Assessing phytase release values for calcium, phosphorus, amino acids and energy in diets for nursery and growing pigs

by

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Abstract

Three experiments were conducted to determine the effect of three fermented amino acids (AA) with their respective biomass compared to crystalline AA on the growth performance of swine (n = 315 nursery pigs) and poultry (n = 1,320 or 2,100 broilers for experiment 2 and 3, respectively). Two experiments using a total of 600 nursery pigs were conducted to determine the available P (aP) release of Smizyme TS G5 2,500 phytase. Additionally, two experiments using a total of 3,483 growing-finishing pigs were conducted to determine the effects of feeding 1,500 phytase units of Ronozyme HiPhos phytase when credited with its corresponding nutrient release values. Experiments 1-3 determined that Trp, Thr, or Val with biomass appeared to be equally bioavailable and as suitable for use as crystalline amino acids in swine and poultry diets. In experiments 4 and 5, increasing phytase from 0 to 1,500 FTU/kg in phosphorus deficient diets improved nursery pig performance and bone ash characteristics. Using average daily gain, feed efficiency, and percentage bone ash values, prediction equations were developed, provided a range of aP release values for Smizyme TS G5 2,500 phytase when fed at levels between 150 and 1,500 FTU/kg in diets for 10- to 21-kg pigs. In Experiment 6, growing-finishing pigs fed diets containing high levels of phytase with full nutrient release values (CaPAA+full NE) until market had decreased growth performance and hot carcass weights compared with those not fed any phytase. Removing the phytase at the end of the grower period sufficiently recovered any loss in performance observed. A similar feed efficiency response was observed in experiment 7 with growing pigs, confirming the hypothesis that full nutrient release values attributed to phytase, especially for NE, were too aggressive and resulted in diets contributing less nutrients than needed to optimize performance.

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Chapter 1 - Effects of amino acid biomass or feed grade amino acids on growth performance of growing swine and poultry

Abstract

Three experiments were conducted to determine the effect of three fermented amino acids (AA) with their respective biomass compared to crystalline AA on the growth performance of swine and poultry. In Exp. 1, 315 barrows (DNA 200 × 400, initially 11.3 ± 0.69 kg) were allotted to 1 of 4 dietary treatments with 5 pigs per pen and 15 or 16 pens per treatment. Dietary treatments included a negative control (16% standardized ileal digestible (SID) Tryptophan:lysine (Trp:Lys) ratio), positive control (21% SID Trp:Lys ratio from crystalline Trp), or diets containing Trp with biomass to provide 21 or 23.5% SID Trp:Lys ratios, respectively. Pigs fed the positive control or low Trp with biomass diet had increased ($P < 0.05$) ADG compared to pigs fed the negative control diet, with pigs fed the high Trp with biomass diet intermediate. Pigs fed the low Trp with biomass diet had increased ($P < 0.05$) G:F compared to the negative control diet, with others intermediate. In Exp. 2, 1,320 one-day-old male broilers (Cobb 500, initially 45.2 g) were allotted to 1 of 4 dietary treatments with 33 birds per pen and 10 pens per treatment. Dietary treatments included a negative control (58/58% Threonine:lysine (Thr:Lys) ratio), positive control (65/66% Thr:Lys ratio from crystalline Thr), or diets containing Thr with biomass to provide 65/66 or 69/70% Thr:Lys ratios in starter and grower diets, respectively. Broilers fed the positive control or Thr with biomass diets had increased ($P < 0.05$) ADG compared to broilers fed the negative control diet. Broilers fed the positive control or the low Thr with biomass diet had increased ($P < 0.05$) G:F compared to the negative control and high Thr with biomass treatments. In Exp. 3, 2,100 one-day-old male broilers (Cobb 500, initially 39.4 g) were allotted to 1 of 4 dietary treatments with 35 birds per pen and 15 pens per

treatment. Dietary treatments included a negative control (59/63% Valine:lysine (Val:Lys) ratio), positive control (75/76% Val:Lys ratio from crystalline Val), or diets containing Val with biomass to provide 75/76 or 84/83% Val:Lys ratios in starter and grower diets, respectively. Broilers fed the positive control or Val with biomass diets had increased ($P < 0.05$) ADG, ADFI, and G:F compared to those fed the negative control diet. In conclusion, Trp, Thr, or Val with their respective biomass appear to be equally bioavailable and a suitable alternative to crystalline AA in swine and poultry diets.

Key Words: biomass, pigs, poultry, threonine, tryptophan, valine

Introduction

Crystalline AA are often used as a replacement for intact protein sources, such as soybean meal, in swine and poultry diets. This allows for a reduction in crude protein (CP) concentration which has both environmental and economic benefits (Miranda et. al., 2015).

The synthesis of crystalline, or feed-grade, AA occurs through the fermentation of bacteria in a culture medium containing carbon, nitrogen, sulfur and phosphorus sources (Leuchtenberger et al., 2005). Due to advances in genome sequencing, most AA can be produced using mutant strains of C. glutamicum or E. coli (Leuchtenberger et al., 2005). Following fermentation, the first step of purification is the separation of the amino acid from the fermented biomass (Herman, 2003). Through this process, residual amounts of CP and indispensable AA are retained in the biomass, which results in a nutrient rich byproduct that often ends up as waste (Almeida et al., 2014). Using the AA rich biomass, before extraction of the specific amino acid, in place of crystalline sources is a viable option because of its opportunity to decrease manufacturing inputs while still providing an AA rich product (Herman, 2003). Lysine sulfate

containing 54.6% L-lysine with fermented biomass is commonly used in swine and poultry diets (Schutte et al., 1994). The relative bioavailability of free lysine from lysine sulfate with fermented biomass has been shown to be equivalent to L-Lysine-HCl (Smiricky-Tjardes et al., 2004; Htoo et al., 2016).

Recently, fermented Tryptophan (Trp), Threonine (Thr), and Valine (Vale) biomass products (CJ Bio America, Downers Grove, IL) have been developed for use in livestock and poultry diets. However, there is limited research available to determine their effectiveness as amino acid sources for growing pigs and broilers. Therefore, our objective was to compare the effect of each fermented amino acid with its respective biomass compared to the crystalline AA form on the growth performance of swine and poultry.

Materials and Methods

Experiment 1: Tryptophan

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in this experiment. The study was conducted at the Kansas State University Segregated Early Weaning Facility in Manhattan, KS. Each pen contained a 4-hole, dry self-feeder and cup waterer for ad libitum access to feed and water.

A total of 315 barrows (Line 200 × 400, DNA, Columbus, NE, initially 11.3 kg) were used in a 21-d growth trial. Pigs were weaned at approximately 21 d of age and following arrival to the research facility, were randomized to pens based on initial BW and fed common starter diets for 21 d. On d 21 after weaning, considered d 0 of the study, pigs were weighed and pens were allotted to 1 of 4 dietary treatments with 5 pigs per pen and 15 or 16 pens per treatment. Dietary treatments consisted of a negative control (16% standardized ileal digestible (SID) Trp:Lys ratio), positive control (21% SID Trp:Lys ratio from crystalline Trp), or diets containing

Trp with biomass to provide 21 or 23.5% SID Trp:Lys ratios (0.104 or 0.156% added biomass, respectively). Diets were corn-soybean meal-based and formulated to contain 1.25% SID Lys. Other AA provided by the Trp biomass were not considered in diet formulation. Ingredient nutrient profiles and SID digestibility coefficients were used from NRC (2012). Diets were formulated to be slightly below or at requirement estimates based on previous data with pigs in this facility so as not to under-estimate the Trp:Lys ratio (Clark et al., 2017a,b). The Trp with biomass (CJ Bio America, Downers Grove, IL) had a granulated, cream-colored appearance and contained 60% Trp (assumed to have 100% SID coefficient) per the supplier's specifications.

All dietary treatments were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS and were formulated to meet or exceed NRC (2012) requirement estimates for nutrients other than Lys and Trp (Table 1-1). Samples of complete diets were collected during bagging of experimental diets with a subsample collected from every fourth bag and pooled into one homogenized sample per dietary treatment. Samples were stored at -20°C until they were subsampled and submitted for analysis (Eurofins Scientific Inc., Des Moines, IA) of complete AA profile (excluding Trp and methionine, method 994.12; AOAC International, 2012), Trp (method 988.15; AOAC International, 2012) and methionine (method 994.12; AOAC International, 2012). Diet samples were also analyzed for CP (method 990.03; AOAC International, 1990).

Data were analyzed as a randomized complete block design using the PROC GLIMMIX procedure of SAS version 9.4 (SAS Institute, Inc., Cary, NC) with pen as the experimental unit. Weight block was included in the model as a random effect. LS Means were applied to estimate the effects of Trp source and level. Results were considered significant at $P \leq 0.05$.

Experiment 2 and 3: Threonine and Valine

The Texas A&M University Institutional Animal Care and Use Committee approved the protocol used in these experiments. The studies were conducted at the Texas A&M University Poultry Research Facility in College Station, TX. Each pen contained a dry tube feeder and four nipple waterers for ad libitum access to feed and water. Minimum ventilation was run to supply necessary air exchange. Additionally, all birds were raised on used litter from two previous flocks.

In Exp. 2, a total of 1,320 one-day-old male broilers (Cobb 500, initially 45.2 g) were used in a 28-d growth study. Upon arrival to the research facility, birds were randomized to pens based on weight and allotted to 1 of 4 dietary treatments with 33 birds per pen and 10 pens per treatment. Dietary treatments were fed in two phases. The starter phase was fed from d 0 to 14 and the grower phase was fed from d 14 to 28. Starter diets consisted of a negative control (58% Thr:Lys ratio), positive control (65% Thr:Lys ratio from crystalline Thr), or diets containing Thr with biomass to provide 65 or 69% Thr:Lys ratios (included at 0.117 or 0.175% of the diet, respectively). Grower diets consisted of a negative control (58% Thr:Lys ratio), positive control (66% Thr:Lys ratio from crystalline Thr), or diets containing Thr with biomass to provide 66 or 70% Thr:Lys ratios (0.113 or 0.170% added biomass, respectively). Diets were corn-soybean meal-based and formulated to contain 1.18 or 1.05% apparent digestible Lys in the starter and grower diets, respectively. Ingredient nutrient profiles and apparent digestibility coefficients were used from NRC (1994). The Thr with biomass (CJ Bio America, Downers Grove, IL) had a granulated, brown cream-colored appearance and contained 75% Thr (assumed to have 100% digestibility coefficient) per the supplier's specifications.

