HCl	0.30	0.38	0.35
DL-Met	0.17	0.20	0.14
L-Thr	0.12	0.16	0.13
L-Val	—	0.05	
Trace mineral premix[d]	0.15	0.15	0.15
Vitamin premix[e]	0.25	0.25	0.25
Choline chloride 60%	0.04	—	—
	100	100	100
Calculated analysis			
Standardized ileal digestibility (SID) AA, %			
Lys	1.40	1.35	1.25
Ile:Lys	58	60	61
Leu:Lys	122	118	125
Met:Lys	33	37	34
Met & Cys:Lys	57	58	56
Thr:Lys	63	63	62
Trp:Lys	19.3	17.8	18.0
Val:Lys	68	69	66
Total Lys, %	1.58	1.50	1.39
CP, %	22.7	22.2	21.8
ME, kcal/kg	3,452	3,400	3,347
NE, kcal/kg	2,556	2,516	2,475
SID Lys:ME, g/Mcal	4.12	4.03	3.79
Ca, %	0.71	0.66	0.56
P, %	0.66	0.62	0.60
Available P[f], %	0.40	0.30	0.25
STTD P, %	0.44	0.36	0.32

[a]The basal batch was used as the major ingredient within each experimental diet. Treatment-specific ingredients were added to the basal batch to achieve final dietary treatments.

[b]International Ingredient Corporation (St. Louis, MO).

[c]HP 300, Hamlet Protein Inc. (Findlay, OH).

[d]Provided per kg of premix: 22,400 mg Mn from manganese oxide, 74,592 mg Fe from iron sulfate, 74,592 mg Zn from zinc sulfate, 11,200 mg Cu from copper sulfate, 201 mg I from calcium iodate, and 201 mg Se from sodium selenite.

[e]Provided per kg premix: 3,584,000 IU vitamin A; 896,000 IU vitamin D3; 17,920 IU vitamin E; 16 mg vitamin B12; 1,792 mg menadione; 3,360 mg riboflavin; 11,200 mg pantothenic acid, 33,600 mg niacin.

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

All dietary treatments within phase were derived from a basal batch of ingredients (Table 2). After manufacturing the basal batch, they were bagged off into eight identical sets (89 kg of Phase 1, 357 kg of Phase 2, and 893 kg of Phase 3 per treatment). For each experimental diet, a subset of bags from the basal 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, and 35th bags, pooled, and used for phytase and nutrient analysis.

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

	Phase 1		Phase 2		Phase 3	
Ingredient, %	Negative control	Positive control	Negative control	Positive control	Negative control	Positive control
Basal mix	96.52	96.52	98.43	98.43	98.75	98.75
Corn	3.35	2.52	1.46	0.63	1.10	0.25
Soybean meal	0.02	0.03	0.01	0.07	—	0.05
Limestone	—	0.73	—	0.08	—	0.08
Monocalcium P	—	0.05	—	0.70	—	0.75
Sand[a]	0.10	0.15	0.10	0.10	0.15	0.13
Phytase[b]	—	—	—	—	—	—
Calculated analysis						
CP, %	22.8	22.8	22.2	22.2	21.2	21.3
Ca, %	0.71	0.85	0.66	0.80	0.56	0.70
P, %	0.66	0.81	0.63	0.77	0.61	0.76
Ca:P ratio	1.07	1.05	1.05	1.04	0.93	0.92

[a]Sand was used to displace phytase in the diet as inclusion rate varied; as a result, the same amount of basal mix in each phase was added to each of the treatment diets.

[b]Natuphos E 5,000 G (BASF Corporation, Florham Park, NJ) was added to the negative control to achieve experimental diets with 0, 500, 1,000, 2,000, 3,000, or 4,000 FTU/kg or was added to positive control diet to achieve experimental diets with 0 or 2,000 FTU/kg. Phytase inclusion was determined using the analyzed concentration, and the phytase contained 5,111,000 FTU/kg.