In Exp. 3, a total of 2,100 one-day-old male broilers (Cobb 500, initially 39.4 g) were used in a 28-d growth study. Upon arrival to the research facility, birds were randomized to pens based on weight and allotted to 1 of 4 dietary treatments with 35 birds per pen and 15 pens per treatment. Dietary treatments were fed in two phases. The starter phase was fed from d 0 to 14 and the grower phase was fed from d 14 to 28. Starter diets consisted of a negative control (59% Val:Lys ratio), positive control (75% Val:Lys ratio from crystalline Val), or diets containing Val with biomass to provide 75 or 84% Val:Lys ratios (0.28 or 0.41% added biomass, respectively). Grower diets consisted of a negative control (63% Val:Lys ratio), positive control (76% Val:Lys ratio from crystalline Val), or diets containing Val with biomass to provide 76 or 83% Val:Lys ratios (0.20 or 0.30% added biomass, respectively). Diets were corn-soybean meal-based and formulated to contain 1.18 or 1.05% apparent digestible Lys in the starter and grower diets, respectively. Ingredient nutrient profiles and apparent digestibility coefficients were used from NRC (1994). The Val biomass (CJ Bio America, Downers Grove, IL) had a granulated, dark brown colored appearance and contained 70% Val (assumed to have 100% digestibility coefficient) per the supplier's specifications.

All dietary treatments for Exp. 2 and 3 were manufactured at Texas A&M University in College Station, TX and were formulated to meet or exceed the Cobb 500 (2017) recommendations (Tables 1-2 and 1-3). Other AA provided by the Thr or Val biomass were not considered in diet formulation. Diets were mixed in a horizontal mixer then conditioned for 15 s prior to pelleting at 75°C. Starter diets were crumbled following pelleting. Complete diet samples were taken during bagging of experimental diets with five 1 kg grab samples collected and pooled into one homogenized sample per dietary treatment. Samples were stored at -20°C until they were split into three equal samples per dietary treatment and submitted for analysis

(ATC Scientific, North Little Rock, AR) of complete AA profile (excluding Trp and methionine, method 994.12; AOAC International, 2012), Trp (method 988.15; AOAC International, 2012) and methionine (method 994.12; AOAC International, 2012). Diet samples were also analyzed for CP (method 990.03; AOAC International, 1990).

Data were analyzed as a randomized complete block design using the GLM procedure of SPSS Statistics version 24.0 (IBM Corporation, Armonk, NY) with pen as the experimental unit. Weight block was included in the model as a random effect. Duncan's Multiple Range Test were applied to estimate the effects of Thr or Val source and level. Mortality was transformed using arcsine prior to statistical analysis. Results were considered significant at $P \leq 0.05$.

Results

Experiment 1: Tryptophan

Analysis of manufactured diets (Table 1-4) resulted in Trp values consistent with diet formulation, as the diet with no crystalline AA had the lowest level of analyzed Trp while the addition of either fermented Trp with biomass or crystalline Trp increased the total analyzed dietary Trp concentration.

Overall (d 0 to 21) pigs fed the 21% Trp:Lys ratio from crystalline Trp or Trp with biomass had increased ($P < 0.05$) ADG compared to those fed the negative control diet, with pigs fed the 23.5% SID Trp:Lys ratio with biomass intermediate (Table 1-5). There was no evidence for difference in overall ADFI but pigs fed the 21% Trp:Lys ratio from Trp with biomass had improved ($P < 0.05$) G:F compared to those fed the negative control diet with the others intermediate.

Experiment 2: Threonine

Analysis of manufactured diets (Table 1-6) resulted in Thr values consistent with diet formulation, as the diet with no crystalline AA had the lowest level of analyzed Thr while the addition of either fermented Thr with biomass or crystalline Thr increased the total analyzed dietary Thr concentration.

Overall (d 0 to 28) broilers fed diets containing crystalline Thr and the two diets containing increasing Thr with biomass had increased ($P < 0.05$) ADG compared to broilers fed the negative control diet (Table 1-7). There was no evidence for difference in overall ADFI but broilers fed diets containing Thr from crystalline Thr or the low Thr with biomass diet had improved ($P < 0.05$) G:F compared to the negative control and high Thr with biomass treatments.

Experiment 3: Valine

Analysis of manufactured diets (Table 1-8) resulted in Val values consistent with diet formulation, as the diet with no crystalline AA had the lowest level of analyzed Val while the addition of either fermented Val with biomass or crystalline Val increased the total analyzed dietary Val concentration.

Overall (d 0 to 28) broilers fed diets containing crystalline Val and the two diets containing increasing Val with biomass had increased ($P < 0.05$) ADG, ADFI, and G:F compared to broilers fed the negative control diet (Table 1-9).

Discussion

Amino acid biomass is formed during the fermentation process. At the conclusion of fermentation, the liquid ferment is concentrated by heating with steam, which results in a wet product that is formed into granules, and then dried in a fluid bed dryer prior to packaging. The final AA with fermented biomass contains an accumulation of both dispensable and

indispensable AA, in addition to CP and carbohydrates. Specifically, the SID coefficients for lysine and methionine, the first limiting amino acid in swine and poultry diets, in dried fermentation biomass is greater when compared to other protein sources such as fish meal (Sulabo et al., 2013). Similarly, Almeida et al (2014), showed that when adding Thr biomass or fish meal as the sole source of AA in nursery pig diets, the SID of CP and indispensable AA, except Trp, was greater ($P < 0.05$) in the Thr with biomass than in fish meal. Additionally, the metabolizable energy values were greater ($P < 0.05$). Further evidence demonstrations that the standard total tract digestibility of phosphorus (P) in fermentation biomass is increased, which suggests that the available P consists of little physic acid (Sulabo et al., 2013). This indicates that AA with biomass has high nutritional value, therefore may be used as an alternative ingredient in nursery pig diets.

Tryptophan is often the second most limiting amino acid in corn-soybean meal diets for growing pigs (Lewis, 2000). Tryptophan is a precursor for the neurotransmitter serotonin, which is believed to play a role in mood, intestinal activity and appetite. Therefore, supplying sufficient Trp in the diet is important for meeting the animal's protein requirements, as well as stimulating feed intake and growth performance. The current NRC (2012) Trp requirement estimate for 11 to 20 kg nursery pigs is 16% of Lys. Goncalves et al (2015) concluded that increasing the SID Trp:Lys ratio up to 21% improved ADG, ADFI, and G:F in 11 to 20 kg nursery pigs, while formulating diets below 18% SID Trp:Lys had negative impacts on performance, when using commercially available crystalline Trp (98.5% Trp). In the current study, using L-Trp with dried fermented biomass demonstrated that the L-Trp is equally bioavailable as commercial crystalline L-Trp, indicating the dried biomass had no negative impacts on performance.

Threonine has long been recognized as the third limiting amino acid for broilers. Warnick et al (1968) demonstrated in a 12% CP semi-purified soybean meal-based diet that lysine and threonine were next limiting essential amino acids after methionine, with valine now recognized as the fourth most limiting (Corzo et. al., 2007). Baker et al (1994) proposed the first "ideal protein" concept for broilers with essential amino acid levels being expressed as a ratio to dietary lysine level. Their initial requirement estimate was 67% and 77% for threonine and valine. Two years after, Kidd et al (1996) demonstrated that increasing dietary threonine improved breast yield, which led to widespread adoption of threonine supplementation in the broiler industry as breast meat became the primary economic driver. The use of crystalline valine, however, didn't gain attention until more recently. Corozo et al (2011) and Miranda et al (2015) demonstrated that broiler diets supplemented with crystalline valine resulted in growth performance and meat yields similar to diets derived from non-crystalline AA sources. The Cobb 500 (2017) recommendations for digestible Thr:lys ratio in broilers for starter, grower, and finisher diets are 65, 66, and 67%. While the Cobb 500 (2017) recommendations for digestible Val:lys in broilers for starter, grower, and finisher diets are 75, 76, and 77%, respectively.

In the present experiments, when growing pigs or poultry were fed diets containing high Trp or Val with biomass, there was a numeric decrease in feed intake when compared to the low added biomass diets. While the reason is unknown, this could be a result of too high of a digestible Trp or Val percentage, creating an imbalance to other AA. In contrast, there was no evidence for difference in performance when pigs or poultry were fed to the AA requirement at the same digestible Trp, Thr, or Val percent from either purified crystalline sources of the AA or from the AA with biomass source.

In conclusion, these data suggest that Trp, Thr, or Val with biomass appear to be equally bioavailable and are as suitable for use as crystalline amino acids in swine and poultry diets.

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Table 1-1 Diet composition, Exp. 1 (as-fed basis)[1]

	Tryptophan source			
	None	Crystalline	Biomass	
Item SID[2] tryptophan:lysine, %:	16	21	21	23.5
Ingredient, %				
Corn	69.51	69.44	69.40	69.34
Soybean meal	25.43	25.44	25.44	25.44
Choice white grease	1.00	1.00	1.00	1.00
Calcium carbonate	0.65	0.65	0.65	0.65
Monocalcium phosphate	1.20	1.20	1.20	1.20
Sodium chloride	0.60	0.60	0.60	0.60
L-Lysine-HCl	0.55	0.55	0.55	0.55
DL-Methionine	0.19	0.19	0.19	0.19
L-Threonine	0.28	0.28	0.28	0.28
L-Tryptophan	---	0.06	---	---
L-Valine	0.15	0.15	0.15	0.15
L-Isoleucine	0.04	0.04	0.04	0.04
Trace mineral premix[3]	0.15	0.15	0.15	0.15
Vitamin premix[4]	0.25	0.25	0.25	0.25
Phytase[5]	0.02	0.02	0.02	0.02
Tryptophan	---	---	0.104	0.156
Total	100	100	100	100
Calculated analysis				
SID Lys, %	1.25	1.25	1.25	1.25
Total Lys, %	1.38	1.38	1.38	1.38
Total Trp, %	0.22	0.29	0.29	0.32
SID amino acid ratios				
Ile:lys	57	57	57	57
Leu:lys	111	111	111	111
Met:lys	36	36	36	36
Met & cys:lys	57	57	57	57
Thr:lys	65	65	65	65
Trp:lys	16	21	21	23.5
Val:lys	70	70	70	70
His:lys	34	34	34	34
ME, kcal/kg	3,333	3,336	3,329	3,329
NE, kcal/kg	2,502	2,502	2,500	2,498
SID Lys:NE, g/Mcal	5.00	4.99	5.00	5.00
Crude protein, %	18.8	18.9	18.8	18.8
Calcium, %	0.72	0.72	0.72	0.72
Phosphorus, %	0.62	0.62	0.62	0.62
Available phosphorus, %	0.42	0.42	0.42	0.42
STTD P, %[7]	0.46	0.46	0.46	0.46

[1] Diets were fed for 21 days from approximately 11 to 23 kg BW.

[2] Standardized ileal digestible.

[3] Provided per kg of premix: 73 g Zn from Zn sulfate; 73 g Fe from iron sulfate; 22 g Mn from manganese oxide; 11 g Cu from copper sulfate; 0.2 g I from calcium iodate; 0.2 g Se from sodium selenite.

[4] Provided per kg of premix: 3,527,399 IU vitamin A; 881,850 IU vitamin D; 17,637 IU vitamin E; 1,764 mg vitamin K; 15.4 mg vitamin B12; 33,069 mg niacin; 11,023 mg pantothenic acid; 3,307 mg riboflavin.

[5] Ronozyme HiPhos 2700 (DSM Nutrition Products, Parsippany, NJ) provided 405 FTU per kg of feed.

[6] CJ Bio America, Downers Grove, IL.

[7] Standardized total tract digestible phosphorus.

Table 1-2 Male broiler starter and growing diet composition, Exp. 2 (as-fed basis)[1]

	Experimental diet	
Item	Starter[2]	Grower[3]
Ingredient, %		
Corn	61.00	66.20
Soybean meal	33.15	27.95
Soybean oil	1.52	1.72
Limestone	1.33	1.27
Monocalcium phosphate	1.61	1.51
Sodium chloride	0.46	0.46
L-Lysine-HCl	0.23	0.23
DL-Methionine	0.30	0.27
L-Threonine	+/-	+/-
Trace mineral premix[4]	0.05	0.05
Vitamin premix[5]	0.13	0.13
Salinomycin – SaCox[6]	0.05	0.05
Cellulose, filler[7]	0.18	0.17
Threonine biomass[8]	+/-	+/-
Total	100	100
Calculated analysis		
Apparent digestible Lys, %	1.18	1.05
Apparent digestible Thr, %	0.68	0.61
Apparent digestible amino acid ratios		
Meth:lys	49	50
Meth & cys:lys	74	76
Arg:lys	108	107
Thr:lys	58	58
Val:lys	75	76
AME[9], kcal/kg	3,036	3,102
Crude protein, %	21.04	18.9
Calcium, %	0.90	0.84
Total phosphorus, %	0.69	0.65
Sodium, %	0.19	0.19

[1] Starter diets were fed from d 0 to 14 from approximately 45.2 to 458 g BW and grower diets were fed from d 14 to 28 from approximately 458 g to 1.55 kg BW.

[2] Starter diets consisted of a negative control (58% Thr:Lys ratio), positive control to provide 65% Thr:Lys ratio from crystalline Thr (0.088% added crystalline Thr), or diets containing Thr with biomass to provide 65 or 69% Thr:Lys ratios (0.117 or 0.175% added biomass, respectively).

[3] Grower diets consisted of a negative control (58% Thr:Lys ratio), positive control to provide 66% Thr:Lys ratio from crystalline Thr (0.085% added crystalline Thr), or diets containing Thr with biomass to provide 66 or 70% Thr:Lys ratios (0.113 or 0.170% added biomass, respectively).

[4] Trace mineral premix per kg of diet: 60.0 mg manganese, 60 mg zinc, 60 mg iron, 7 mg copper, 0.4 mg iodine, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[5] Vitamin premix per kg of diet: 7700 IU vitamin A, 5500 ICU vitamin D3, 55 IU vitamin E, 1.5 mg vitamin K-3, 0.01 mg B12, 6.6 mg riboflavin, 38.5 mg niacin, 9.9 mg d-pantothenic acid, 0.88 mg folic acid, 2.75 mg pyroxidine, 1.54 mg thiamine, 0.08 mg biotin.

[6] Active drug ingredient salinomycin sodium, 132 g/kg (66 mg/kg inclusion; Huvepharma, Peachtree City, GA). For the prevention of coccidiosis caused by Eimeria tenella, Eimeria necatrix, Eimeria acervulina, Eimeria maxima, Eimeria brunetti and Eimeria mivati.

[7] Cellulose, filler was used to equalize the addition of crystalline Thr and Thr biomass across experimental diets.

[8] CJ Bio America, Downers Grove, IL.

[9] Apparent metabolizable energy.

Table 1-3 Male broiler starter and grower diet composition, Exp. 3 (as-fed basis)[1]

	Experimental diet	
Item	Starter[2]	Grower[3]
Ingredient, %		
Corn	69.53	72.76
Soybean meal	23.46	21.00
Soybean oil	0.55	0.70
Limestone	1.40	1.36
Monocalcium phosphate	1.61	1.49
Sodium chloride	0.45	0.45
L-Lysine-HCl	0.49	0.40
L-Methionine	0.42	0.35
L-Threonine	0.26	0.20
L-Tryptophan	0.02	0.02
L-Isoleucine	0.21	0.16
L-Arginine	0.29	0.21
L-Valine	+/-	+/-
Trace mineral premix[4]	0.05	0.05
Vitamin premix[5]	0.13	0.13
Salinomycin – SaCox[6]	0.05	0.05
Cellulose, filler[7]	0.41	0.30
Choline chloride	0.05	0.05
Copper sulfate	0.05	0.05
Glycine	0.39	0.27
Valine biomass[8]	+/-	+/-
Total	100	100
Calculated analysis		
Apparent digestible Lys, %	1.18	1.05
Apparent digestible Val, %	0.70	0.66
Apparent digestible amino acid ratios		
Met:lys	58	58
Met & cys:lys	84	77
Arg:lys	107	107
Thr:lys	69	70
Val:lys	59	63
AME[9], kcal/kg	3,042	3,086
Crude protein, %	19.25	17.70
Calcium, %	0.88	0.84
Total phosphorus, %	0.44	0.42
Sodium, %	0.18	0.18

[1] Starter diets were fed from d 0 to 14 from approximately 39.4 g to 0.44 kg BW and grower diets were fed from d 14 to 28 from approximately 0.44 to 1.64 kg BW.

[2] Starter diets consisted of a negative control (59% Val:Lys ratio), positive control to provide 75% Val:Lys ratio from crystalline Val (0.20% added crystalline Val), or diets containing Val with biomass to provide 75 or 84% Val:Lys ratios (0.28 or 0.41% added biomass, respectively).

[3] Grower diets consisted of a negative control (63% Val:Lys ratio), positive control to provide 76% Val:Lys ratio from crystalline Val (0.15% added crystalline Val), or diets containing Val with biomass to provide 76 or 83% Val:Lys ratios (0.20 or 0.30% added biomass, respectively).

[4] Trace mineral premix added at this rate yields 60.0 mg manganese, 60 mg zinc, 60 mg iron, 7 mg copper, 0.4 mg iodine, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

[5] Vitamin premix added at this rate yields 7700 IU vitamin A, 5500 ICU vitamin D3, 55 IU vitamin E, 1.5 mg vitamin K-3, 0.01 mg B12, 6.6 mg riboflavin, 38.5 mg niacin, 9.9 mg d-pantothenic acid, 0.88 mg folic acid, 2.75 mg pyroxidine, 1.54 mg thiamine, 0.08 mg biotin per kg diet.

[6] Active drug ingredient salinomycin sodium, 132 g/kg (66 mg/kg inclusion; Huvepharma, Peachtree City, GA). For the prevention of coccidiosis caused by Eimeria tenella, Eimeria necatrix, Eimeria acervulina, Eimeria maxima, Eimeria brunetti and Eimeria mivati.

[7] Cellulose, Filler (wt:wt) was used to equalize the addition of crystalline Thr and Thr biomass across experimental diets.

[8] CJ Bio America, Downers Grove, IL.

[9] Apparent metabolizable energy.

Table 1-4 Chemical analysis of diets, Exp. 1 (as-fed basis)[1]

	Experimental diet				
Tryptophan source:	None	Crystalline	Biomass		
Item, %[4] SID[2] tryptophan:lysine, %:	16	21	21	23.5	Trp Biomass[3]
Crude protein	18.19	17.31	18.56	19.13	77.69
Lysine	1.29	1.31	1.23	1.49	0.79
Isoleucine	0.80	0.72	0.71	0.79	0.84
Leucine	1.62	1.49	1.48	1.59	1.41
Methionine	0.48	0.46	0.42	0.45	0.18
Threonine	0.91	0.88	0.89	0.99	0.86
Tryptophan	0.22	0.27	0.29	0.33	52.56
Valine	1.03	0.96	0.92	0.99	1.05
Histidine	0.48	0.45	0.45	0.50	0.38
Phenylalanine	0.90	0.80	0.81	0.89	0.73
Arginine	1.13	1.04	1.05	1.13	---

[1] Diets were fed for 21 d from approximately 11 to 23 kg BW.

[2] Standardized ileal digestible.

[3] CJ Bio America, Downers Grove, IL.

[4] A sample of all experimental diets and the Trp with biomass were submitted for AA analysis and crude protein (Eurofins Scientific Inc., Des Moines, IA).

Table 1-5 Effects of using tryptophan biomass as a source of tryptophan on nursery pig performance, Exp.1[1]

	Experimental diet					
Tryptophan source:	None	Crystalline	Biomass[3]			
Item SID[2] tryptophan:lysine, %:	16	21	21	23.5	SEM	Probability, $P <$
BW, kg						
d 0	11.3	11.3	11.2	11.3	0.25	0.723
d 21	22.8[b]	23.5[a]	23.5[ab]	23.0[ab]	0.37	0.019
d 0 to 21						
ADG, g	549[b]	576[a]	580[a]	556[ab]	8.9	0.004
ADFI, g	836	865	854	833	18.0	0.132
G:F	0.658[b]	0.667[ab]	0.680[a]	0.668[ab]	0.0077	0.045

[ab] Values with different superscripts differ, $P < 0.05$.

[1] A total of 315 barrows (DNA 200 × 400, initially 11.3 kg) were used in a 21-d nursery study with 5 pigs per pen and 15 or 16 pens per treatment.

[2] Standardized ileal digestible.

[3] CJ Bio America, Downers Grove, IL.

Table 1-6 Chemical analysis of starter and grower diets, Exp. 2 (as-fed basis)

		Starter diet[1]				Grower diet[2]			
	Threonine source:	None	Crystalline	Biomass		None	Crystalline	Biomass	
Item, %[3]	Apparent digestible threonine:lysine, %:	58	65	65	69	58	66	66	70
Crude protein		20.23	21.69	21.64	21.41	18.52	19.06	18.89	18.57
Lysine		1.35	1.29	1.31	1.34	1.22	1.23	1.22	1.18
Isoleucine		0.90	0.87	0.85	0.93	0.81	0.81	0.81	0.75
Leucine		1.77	1.78	1.73	1.85	1.65	1.67	1.68	1.55
Methionine		0.53	0.61	0.54	0.57	0.54	0.49	0.50	0.53
Threonine		0.79	0.84	0.84	0.92	0.72	0.79	0.79	0.80
Tryptophan		0.31	0.28	0.33	0.31	0.28	0.26	0.27	0.29
Valine		1.00	1.02	0.97	1.06	0.95	0.96	0.94	0.88
Arginine		1.46	1.43	1.43	1.57	1.32	1.36	1.28	1.20

[1] Starter diets were fed from d 0 to 14 from approximately 45.2 to 458 g BW.