A total of 360 barrows (DNA 200 × 400; initially 5.9 ± 0.1 kg and 21 d of age) were used in a 42 d growth trial. Pigs were randomly allotted to pens and then pens of pigs were blocked by weight and randomly allotted to one of eight dietary treatments. There were five pigs per pen and nine replications (pens) per treatment. Diets were fed in three phases from d 0 to 7, 7 to 21, and 21 to 42. During the experiment, pigs and feeders were weighed every 7 d to determine ADG, ADFI, and G:F. On d 42 of the study, the median weight pig in each pen was euthanized via captive bolt and fibulas were collected to determine bone ash values. Once collected, all fibulas were stored at −20 °C for 7 d. To determine bone ash concentrations, bones were autoclaved at 121 °C for 40 min under 6.82 kg of pressure. Adhering tissue and cartilage caps were 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 percentage bone ash.

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 (Table 4). In addition, ingredients containing Ca or P were analyzed (Ward Laboratories, Kearney, NE) in duplicate prior to manufacturing diets to determine nutrient loading values. One sample per treatment was sent to another commercial feed laboratory (Eurofins Scientific Inc., Des Moines, IA) for complete diet phytase analysis (ISO 30024:2009).

Data Analysis

All data (pen means or bone values) 3 SD outside the mean of each response criteria were evaluated as outliers. In Phase 1, there were four pen outliers and one outlier each for Phase 2 and Phase 3. The outliers were the result of mortality or unthrifty pigs being removed. The pens deemed as outliers were removed within each respective phase but remained for all other data analysis.

Data were analyzed as a randomized complete block design with pen as the experimental unit. Barn was treated as a random effect. Contrast coefficients for phytase concentrations were adjusted to account for the unequal treatment spacing on phytase inclusion using the IML procedure in SAS. Pre-planned contrast statements were used to determine the linear and quadratic responses to phytase. A pairwise comparison was used to compare the PC and PC + 2,000 FTU phytase treatments to test for an extra-phosphoric effect. Another pairwise comparison was used to compare the NC + 500 FTU/kg and the PC control to confirm the estimated release of Natuphos E 5,000 G. A third pairwise comparison was used to compare the NC and the PC. Analysis of variance was performed using the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC). Results were significant with P values ≤ 0.05 and were considered tendencies with P values > 0.05 and ≤ 0.10.

Table 4. Analyzed composition of experimental diets (as-fed basis)[a]

	Analyzed composition				
Diets	CP, %	Ca, %	P, %	Ca:P	Phytase, FTU/kg
Phase 1					
NC[b]	21.8	0.88	0.61	1.44	< 60
NC + 500 FTU	22.3	0.87	0.64	1.36	612
NC + 1,000 FTU	22.1	0.89	0.63	1.41	1,100
NC + 2,000 FTU	22.1	0.90	0.64	1.40	2,060
NC + 3,000 FTU	22.4	0.93	0.64	1.45	3,880
NC + 4,000 FTU	22.2	0.85	0.60	1.42	5,270
PC[c]	21.8	1.10	0.76	1.45	< 60
PC + 2,000 FTU	22.4	1.07	0.80	1.34	2,580
Phase 2					
NC	21.8	0.75	0.59	1.27	< 60
NC + 500 FTU	21.6	0.78	0.58	1.34	650
NC + 1,000 FTU	21.3	0.83	0.61	1.36	1,350
NC + 2,000 FTU	21.9	0.84	0.63	1.33	2,590
NC + 3,000 FTU	22.6	0.75	0.56	1.33	3,630
NC + 4,000 FTU	22.6	0.89	0.67	1.33	5,200
PC	21.6	1.01	0.74	1.36	< 60
PC + 2,000 FTU	22.2	0.94	0.75	1.25	2,560
Phase 3					
NC	20.8	0.75	0.63	1.19	< 60
NC + 500 FTU	22.0	0.75	0.61	1.23	536
NC + 1,000 FTU	21.6	0.73	0.60	1.22	1,190
NC + 2,000 FTU	21.5	0.78	0.61	1.28	2,280
NC + 3,000 FTU	21.9	0.70	0.60	1.17	3,710
NC + 4,000 FTU	21.8	0.70	0.63	1.11	4,660
PC	21.9	0.87	0.77	1.13	62
PC + 2,000 FTU	22.2	0.87	0.77	1.13	2,110

[a]Seven subsamples were pooled and proximate analysis was performed in triplicate by a commercial laboratory (Ward Laboratories, Kearney, NE). In addition, phytase analysis was conducted in duplicate to determine complete diet phytase concentrations at another commercial laboratory (Eurofins Scientific Inc., Des Moines, IA).