[2] Grower diets were fed from d 14 to 28 from approximately 458 g to 1.55 kg BW.

[3] A sample of all experimental diets were submitted for AA analysis and crude protein (Eurofins Scientific Inc., Des Moines, IA).

Table 1-7 Effects of using threonine biomass as a source of threonine on male broiler performance, Exp. 2[1]

		Experimental diet					
	Threonine source:	None	Crystalline	Biomass[2]		PSEM	Probability, $P <$
Item	Apparent digestible threonine:lysine, %[3]:	58/58	65/66	65/66	69/70		
BW							
d 0, g		45.2	45.1	45.2	45.2	0.04	0.683
d 28, kg		1.524[b]	1.562[a]	1.563[a]	1.546[ab]	0.0066	0.038
d 0 to 28							
ADG, g		52.8[b]	54.2[a]	54.2[a]	53.6[a]	0.38	0.041
ADFI, g		80.9	81.3	81.6	82.0	0.35	0.486
G:F		0.671[b]	0.685[a]	0.685[a]	0.677[ab]	0.0029	0.006

[ab] Values with different superscripts differ, $P < 0.05$.

[1] A total of 1,320 male broilers (Cobb 500, initially 45.2 g) were used in a 28-d growth study with 33 birds per pen and 10 pens per treatment.

[2] CJ Bio America, Downers Grove, IL.

[3] Apparent digestible threonine:lysine ratio in the starter/grower phase.

Table 1-8 Chemical analysis of starter and grower diets, Exp. 3 (as-fed basis)

		Starter diet[1]				Grower diet[2]			
	Valine source:	None	Crystalline	Biomass		None	Crystalline	Biomass	
Item, %[3]	Apparent digestible valine:lysine, %:	59	75	75	84	63	76	76	83
Crude protein		18.25	18.69	19.00	18.50	16.25	17.06	16.56	17.69
Lysine		1.25	1.35	1.35	1.39	1.09	1.27	1.16	1.22
Isoleucine		0.83	0.84	0.86	0.86	0.76	0.79	0.73	0.78
Leucine		1.46	1.40	1.46	1.39	1.36	1.39	1.25	1.40
Methionine		0.60	0.60	0.62	0.62	0.55	0.57	0.58	0.55
Threonine		0.86	0.87	0.91	0.87	0.79	0.80	0.76	0.78
Tryptophan		0.25	0.25	0.27	0.26	0.22	0.22	0.22	0.23
Valine		0.80	0.95	0.98	1.06	0.74	0.88	0.84	0.96
Arginine		1.28	1.29	1.33	1.31	1.16	1.20	1.12	1.6

[1] Starter diets were fed from d 0 to 14 from approximately 39.4 g to 0.44 kg BW.

[2] Grower diets were fed from d 14 to 28 from approximately 0.44 to 1.64 kg BW.

[3] A sample of all experimental diets were submitted for AA analysis and crude protein (Eurofins Scientific Inc., Des Moines, IA).

Table 1-9 Effects of using valine biomass as a source of valine on male broiler performance, Exp. 3[1]

	Experimental diet					
Valine source:	None	Crystalline	Biomass[2]		PSEM	Probability, $P <$
Item / Apparent digestible valine:lysine, %[3]:	59/63	75/76	75/76	84/83		
BW						
d 0, g	39.4	39.4	39.5	39.3	0.03	0.764
d 28, kg	1.551[b]	1.665[a]	1.684[a]	1.662[a]	0.0088	<0.001
d 0 to 28						
ADG, g	54.0[b]	58.1[a]	58.7[a]	58.0[a]	0.34	<0.001
ADFI, g	78.0[b]	81.4[a]	82.4[a]	81.1[a]	0.39	<0.001
G:F	0.711[b]	0.729[a]	0.730[a]	0.728[a]	0.0031	<0.001

[ab] Values with different superscripts differ, $P < 0.05$.

[1] A total of 2,100 male broilers (Cobb 500, initially 39.4 g) were used in a 28-d growth study with 35 birds per pen and 15 pens per treatment.

[2] CJ Bio America, Downers Grove, IL.

[3] Apparent digestible valine:lysine ratio in the starter/grower phase.

Chapter 2 - Determining the Phosphorus Release of Smizyme TS G5 2,500 Phytase in Diets for Nursery Pigs

Abstract

Two experiments were conducted to determine the available P (aP) release of Smizyme TS G5 2,500 (Origination, LLC., Maplewood, MN) phytase. Pigs were weaned at approximately 21-d of age, randomly allotted to pens based on initial body weight (BW) and fed a common diet. On d 21 post-weaning, pens were blocked by BW and randomly allotted to 1 of 8 (Exp. 1) or 7 (Exp. 2) dietary treatments with 5 pigs per pen and 8 pens per treatment. Treatments were formulated to include increasing aP from either inorganic P (0.12, 0.18, or 0.24% in Exp. 1 and 0.11, 0.19, or 0.27% in Exp. 2 from monocalcium P) or increasing phytase (150, 250, 500, 750, or 1,000 FTU/kg in Exp. 1 and 250, 500, 1000, or 1,500 FTU/kg in Exp. 2). Prior to beginning the 21-d studies, all pigs were fed the lowest inorganic P diet for a 3-d period. At the conclusion of each experiment, the pig closest to the pen mean BW was euthanized and fibulas were collected to determine bone ash weight and percentage bone ash. Fibulas were processed using defatted bone mineral procedures. In both experiments, pigs fed increasing aP from inorganic P had increased (linear, $P < 0.01$) ADG, G:F, and final BW. Additionally, pigs fed diets with increasing phytase had increased (Exp. 1 linear, $P < 0.01$, Exp. 2 linear and quadratic, $P < 0.05$) performance across all growth response criteria. For bone composition, pigs fed increasing aP from inorganic P had increased bone ash weights (linear, $P < 0.01$) and percentage bone ash (Exp. 1 quadratic, $P < 0.01$, Exp. 2 linear, $P < 0.01$). Similarly, pigs fed increasing phytase had increased bone ash weights (linear, $P < 0.01$) and percentage bone ash (Exp. 1 linear, $P < 0.01$, Exp. 2 linear and quadratic, $P < 0.05$). The percentage aP released from Smizyme TS G5 2,500 for both experiments varied depending on the response criteria used. As the amount of phytase in

the diet increased, the calculated aP release increased when ADG (Exp. 1 linear, $P < 0.01$; Exp. 2 linear and quadratic, $P < 0.01$), G:F (linear, $P < 0.01$), or percentage bone ash (Exp. 1 linear and quadratic, $P < 0.05$; Exp. 2 linear, $P < 0.01$) were used a the predictor variable. When combining the data from Exp. 1 and 2, the aP release prediction equations for Smizyme TS G5 2,500 are aP=(0.197×FTU)÷(584.956+FTU), aP=(0.175×FTU)÷(248.348+FTU), and aP=(0.165×FTU)÷(178.146+FTU) when using ADG, G:F, and percentage bone ash, respectively as the predictor variable.

Key Words: bone ash, nursery pig, phytase, phosphorus

Introduction

Phytase is an enzyme commonly added to swine diets to improve the digestibility of phytate-bound-phosphorus. Phytate or phytic acid is a six-fold dihydrogen phosphate ester of inositol that is the major storage form (60 to 82%) of phosphorus (P) found in feedstuffs of plant origin (Ravindran et al., 1994). Pigs and other monogastric animals do not synthesize adequate levels of endogenous phytase to effectively cleave the phosphates from the phytate (Selle and Ravindran, 2008). Therefore, the P found in corn-soybean meal-based diets has limited availability, requiring nutritionists to add P from inorganic sources, such as monocalcium phosphate, in order to meet P requirements. Alternatively, adding phytase from an exogenous source has been shown to improve the hydrolysis of phytic acid, making P more available for absorption (Simons et al., 1990, Cromwell et al., 1993). This allows nutritionists to reduce dietary inorganic P without compromising growth, while subsequently improving diet costs and decreasing the amount of P excreted in swine waste (Simons et al., 1990; Selle and Ravindran, 2008; Lei et al., 2013).

Phytase was first commercialized in 1991 and nearly 20 years later it was coined one of the most significant discoveries in animal nutrition (Cromwell, 2009; Lei et al., 2013). While there are many commercially available phytase sources that have already undergone evaluation to determine their available P (aP) release, new phytase sources must be analyzed to determine their efficacy (Jones et al., 2010; Goncalves et al., 2016; Gourley et al., 2018). Smizyme TS G5 2,500 (Origination, LLC., Maplewood, MN) is a newly available bacterial derived 6-phytase produced through the fermentation of E. coli. Little research has been conducted to demonstrate its efficacy for use in the U.S swine industry. Therefore, the objective of these studies was to evaluate the effects of Smizyme TS G5 2,500 phytase (Origination, LLC., Maplewood, MN) on the growth performance and bone ash of 10- to 21-kg nursery pigs to develop an aP release curve.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocol used in these experiments. Ingredients containing Ca and P were analyzed (Ward Laboratories, Inc., Kearney, NE) in duplicate prior to the manufacturing of experimental diets to determine nutrient loading values used for formulation (Table 2-1). Additionally, phytase was acquired for each experiment and the analyzed (Eurofins Scientific Inc., Des Moines, IA) activity was 2,630,000 FTU/kg (Exp.1) and 2,314,000 FTU/kg (Exp. 2). Diets were corn-soybean meal-based and contained 1.24% SID Lys with other amino acids set to meet or exceed NRC (2012) requirement estimates. All diets were formulated to contain a Ca:P ratio of 1.10:1 with no allowance for release of Ca by phytase. All dietary treatments were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS.

Diet Manufacturing

At the time of feed manufacturing, 8 (Exp. 1) or 7 (Exp. 2) identical 907 kg batches of basal diet were produced and packaged in 22.7 kg bags (Table 2-2). For each experimental diet, a subset of bags from the basal diet was added to the mixer along with treatment specific ingredients to achieve the final experimental diets (Table 2-3 and 2-4). Complete diet samples were taken during bagging of experimental diets with a subsample collected from every fourth bag and pooled into one homogenized sample per dietary treatment. After homogenization, each diet sample was ground to reduce particle size and then divided into three separate sub-samples per dietary treatment. Samples were stored at -20°C until they were submitted for phytase and nutrient analysis.

Animals and Housing

Experiments were 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 nipple waterer for ad libitum access to feed and water.

A total of 320 (Exp 1) or 280 (Exp 2) pigs (DNA 241 × 600, initially 10 kg) were used in 21-d growth trials. Pigs were weaned at approximately 21 d of age, randomly allotted to pens based on initial BW, and fed a common starter diet. On d 21 post-weaning, considered d 0 of the studies, pens were blocked by BW and randomly allotted to 1 of 8 (Exp. 1) or 7 (Exp. 2) dietary treatments with 5 pigs per pen (2 barrows and 3 gilts or 3 barrows and 2 gilts) and 8 pens per treatment. In Exp. 1, treatments consisted of 3 diets with increasing (0.12, 0.18, or 0.24%) inorganic P from monocalcium P, or 5 diets with increasing (150, 250, 500, 750, or 1,000 FTU/kg) phytase added to the diet containing 0.12% aP. In Exp. 2, treatments consisted of 3

diets with increasing (0.11, 0.19, or 0.27%) inorganic P from monocalcium P, or 4 diets with increasing (250, 500, 1,000, or 1,500 FTU/kg) phytase added to the diet containing 0.11% aP. In both trials the source of phytase was Smizyme TS G5 2,500 (Origination, LLC., Maplewood, MN), a microbial phytase derived from the fermentation of E. coli. Prior to beginning the 21-d studies, all pigs were fed the lowest inorganic P diet for a 3-d period (d 18 to 21 post-weaning).

During the experiments, pigs and feeders were weighed every 7 d to determine ADG, ADFI, and G:F. At the conclusion of each study, the pig closest to the pen mean BW was euthanized via penetrating captive bolt and fibulas were collected to determine bone ash weight and percentage bone ash (32 barrows and 32 gilts (Exp. 1) or 28 barrows and 28 gilts (Exp. 2)). After collection, bones were individually placed in plastic bags with permanent identification and stored at -20°C until processing. On the day of processing, bones were autoclaved for 1 hour at 121°C. After cooling, any leftover extraneous soft tissue including cartilage caps was cleaned from the fibulas. A total of 64 (Exp. 1) or 56 (Exp. 2) fibulas (one from each pig) were placed in Soxhlet extractors containing petroleum ether for 7 d as a means of removing water and fat. They were then dried at 105°C for 24 h, and ashed at 600°C for 24 h.

Chemical Analysis

Three samples per dietary treatment from the pooled feed samples were sent to a commercial laboratory (Ward Laboratories, Inc., Kearney, NE) for duplicate analysis of crude protein (AOAC 990.03, 2006), Ca (AOAC 985.01, 2006), and P (AOAC 985.01, 2006). Additionally, one sample of each diet containing phytase was submitted for complete phytase analysis (Eurofins Scientific Inc., Des Moines, IA) using the AOAC official method 2000.12.

Statistical Analysis

Studentized residuals were evaluated for pen means or individual bone ash measurements to ensure data met the assumption of normal distribution. 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 version 9.4 (SAS Institute, Inc., Cary, NC). Treatment was considered a fixed effect and weight block a random effect. Linear and quadratic contrasts were evaluated within increasing inorganic P or phytase treatments. Contrast coefficients were adjusted to account for unequal spacing in phytase doses.

For pens of pigs fed the inorganic P diets, the marginal intake of aP per day was calculated for each pen using the equation: dietary aP% minus 0.12% (aP in the basal diet, Exp. 1) or 0.11% (aP in the basal diet, Exp. 2) multiplied by ADFI. A standard curve was then developed for each response criteria using the marginal aP release as the predictor variable. The equation for the standard curve was used to calculate aP release from 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.

A mixed model ANOVA with weight block as a random effect was performed to evaluate aP release as a function of phytase dosage, assuming an intercept of no aP release for the control diet without phytase. All release values were calculated using formulated P and phytase levels. The GLIMMIX procedure of SAS version 9.4 (SAS Institute, Inc., Cary, NC) was used for the analysis.

Release values were combined from the two experiments and were used in a non-linear regression to fit a model predicting aP release curves dependant on phytase dosage as a continuous variable using the individual pen data. Separate aP release curves were developed

using aP release derived from ADG, G:F, and percentage bone ash data. Model parameters were estimated using the nls function from the stats package in R (version 3.5.1 (2018-07-02)). Results were considered to be significant with P-values ≤ 0.05 and were considered marginally significant with P-values > 0.05 and ≤ 0.10.

Results

Chemical Analysis

Analysis of manufactured diets resulted in crude protein, Ca and P values that were reasonably consistent with formulation (Table 2-3 and 2-4). Phytase analysis of complete diets showed a stepwise increase in phytase concentration.

Experiment 1

From d 0 to 21, pigs fed increasing aP from inorganic P had improved (linear, $P < 0.001$; Table 2-5) ADG, G:F, and final BW, with a tendency for increased ADFI (linear, $P = 0.080$). Additionally, pigs fed increasing aP from phytase had improved (linear, $P < 0.010$) performance across all growth response criteria measured.

For bone composition, pigs fed increasing aP from inorganic P had increased (linear, $P < 0.001$) bone ash weights, resulting in increased (quadratic, $P < 0.001$) percentage bone ash, while those fed increasing phytase had increased bone ash weights (linear, $P < 0.001$) and percentage bone ash (linear, $P < 0.001$ and quadratic, $P = 0.066$).

The percentage aP released from Smizyme TS G5 2,500 varied depending on the response criteria (Table 2-6). As the amount of phytase in the diet increased, the calculated aP release increased linearly ($P < 0.001$) when using ADG, G:F, or bone ash weight as the indicator of release. When using percentage bone ash as the indicator of release, aP increased in a linear and quadratic fashion (linear, $P < 0.001$ and quadratic $P = 0.028$).

Experiment 2

From d 0 to 21, pigs fed increasing aP from either inorganic P (linear, $P < 0.001$; Table 2-7) or phytase (linear, $P < 0.001$ and quadratic, $P < 0.05$) had improved performance across all growth response criteria measured.

For bone composition, pigs fed increasing aP from inorganic P had increased (linear, $P < 0.001$) bone ash weights and percentage bone ash, while those fed increasing phytase had increased bone ash weights (linear, $P < 0.001$ and quadratic, $P = 0.061$) and percentage bone ash (linear, $P < 0.001$ and quadratic, $P = 0.035$).

Similar to Exp. 1, the percentage aP released from Smizyme TS G5 2,500 in Exp. 2 varied depending on the response criteria used (Table 2-8). As the amount of phytase in the diet increased, the calculated aP release increased when using ADG (linear and quadratic, $P < 0.001$), G:F (linear, $P < 0.001$ and quadratic $P = 0.066$), or bone ash weight (linear, $P < 0.001$ and quadratic $P = 0.059$) as the indicator of release. Likewise, when using percentage bone ash, the calculated aP release increased linearly ($P < 0.001$) with a quadratic tendency ($P = 0.055$).

Combining the release values for both experiments, the release equations for Smizyme TS G5 2,500 are $aP=(0.197\times FTU)\div(584.956+FTU)$, $aP=(0.175\times FTU)\div(248.348+FTU)$, and $aP=(0.165\times FTU)\div(178.146+FTU)$ when ADG, G:F, and percentage bone ash, respectively were used as the predictor variable (Figure 1).

Discussion

Smizyme TS G5 used in the present studies is a bacterial derived 6-phytase produced through the fermentation of E. coli in a Pichia Pastoris yeast medium. The efficacy of different phytase sources to release P is affected by the solubility of phytate bound P in feedstuffs, which influences its susceptibility to hydrolysis (Létourneau-Montminy et al., 2012). Additionally, the

position of hydrolysis onset, preferered pH conditions, and temperature stability of the phytase further impact its efficacy (Humer et al., 2015). In vitro studies have shown that the optimal pH for E. coli derived phytase activity is between 2 and 4.5 (Adeola et al., 2004). A model conducted by Létourneau-Montminy et al. (2011) indicated that microbial phytases are largely active in the upper portion of the digestive tract, or stomach of the pig, where more acidic conditions are found. Because P solubility, hydrolysis, and absorption are affected by the physicochemical conditions along the digestive tract, microbial phytase activity is observed to significantly decrease in the small intestine due to increasing pH (Létourneau-Montminy et al., 2011). Rather, the majority of free P achieved from phytate hydrolysis in the stomach is absorbed in the small intestine. Any remaining concentrations of phytate bound P cleaved in the hindgut are of little physciological importance because P released in the hindgut is not absorbed (Rutherfurd et al., 2014; Humer et al., 2015).

Phosphorus is involved in many biochemical pathways, including soft tissue growth and skeletal integrity (Crenshaw, 2001). Jendza et al. (2006) evaluated the efficacy of an E. coli-derived phytase to determine its equivalency relative to P from monosodium phosphate. They determined that the equivalency requirement to maximize bone mineralization is more sensitive than the requirement needed to maximize growth performance. Several other studies have confirmed that lean growth and structural integrity are often independent of one another (Augspurger et al., 2003; Veum et al., 2006), therefore providing different aP release predictions based on response criteria (Jones et al., 2010; Gourley et al., 2018). In our studies, we also observed that the aP release with incrementally increasing phytase was different depending on the response criteria measured. It was also observed that as the inclusion of inorganic P or

phytase in the diet increased, percentage bone ash increased. This confirms that changes in nutritional status have an impact on bone ash percent (Crenshaw, 2001).

Multiple experiments have been conducted to determine the efficacy of microbial phytases on mineral digestibility, growth performance, or bone characteristics (Adeola et al., 2004; Jendza et al., 2006; Veum et al., 2006; Jones et al., 2010;). By adding graded levels of inorganic P or exogenous phytase to P deficient diets and evaluating percentage bone ash, phytase activity and aP release can be determined. Due to differences in phytase characteristics and stability, phytase sources often differ in the amount of P released per FTU included in the diet (Goncalves et al., 2016). One FTU can be defined as the amount of enzyme activity that liberates 1 μmol inorganic orthophosphate per minute from 0.0051 mol L-1 sodium phosphate at a pH of 5.5 and 37°C (AOAC, 2006). Numerous analytical methods have been developed to determine phytase activity, including the commonly used AOAC Official Method 2000.12 (Jones et al., 2010). However, due to the intrinsic differences between phytase sources, it is important when new or enhance phytases enter the marketplace to evaluate their unique aP release.