[b]Negative Control.

[c]Positive Control.

RESULTS

Chemical Analysis

Analysis of CP and P of the experimental diets were similar to those expected from diet formulation; however, Ca in the final diets analyzed greater than expected. This was not anticipated since all ingredients containing Ca were analyzed and those values were used in diet formulation. Analyzed phytase increased as phytase addition increased as anticipated, but was greater than expected across all diets (Table 4).

Growth Performance

From d 0 to 7 and 7 to 21, there were no differences observed for growth performance among pigs fed any of the dietary treatments (Table 5). From d 21 to 42, increasing phytase tended to increase (quadratic, $P = 0.078$) ADG up to 1,000 FTU/kg and (linear, $P = 0.095$) ADFI. In addition, G:F improved (quadratic, $P = 0.001$) when increasing phytase up to 500 FTU/kg, but then decreased with the addition of 4,000 FTU/kg. When comparing the NC diet with 500 FTU/kg phytase and the PC diet formulated to have the same aP, pigs fed the PC diet had increased ($P < 0.05$) ADG and ADFI; however, pigs fed the NC with 500 FTU/kg of phytase had improved ($P = 0.047$) G:F. Among pigs fed the two positive control diets, including phytase at 2,000 FTU improved ($P = 0.047$) G:F. Pigs fed the PC had increased ($P = 0.038$) ADG and ($P = 0.049$) ADFI compared to those fed the NC.

From d 0 to 42, pigs fed increasing phytase tended to have increased (quadratic, $P = 0.064$) ADG up to 1,000 FTU/kg resulting in heavier (linear, $P = 0.082$) ending BW. Increasing phytase improved (quadratic, $P = 0.008$) G:F up to 500 FTU/kg, but then decreased with the inclusion of

Table 5. Effect of high doses of Natuphos E 5,000 G on nursery pig growth performance and bone ash values[a]

	Negative Control[b]						Positive Control[c]			*P* <				
										Negative Control				
Item	0	500	1,000	2,000	3,000	4,000	0	2,000	SEM	Linear	Quadratic	NC vs. PC	NC + 500 vs. PC[d]	PC vs. PC + 2,000
BW, kg														
d 0	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9	0.01	0.250	0.818	0.687	0.230	0.421
d 42	21.5	21.4	22.6	22.2	22.5	22.2	22.7	22.6	0.38	0.082	0.128	0.035	0.021	0.921
d 0 to 7														
ADG, g	64	74	84	72	77	83	83	75	9.6	0.293	0.785	0.145	0.475	0.512
ADFI, g	112	114	124	108	118	129	123	115	6.9	0.165	0.347	0.248	0.332	0.403
G:F, g/kg	562	645	675	666	636	640	665	616	57.1	0.558	0.228	0.157	0.785	0.499
d 7 to 21														
ADG, g	276	271	297	277	295	291	295	285	14.9	0.274	0.763	0.333	0.215	0.630
ADFI, g	349	345	363	352	361	362	369	349	13.9	0.343	0.851	0.250	0.173	0.241
G:F, g/kg	791	782	816	786	814	802	795	818	18.1	0.456	0.774	0.866	0.595	0.352
d 21 to 42														
ADG, g	541	541	569	569	568	557	577	581	13.5	0.192	0.078	0.038	0.048	0.847
ADFI, g	820	790	844	837	838	847	871	849	19.6	0.095	0.644	0.049	0.003	0.398
G:F, g/kg	659	685	674	680	678	658	663	685	7.8	0.493	0.001	0.696	0.047	0.047
d 0 to 42														
ADG, g	369	377	396	387	395	381	400	398	10.2	0.314	0.064	0.027	0.107	0.864
ADFI, g	540	529	562	551	559	555	580	561	13.5	0.188	0.427	0.029	0.007	0.289
G:F, g/kg	684	713	705	702	707	686	689	709	7.8	0.616	0.008	0.571	0.032	0.060
Bone ash, g[e]	1.94	2.30	2.35	2.56	2.53	2.25	2.42	2.51	0.093	0.012	0.001	0.001	0.374	0.465
Bone ash, %	44.2	45.2	47.1	48.0	48.4	49.1	47.0	51.3	0.007	0.001	0.078	0.010	0.099	0.001

[a]A total of 360 barrows (DNA 200 × 400, initially 5.9 ± 0.1 kg) were used in a 42 d growth study with five pigs per pen and nine pens per treatment (Natuphos E 5,000 G, BASF Corporation, Florham Park, NJ).