Currently, there is limited research regarding the use of Smizyme phytase in swine diets. Arredondo et al. (2019) conducted a 12-d study to evaluate the apparent total tract digestibility (ATTD) of nutrients in growing pigs fed 250 to 2,500 FTU/kg Smizyme TS G5 in P-deficient diets. Results indicated that as phytase dose increased, the ATTD of Ca and P, and the standardized total tract digestibility of P increased in a linear and quadratic fashion ($P < 0.01$). In a broken line analysis 1,041 and 1,107 FTU were needed to maximize the ATTD of Ca and P, respectively. Similarly, in the present studies when evaluating growth performance and bone ash, increasing phytase inclusion from 150 to 1,500 FTU resulted in linear and quadratic

improvements in ADG, G:F and percentage bone ash. When increasing phytase concentration up to 1,500 FTU a quadratic response was observed, indicating diminishing marginal improvements as phytase dose increases beyond 1,000 FTU. Zhang et al. (2000) also reported that growth criteria and bone ash percent response to phytase are better represented by a non-linear equation.

These studies have provided a range of aP release for Smizyme TS G5 2,500 phytase in nursery pigs weighing 10- to 21-kg when fed at levels between 150 and 1,500 FTU/kg. In summary, the magnitude of aP release at different FTU inclusion rates depends on the response criteria measured. When comparing the amount of phytase needed to reach a particular aP release value in the diet (Goncalves et al., 2016), Smizyme TS G5 2,500 appears to have a similar aP release to other commercially available phytase sources based on manufacture recommendation.

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Table 2-1 Analyzed ingredient composition[1]

	Experiment 1		Experiment 2	
Ingredient	Ca, %	P, %	Ca, %	P, %
Corn	0.07	0.24	0.06	0.20
Soybean meal	0.56	0.64	0.60	0.64
Limestone	40.24	0.03	40.28	0.06
Monocalcium P	17.90	20.23	17.42	20.66

[1]Ingredient samples were taken from the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS, and submitted for analysis (Ward Laboratories, Inc., Kearney, NE).

Table 2-2 Composition of basal mix (as-fed)[1]

Item	Experiment 1 and 2
Ingredient, %	
Corn	64.39
Soybean meal	34.05
Sodium chloride	0.61
L-Lysine-HCl	0.30
DL-Methionine	0.12
L-Threonine	0.12
L-Valine	0.01
Trace mineral premix	0.15
Vitamin premix	0.25
Calculated analysis	
Standardized ileal digestible (SID) amino acids	
Lysine, %	1.24
Isoleucine:lysine	64
Leucine:lysine	131
Methionine:lysine	33
Methionine and cystine:lysine	57
Threonine:lysine	64
Tryptophan:lysine	19
Valine:lysine	70
Histidine:lysine	42
Total lysine, %	1.41
Metabolizable energy, kcal/kg	3,324
Net energy (NE), kcal/kg	2,444
SID lysine:NE, g/Mcal	5.06
Crude protein, %	22.0
Calcium, %[2]	---
Phosphorus, %[3]	---
Available phosphorus, %	0.07
STTD P, %[4]	0.16

[1] The basal batch was used as the major ingredient in each experimental diet.
[2] The calculated analysis of Ca in Exp. 1 and 2 was 0.31 and 0.32%, respectively.
[3] The calculated analysis of P in Exp. 1 and 2 was 0.37 and 0.35%, respectively.
[4] STTD P = Standardized total tract digestible phosphorus.

Table 2-3 Composition of experimental diets (as-fed basis), Exp. 1[1]

	Inorganic P			Phytase[2]				
Ingredient, %	0.12%	0.18%	0.24%	150	250	500	750	1,000
Basal mix	98.75	98.75	98.75	98.75	98.75	98.75	98.75	98.75
Limestone	0.30	0.32	0.35	0.29	0.29	0.29	0.29	0.29
Monocalcium P	0.25	0.53	0.85	0.25	0.25	0.25	0.25	0.25
Sand[3]	0.71	0.41	0.05	0.70	0.70	0.69	0.68	0.67
Phytase[4]	---	---	---	0.006	0.009	0.019	0.029	0.038
Calculated analysis								
Crude protein, %	21.7	21.7	21.7	21.7	21.7	21.7	21.7	21.7
Ca, %	0.46	0.52	0.59	0.46	0.46	0.46	0.46	0.46
P, %	0.42	0.48	0.54	0.42	0.42	0.42	0.42	0.42
Phytase, FTU/kg	---	---	---	150	250	500	750	1000
Ca:P ratio	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Analyzed composition[5]								
Crude protein, %	21.9	22.0	21.8	21.5	21.8	21.9	21.0	21.6
Ca, %	0.44	0.43	0.54	0.38	0.43	0.40	0.46	0.41
P, %	0.45	0.48	0.56	0.43	0.41	0.43	0.44	0.44
Phytase, FTU/kg[6,7]	---	---	---	190	310	500	790	850
Ca:P ratio	0.98	0.90	0.96	0.88	1.05	0.93	1.05	0.93

[1]Diets were fed for 21 d from approximately 10- to 23-kg.

[2]Smizyme TS G5 2,500 (Origination, LLC., Maplewood, MN).

[3]Sand was used to equalize the addition of the sum of limestone, monocalcium P, and phytase across experimental diets.

[4]Phytase premix was analyzed for phytase level and contained 2,630,000 FTU/kg (Eurofins Scientific Inc., Des Moines, IA).

[5]Complete diet samples were taken during bagging of experimental diets with a subsample collected from every fourth bag and pooled into one homogenized sample per dietary treatment. After homogenization, each diet was placed in a grinder to reduce particle size and then divided into three separate samples per dietary treatment. Samples were stored at -20°C until they were submitted for duplicate analysis of crude protein, Ca, and P (Ward Laboratories, Inc., Kearney, NE).

[6] One sample of each diet containing phytase was submitted to Eurofins Scientific Inc. (Des Moines, IA) for complete phytase analysis.

[7] AOAC. 2000. Official Methods of Analysis of AOAC International. 17th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.

Table 2-4 Composition of experimental diets (as-fed basis), Exp. 2[1]

	Inorganic P			Phytase[2]			
Ingredient, %	0.11%	0.19%	0.27%	250	500	1,000	1,500
Basal mix	98.77	98.77	98.77	98.77	98.77	98.77	98.77
Limestone	0.18	0.23	0.28	0.18	0.18	0.18	0.18
Monocalcium P	0.20	0.55	0.95	0.20	0.20	0.20	0.20
Sand[3]	0.85	0.45	0.00	0.84	0.83	0.81	0.78
Phytase[4]	---	---	---	0.011	0.022	0.043	0.065
Calculated analysis							
Crude protein, %	21.7	21.7	21.7	21.7	21.7	21.7	21.7
Ca, %	0.42	0.50	0.59	0.42	0.42	0.42	0.42
P, %	0.38	0.46	0.54	0.38	0.38	0.38	0.38
Phytase, FTU/kg	---	---	---	250	500	1,000	1,500
Ca:P ratio	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Analyzed composition[5]							
Crude protein, %	21.2	20.7	20.9	20.9	21.0	21.5	21.1
Ca, %	0.38	0.49	0.59	0.41	0.37	0.38	0.37
P, %	0.38	0.44	0.53	0.38	0.37	0.39	0.37
Phytase, FTU/kg[6,7]	---	---	---	265	470	1,000	1,450
Ca:P ratio	0.99	1.10	1.12	1.07	1.00	0.97	0.98

[1]Diets were fed for 21 d from approximately 10- to 22-kg.

[2]Smizyme TS G5 2,500 (Origination, LLC., Maplewood, MN).

[3]Sand was used to equalize the addition of the sum of limestone, monocalcium P, and phytase across experimental diets.

[4]Phytase premix was analyzed for phytase level and contained 2,314,000 FTU/kg (Eurofins Scientific Inc., Des Moines, IA).

[5]Complete diet samples were taken during bagging of experimental diets with a subsample collected from every fourth bag and pooled into one homogenized sample per dietary treatment. After homogenization, each diet was placed in a grinder to reduce particle size and then divided into three separate samples per dietary treatment. Samples were stored at -20°C until they were submitted for duplicate analysis of crude protein, Ca, and P (Ward Laboratories, Inc., Kearney, NE).

[6] One sample of each diet containing phytase was submitted to Eurofins Scientific Inc. (Des Moines, IA) for complete phytase analysis.

[7] AOAC. 2000. Official Methods of Analysis of AOAC International. 17th ed. Assoc. Off. Anal. Chem., Gaithersburg, MD.

Table 2-5 Effects of increasing aP from inorganic P or Smizyme TS G5 G5 2,500 phytase on nursery pig growth performance and bone ash values, Exp. 1[1,2]

	Inorganic P, % aP[3]			Phytase, FTU/kg[4]						Inorganic P		Phytase	
Item	0.12%	0.18%	0.24%	150	250	500	750	1,000	SEM[5]	Linear	Quadratic	Linear	Quadratic
BW, kg													
d 0	10.4	10.5	10.4	10.3	10.4	10.4	10.4	10.4	0.17	0.548	0.284	0.693	0.671
d 21	21.4	22.7	23.1	21.9	22.2	22.9	23.2	23.6	0.41	<0.001	0.162	<0.001	0.259
d 0 to 21													
ADG, g	523	585	605	551	563	593	608	629	14.6	<0.001	0.198	<0.001	0.273
ADFI, g	882	944	937	868	910	923	931	947	24.8	0.080	0.204	0.007	0.725
G:F, g/kg	592	624	647	633	619	643	655	665	9.9	<0.001	0.732	<0.001	0.190
Bone ash, g[6]	0.58	0.66	0.81	0.68	0.71	0.75	0.74	0.92	0.031	<0.001	0.344	<0.001	0.683
Bone ash, %[6]	45.1	44.6	51.8	48.7	49.2	50.0	51.5	52.2	0.890	<0.001	0.001	<0.001	0.066

[1] A total of 320 nursery pigs (DNA 241 × 600, initially 10.4 kg body weight (BW)) were used in a 21-day growth study with 5 pigs per pen and 8 pens per treatment.

[2] ADG = average daily gain; ADFI = average daily feed intake; G:F = gain-to-feed ratio.

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

[4] Smizyme TS G5 2,500, Origination, LLC., Maplewood, MN.

[5] SEM = standard error of the mean.

[6] One pig per pen (8 pens per treatment) was euthanized and fibulas were collected to determine bone ash weight and percentage bone ash. A total of 64 fibulas (one from each animal) were autoclaved for 1 h and then placed in Soxhlet extractors containing petroleum ether for 7 d as a means of removing water and fat. They were then dried at 105°C for 24 h, and ashed at 600°C for 24 h.

Table 2-6 Calculated aP release values based on different response criteria, Exp. 1[1,2]

	Phytase, FTU/kg[3]						Probability, *P* <	
Item	150	250	500	750	1,000	SEM[4]	Linear	Quadratic
ADG	0.031	0.052	0.094	0.109	0.139	0.019	<0.001	0.184
G:F	0.098	0.058	0.117	0.148	0.166	0.022	<0.001	0.096
Bone ash weight[5]	0.066	0.078	0.104	0.096	0.191	0.017	<0.001	0.847
Percentage bone ash[5]	0.095	0.102	0.114	0.142	0.149	0.014	<0.001	0.028

[1]The marginal intake of available P (aP) per day was calculated for each pen using the equation: dietary aP% minus 0.12% (the aP in the basal diet) multiplied by average daily feed intake. A standard curve was then developed for each response criteria using the marginal aP release as the predictor variable. The equation for the standard curve was used to calculate aP release from each pen fed the different phytase dosages based on the observed value for each response criteria.

[2] ADG = average daily gain; G:F = gain-to-feed ratio.

[3] Smizyme TS G5 2,500 (Origination, LLC., Maplewood, MN).

[4] SEM = standard error of the mean.

[5] One pig per pen (8 pens per treatment) was euthanized and fibulas were collected to determine bone ash weight and percentage bone ash. A total of 64 fibulas (one from each animal) were autoclaved for 1 h and then placed in Soxhlet extractors containing petroleum ether for 7 d as a means of removing water and fat. They were then dried at 105°C for 24 h, and ashed at 600°C for 24 h.

Table 2-7 Effects of increasing aP from inorganic P or Smizyme TS G5 2,500 phytase on nursery pig growth performance and bone ash values, Exp. 2[1,2]

	Inorganic P, % aP[3]			Phytase, FTU/kg[4]					Inorganic P		Phytase	
Item	0.11%	0.19%	0.27%	250	500	1,000	1,500	SEM[5]	Linear	Quadratic	Linear	Quadratic
BW, kg												
d 0	10.4	10.3	10.4	10.3	10.3	10.4	10.3	0.15	0.952	0.252	0.963	0.768
d 21	20.6	21.9	23.4	21.5	22.4	22.6	22.9	0.34	<0.001	0.881	<0.001	<0.001
d 0 to 21												
ADG, g	485	553	620	532	574	581	600	10.8	<0.001	0.957	<0.001	<0.001
ADFI, g	810	874	937	843	888	910	914	20.0	<0.001	0.979	<0.001	0.031
G:F, g/kg	600	633	664	631	646	639	658	7.2	<0.001	0.853	<0.001	0.045
Bone ash, g[6]	0.60	0.70	0.87	0.69	0.74	0.85	0.87	0.032	<0.001	0.449	<0.001	0.061
Bone ash, %[6]	47.3	50.1	53.5	50.4	50.5	52.5	52.9	0.694	<0.001	0.696	<0.001	0.035

[1] A total of 280 nursery pigs (DNA 241 × 600, initially 10.3 kg body weight (BW)) were used with 5 pigs per pen and 8 pens per treatment.

[2] ADG = average daily gain; ADFI = average daily feed intake; G:F = gain-to-feed ratio.

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

[4] Smizyme TS G5 2,500, Origination, LLC., Maplewood, MN.

[5] SEM = standard error of the mean.

[6] One pig per pen (8 pens per treatment) was euthanized and fibulas were collected to determine bone ash weight and percentage bone ash. A total of 56 fibulas (one from each animal) were autoclaved for 1 h and then placed in Soxhlet extractors containing petroleum ether for 7 d as a means of removing water and fat. They were then dried at 105°C for 24 h, and ashed at 600°C for 24 h.

Table 2-8 Calculated aP release values based on different response criteria, Exp. 2[1,2]

	Phytase, FTU/kg[3]					Probability, *P* <	
Item	250	500	1,000	1,500	SEM[4]	Linear	Quadratic
ADG	0.057	0.107	0.112	0.136	0.013	<0.001	<0.001
G:F	0.083	0.123	0.100	0.154	0.022	<0.001	0.066
Bone ash weight[5]	0.060	0.094	0.165	0.173	0.023	<0.001	0.059
Percentage bone ash[5]	0.088	0.091	0.143	0.152	0.024	<0.001	0.055

[1]The marginal intake of available P (aP) per day was calculated for each pen using the equation: dietary aP% minus 0.11% (the aP in the basal diet) multiplied by average daily feed intake. A standard curve was then developed for each response criteria using the marginal aP release as the predictor variable. The equation for the standard curve was used to calculate aP release from each pen fed the different phytase dosages based on the observed value for each response criteria.

[2] ADG = average daily gain; G:F = gain-to-feed ratio.

[3] Smizyme TS G5 2,500 (Origination, LLC., Maplewood, MN).

[4] SEM = standard error of the mean.

[5] One pig per pen (8 pens per treatment) was euthanized and fibulas were collected to determine bone ash weight and percentage bone ash. A total of 56 fibulas (one from each animal) were autoclaved for 1 h and then placed in Soxhlet extractors containing petroleum ether for 7 d as a means of removing water and fat. They were then dried at 105°C for 24 h, and ashed at 600°C for 24 h.

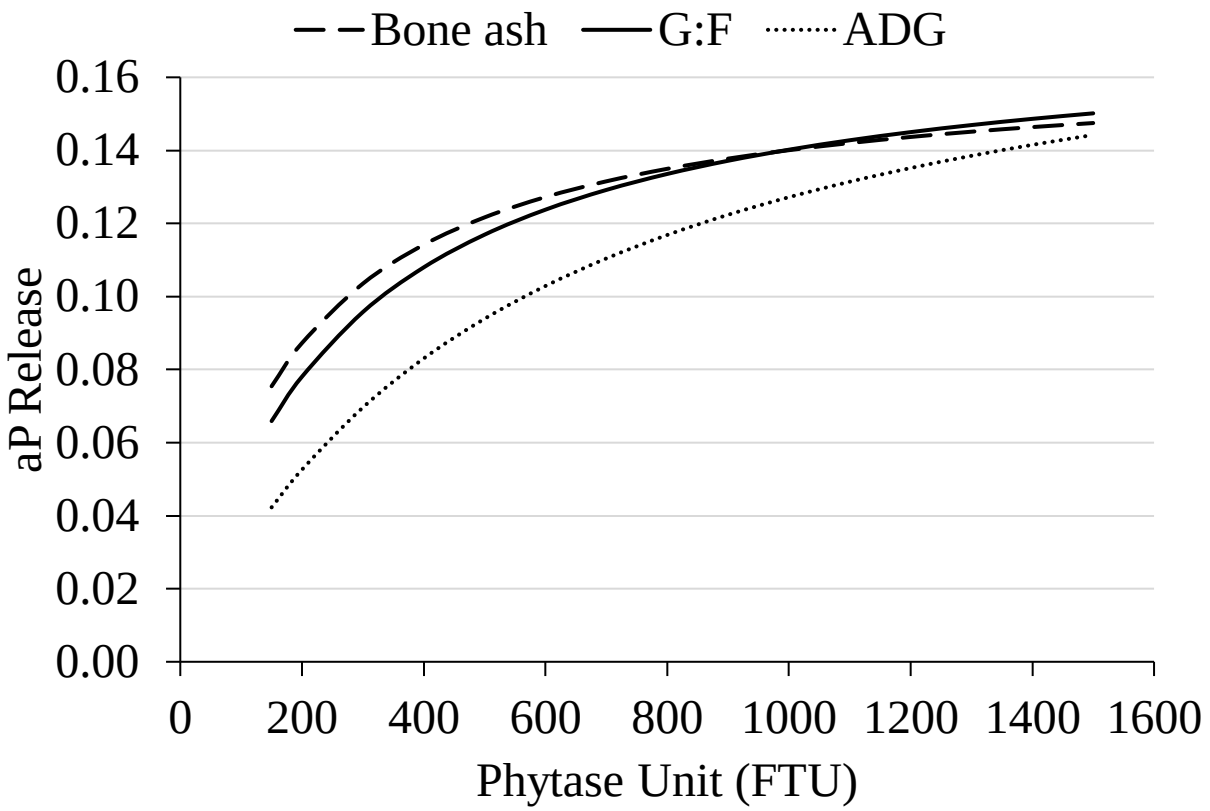

Figure 2-1 Available P release curve for Smizyme TS G5 2,500 phytase as predicted by ADG, G:F, and percentage bone ash.

Chapter 3 - Assessing the current phytase release values for calcium, phosphorus, amino acids and energy in diets for growing-finishing pigs

Abstract

Two experiments were conducted to determine the effects of feeding 1,500 phytase units (FYT/kg; Ronozyme HiPhos 2,500; DSM Nutritional Products, Inc., Parsippany, NJ) when credited with its corresponding nutrient release values to growing-finishing pigs. The assumed phytase release values were 0.146% standardized total tract digestible (STTD) P, 0.102% STTD Ca, 8.6 kcal/kg of NE, and 0.0217, 0.0003, 0.0086, 0.0224, 0.0056, 0.0122, and 0.0163% standardized ileal digestible Lys, Met, Met+Cys, Thr, Trp, Ile, and Val, respectively. In Exp. 1, 1,215 pigs (PIC 359 × Camborough, initially 28.0 ± 0.46 kg) were used. Pens were assigned to 1 of 3 dietary treatments with 27 pigs per pen and 15 pens per treatment. Experimental diets consisted of a control with no added phytase or diets with 1,500 FYT fed either in the grower period (d 0 to 57) then switched to the control diet until market or fed throughout the entire study (d 0 to market). Diets containing added phytase were adjusted based on the supplier-provided expected nutrient release values. During the grower period, pigs fed the control diet with no added phytase had increased ($P < 0.05$) ADG and G:F compared with pigs fed added phytase. Overall, pigs fed either the control or phytase only in the grower period had increased ($P < 0.05$) ADG and G:F compared to pigs fed phytase until market. In Exp. 2, 2,268 pigs (PIC 337 × 1050, initially 28.5 ± 1.96 kg) were used. There were 6 dietary treatments with 27 pigs per pen and 14 pens per treatment. Experimental diets consisted of a control with no added phytase or 5 diets with 1,500 FYT assuming nutrient release values for Ca and P; Ca, P, and AA; Ca, P, AA, and

half of the suggested NE; Ca, P, AA, and full NE; or no nutrient release. Overall, there was no evidence for difference in ADG or ADFI among treatments; however, pigs fed the diet containing 1,500 FYT assuming no nutrient release had improved ($P < 0.05$) G:F compared to pigs fed diets containing 1,500 FYT assuming either Ca and P or Ca, P, AA, and full NE release, with others intermediate. In summary, pigs fed phytase added diets accounting for full nutrient release values in both experiments had the poorest performance. This suggests that using all of the nutrient release values attributed to this source of phytase was too aggressive and resulted in lower nutrient concentrations than needed to optimize performance.