[b]Negative control diets were formulated with 0.40, 0.30, or 0.25% aP from inorganic P for Phases 1, 2, and 3, respectively. Phytase was added at 0, 500, 1,000, 2,000, 3,000, 4,000 FTU/kg to the negative control diet to achieve final experimental diets.

[c]Positive control diets were formulated with 0.55, 0.45, or 0.40% aP from inorganic P for Phases 1, 2, and 3, respectively. Phytase was added at either 0 or 2,000 FTU/kg to the positive control diet to achieve final experimental diets.

[d]NC diets were formulated to be the PC minus 0.15% P and 0.14% Ca released by 500 FTU/kg Natuphos E suggested by the manufacturer. The NC + 500 FTU and PC treatments were compared to confirm the estimated release value.

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

4,000 FTU/kg. Pigs fed the NC diet with 500 FTU/kg phytase and PC diets were formulated to be equivalent in available Ca and P. When comparing these diets, pigs fed the positive control diet had increased ($P = 0.007$) ADFI; however, pigs fed the NC with 500 FTU/kg phytase diet had improved ($P = 0.032$) G:F. Adding 2,000 FTU/kg phytase to the positive control diet did not influence ADG or ADFI, but tended to improve ($P = 0.060$) G:F. Pigs fed the NC had poorer ($P \leq 0.030$) ADG and ADFI compared to the PC diet, but no difference in G:F was observed.

Pigs fed increasing phytase concentrations up to 2,000 FTU/kg had increased (quadratic, $P < 0.001$) bone ash weights, with a decrease observed in pigs fed 4,000 FTU/kg phytase. In addition, percentage bone ash values increased (linear, $P < 0.001$) as phytase increased. There was a tendency for pigs fed the PC diet to have greater ($P = 0.099$) percentage bone ash compared to the NC diet containing 500 FTU/kg of phytase. Pigs fed the PC diet with phytase had increased ($P = 0.001$) percentage bone ash compared to pigs were fed the PC diet without phytase. Finally, pigs fed the PC diet had greater ($P = 0.010$) bone ash weight and percentage compared to pigs fed the NC diet.

DISCUSSION

Commercially produced microbially derived phytase is one of the most significant enzyme discoveries used in swine diets (Cromwell, 2009). Since the early 1990's, it has been used to efficiently make P, that is bound in the form of phytate, available to monogastrics. Many commercial phytases are available for use in swine diets; however, phytase enzymes differ based on the origin, specificity and configuration (Rodehutscord and Rosenfelder, 2016). Thus, each product should have its own unique available

P release curve to be used in formulation. Many products have already undergone studies to determine specific phytase release curves (Kornegay and Qian, 1996; Kerr et al., 2010; Jones et al., 2010). Recently, Gourley et al. (2017) determined the available P release curve for Natuphos E 5,000 G. When using concentrations between 150 and 1,000 FTU/kg and utilizing percentage bone ash as the response criteria, aP release for up to 1,000 FTU/kg of Natuphos E 5,000 was predicted by the equation: aP release = 0.000212 × FTU/kg phytase.

Based on the linear response to increasing Natuphos E up to 1,000 FTU/kg (Gourley et al., 2017), the current study aimed to evaluate growth performance and bone ash when adding phytase above 1,000 FTU. The current study revealed a quadratic increase for growth performance (ADG and G:F) up to 1,000 FTU/kg of phytase in the NC, with no further improvement or a decrease in performance when included up to 4,000 FTU/kg phytase. Few studies are available on the effects of Natuphos E, and to our knowledge this is the first study to demonstrate high concentration release values of Natuphos E. Kornegay and Qian (1996) observed that with an older generation of Natuphos, breakpoints for growth performance were between 750 and 1,050 FTU/kg. The total P levels in the current experiment NC diets were slightly below the NRC (2012) requirement estimate for each nursery phase. In a recent study Vier et al. (2016) formulated diets from 80 to 160% of the NRC (2012) STTD P requirement estimate and determined that growth was linear up to 160% for a 15 to 25 kg pig, which would suggest that pigs were still below the P requirement needed to maximize growth performance. The quadratic response to phytase in the current study could be explained in part by releasing maximum P at 1,000 FTU/kg to optimize growth performance, with no additional benefit in growth performance when more phytate-bound P was released.

The current study showed a linear increase in bone ash weight and percentage bone ash as phytase increased from 0 to 4,000 FTU/kg. Our study would suggest that the requirement to improve percentage bone ash is greater than what is needed to maximize growth performance in the pig. This is like other studies (Kornegay and Thomas, 1981; Mahan, 1982) that observed the P and Ca requirement to maximize bone development is greater than the requirement for growth performance. However, there was no indication that the amount needed for maximum bone development influences structural soundness.

Kies et al. (2006) observed an improvement in growth performance and digestibility of minerals when phytase (Natuphos) was included up to 15,000 FTU/kg in P-deficient diets. Similarly, Zeng et al. (2014) also observed improved growth performance, mineral digestibility, and bone ash weight as phytase (Phyzyme XP) increased up to 20,000 FTU/kg in P-deficient diets. Because the P requirement would be met at a low addition of phytase, it is suggested that the additional benefit in performance is not coming from P, but rather a release of AA, energy, and other minerals (Selle and Ravindran, 2008). Beers and Jongbloed (1992) were the first to observe an improvement in growth performance when phytase was included in P-sufficient diets, again suggesting the improvement in growth was due to increased digestibility of other nutrients rather than of P. The current study would disagree with these results, where phytase added at 2,000 FTU/kg in a P-sufficient diet did not provide a benefit in ADG or ADFI; however, there was a tendency observed for an improvement in G:F.

A review by Adeola and Cowieson (2011) suggests that when phytate is present in the gut, AA, vitamins and minerals, energy viability, and absorption are reduced. Phytate:protein complexes can form due to an electrostatic attraction between molecules, which can reduce the amount of AA available for absorption. In addition, intact phytate that reaches the duodenum will seek out divalent cations, such as Ca, and create insoluble precipitates where its absorption is reduced (Cowieson et al., 2009). The reduction of Ca from these precipitates further reduces the ability for endogenous processes to proceed and can negatively impact pig performance. Therefore, it is thought that phytase could help to release nutrients other than P that are unavailable to the pig due to high concentrations of phytate.

Providing high concentrations of phytase is also suggested to influence *myo*-inositol availability for the pig. The phytase enzyme works to catalyze the hydrolysis of phytate to inorganic phosphate (PO_4) and *myo*-inositol (Humer et al., 2015). Although there is no requirement for *myo*-inositol, metabolically it is converted to glucose, and is a structural component of phosphoinositides, which regulate amylase secretion, insulin release, smooth muscle contraction, and liver glycogenolysis (McDowell, 2000). Although feeding P above the pig's requirement may not improve growth performance, perhaps the additional *myo*-inositol release could help increase metabolic functions within the pig.

However, because the pig can synthesize *myo*-inositol endogenously, it becomes difficult to determine whether it is release from phytate has a beneficiary role (McDowell, 2000). The current study observed a tendency for an extra-phosphoric effect when phytase was added to the positive control (formulated to meet the Ca and P requirements), with a tendency to improve G:F. Further research is needed to fully determine if Natuphos E does induce "extra-phosphoric" effects and to confirm the benefit of additional *myo*-inositol release due to high concentrations of phytase, and its impact within the pig.

Overall, our study found growth performance improved as added dietary phytase increased up to 1,000 FTU/kg. Because pigs fed the NC + 500 FTU phytase and the PC did not have similar growth performance or bone ash, we can conclude that the release value of 500 FTU/kg Natuphos E used in formulation overestimated the P release. A tendency for improved G:F was observed as phytase was added to the positive control diet when P and Ca were formulated at commercial industry levels. Lastly, the addition of phytase continued to increase percentage bone ash in the NC and when added to the PC, although there was little improvement in growth performance.

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