Key Words: phytase, growing-finishing pigs, super-dosing

Introduction

Approximately 60 to 80% of phosphorus (P) in feedstuffs of plant origin is stored in phytic acid, typically in the form of phytate (Eeckhout et al., 1994). Pigs poorly utilize phytate-bound-phosphorus because they lack sufficient endogenous phytase. Therefore, phytate is commonly known as an antinutritional factor in swine diets, as it reduces the digestibility of P (Swick et al., 1992). Adding exogenous phytase to swine diets has the ability to dephosphorylate phytate in a stepwise manner and liberate P. In response, P availability to the pig increases and the need for dietary inclusion of inorganic P decreases (Selle and Ravindran, 2008).

Previous data has demonstrated that phytase, when provided above conventional levels (500 to 1,000 FYT/kg) in the diet (Adeola et al., 2011), can also exert extra-phosphoric effects, improving the digestibility of nutrients other than P (Cowieson et al., 2011). When phytate reaches the digestive tract, it takes on an electro-negative charge which allows phytate to bind and form stable insoluble complexes with minerals, AA, and lipids, decreasing their absorption

(Woyengo et al., 2013). High levels of added phytase has been observed to enhance the digestibility and absorption of these nutrients through a dissociation of such complexes (Selle and Ravindran, 2008; Adeola et al., 2011).

If added phytase indeed releases other nutrients in addition to P, crediting phytase with nutrient release values should allow nutritionists to decrease diet costs by reducing the amount of inorganic P and both feed-grade and intact AA used in formulation. However, there is limited data to confirm that the use of current nutrient release values, other than P, will maintain growth performance. Therefore, the objective of these experiments was to investigate the effects of feeding diets containing 1,500 FYT of phytase when credited with additional nutrient release values above Ca and P in diets for growing-finishing pigs.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved the protocols used in these experiments. Each trial was conducted at a commercial research wean-to-finish site in southwestern Minnesota. Barns were naturally ventilated and double-curtain-sided. Pens had completely slatted flooring and deep pits. Each pen was equipped with a 5-hole stainless steel feeder and cup waterer to allow ad libitum access to feed and water. Additionally, the facility was equipped with an automated feeding system (FeedPro; Feedlogic Corp., Willmar, MN) capable of measuring and recording daily feed additions to individual pens.

Experiment 1

A total of 1,215 barrows and gilts (PIC 359 × Camborough, initially 28 kg) were used in a 126-d growth trial. At placement (15.9 kg), pigs were fed a common diet containing 0.66% total Ca and 0.42% STTD P until the initiation of the trial. On d 0 of the study, pens of mixed gender pigs were blocked by body weight (BW) and randomly assigned to 1 of 3 dietary

treatments in a randomized complete block design, with BW used as a blocking factor. There were 27 pigs per pen and 15 replicate pens per treatment, with gender balanced throughout. The 3 dietary treatments consisted of a control with no added phytase, or 2 treatments with 1,500 FYT of phytase fed either in the grower period (d 0 to 57) then switched to control diet until market, or phytase fed throughout the entire grower and finisher period (d 0 to market).

The experimental diets were corn-soybean-meal–distiller's dried grains with solubles (DDGS)-based and fed in 4 different phases (Tables 3-1 and 3-2). Phase 1 diets were fed from d 0 to 29 (28 to 51 kg); phase 2 diets were fed from d 29 to 57 (51 to 74 kg); phase 3 diets were fed from d 57 to 85 (74 to 99 kg); and phase 4 diets were fed from d 85 to 126 (99 to 135 kg). Ronozyme HiPhos 2,500 (DSM Nutritional Products, Inc., Parsippany, NJ) was included in the phytase-containing diets with assumed release values of 0.146% standardized total tract digestible (STTD) P, 0.102% STTD Ca, 10.9 kcal/kg of ME, 8.6 kcal/kg of NE, and 0.0217, 0.0003, 0.0086, 0.0224, 0.0056, 0.0122, and 0.0163% standardized ileal digestible Lys, Met, Met +Cys, Thr, Trp, Ile, and Val, respectively. Phytase nutrient release values were provided by the manufacturer. Ingredient nutrient values were obtained from laboratory results of a previous trial in the same facility for corn, soybean meal, and vitamin trace mineral mix or from NRC (2012) for monocalcium P and calcium carbonate. Digestibility coefficients for P and AA were obtained from NRC (2012) and the digestibility coefficients for Ca were obtained from the literature (González-Vega et al., 2013, 2015; Stein, 2016). The diets were formulated to contain adequate STTD P across the dietary treatments in all phases based on the estimated requirement previously determined in the research facility (Vier et al., 2019). All diets were balanced for a STTD Ca:STTD P of 1.55:1.

Pens of pigs were weighed, and feed disappearance was recorded approximately every 14 d to determine ADG, ADFI, and G:F. On d 99, the 2 heaviest pigs in each pen were selected, weighed, and marketed according to standard farm procedures. On d 126, final pen weights were taken, and pigs were individually tattooed with the specific pen identity on the shoulder to allow for carcass measurements to be recorded on a pen basis. These pigs were transported to a commercial packing plant in southwestern Minnesota (JBS Swift and Company, Worthington, MN) for processing and carcass data collection. Carcass measurements included hot carcass weight (HCW), loin depth, backfat depth, and percentage lean. Loin depth and backfat depth were measured with an optical probe inserted between the third and fourth last rib (counting from the ham end of the carcass) at a distance approximately 7 cm from the dorsal midline. Percentage carcass yield was calculated by dividing the average pen HCW by the average final live weight at the farm.

Experiment 2

A total of 2,268 barrows and gilts (PIC 337 × 1050, initially 28.5 kg) were used from 2 groups of pigs (1,134 in group 1 and 1,134 in group 2) in a 55-d growth trial. At placement (23.0 and 16.8 kg, respectively), pigs were fed a common diet containing 0.66% total Ca and 0.42% STTD P until the initiation of the trial. On d 0 of the study, pens of mixed gender pigs were blocked by BW and randomly allotted to 1 of 6 dietary treatments with 27 pigs per pen and 14 replicate pens per treatment (7 pens per group), with gender balanced throughout. Diets were fed in 2 different phases. Phase 1 diets were fed from d 0 to 29 (28.5 to 51.2 kg) and phase 2 diets were fed from d 29 to 55 (51.2 to 72.4 kg). Treatments consisted of a control diet with inorganic P from monocalcium P and no added phytase, or 5 diets with 1,500 FYT/kg assuming the same release values as those used in Exp. 1, but only accounting release values for Ca and P (CaP);

Ca, P, and AA (CaPAA); Ca, P, AA, and half of the suggested net energy (CaPAA+half NE); Ca, P, AA, and full NE (CaPAA+full NE); or no nutrient release to form the 5 treatment diets (Table 3-3 and 3-4).

The experimental diets were corn-soybean-meal–DDGS-based with Ronozyme HiPhos 2,500 as the phytase source. The same ingredient loading values and digestibility coefficients for P and Ca in Exp. 1 were used. All diets were formulated to contain adequate STTD P across the dietary treatments in both phases based on the estimated requirement previously determined in the research facility (Vier et al., 2019). The STTD Ca:STTD P ratio for all diets was 1.60:1. Pens of pigs were weighed, and feed disappearance was recorded approximately every 14 d to determine ADG, ADFI, and G:F.

Feed Sampling

Experimental diets for both experiments were manufactured at the New Horizon Farms Feed Mill (Pipestone, MN) and fed in meal form. Representative samples of treatment diets were taken from 6 feeders per dietary treatment 3 d after the beginning and 3 d before the end of each phase and stored at -20°C. After blending, subsamples were analyzed in duplicates for dry matter (DM; AOAC 934.01), crude protein (CP; AOAC 990.03, 2006), Ca (AOAC 985.01, 2006), and P (AOAC 985.01, 2006) and average values were reported (Table 3-1 through 3-4; Ward Laboratories, Inc., Kearney, NE).

Statistical Analysis

In Exp. 1, data were analyzed as a randomized complete block design, with pen considered the experimental unit and BW the blocking factor and evaluated as a random effect. Although fed the same diet in the grower phase, pigs were initially allotted to 3 individual treatments. The study was structured as a one-way treatment structure with dietary treatment as

the factor level. Because carcass characteristics were recorded on an individual pig basis, a random effect of block by treatment was used to identify the pen as the experimental unit. Pairwise comparisons were conducted, and means were reported as least-square means. Statistical models were fitted using GLIMMIX procedure of SAS (Version 9.3, SAS Institute Inc., Cary, NC). Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 \leq P \leq 0.10$.

In Exp. 2, similar to Exp. 1 pens were assigned to the 6 treatments based on average pig BW as a blocking factor. Therefore, data were analyzed as a randomized complete block design for one-way ANOVA with pen as the experimental unit in a similar manner as Exp. 1 with treatment as fixed effect, and weight block as random effect. Pairwise comparisons were conducted, and means were reported as least-square means with a Tukey-Kamer adjustment. The pairwise comparisons were only evaluated if the overall treatment F-test was significant ($P \leq 0.05$). For the analysis, the lmer function from the lme4 package in R (version 3.5.1 (2018-07-02)) was used, with pen considered the experimental unit. Results were considered significant at $P \leq 0.05$ and marginally significant at $0.05 \leq P \leq 0.10$.

Results

Experiment 1

During the grower period, which corresponds to phases 1 and 2 (d 0 to 57), pigs fed the control diet with no added phytase had increased ($P < 0.05$) ADG compared to pigs fed phytase only in the grower period, with pigs fed phytase in grower and finisher intermediate (Table 3-5). There was no evidence ($P > 0.10$) for difference in ADFI between treatments. Pigs fed the phytase-containing diets had decreased ($P < 0.01$) G:F compared to pigs fed the control.

During the finisher period, which corresponds to phases 3 and 4 (d 58 to 126), ADG was similar for pigs fed either the control or added phytase in the grower period. The ADG for these treatments was greater ($P < 0.05$) than that of pigs fed phytase in the grower and finisher. There was no evidence ($P > 0.10$) for difference in ADFI between treatments. As a result, pigs fed either the control or phytase in the grower period had increased G:F ($P < 0.05$) compared to pigs fed phytase in the grower and finisher. Overall (d 0 to 126), pigs fed either the control or phytase only in the grower period had increased ($P <0.05$) ADG and G:F compared to pigs fed phytase in the grower and finisher, with no evidence ($P > 0.10$) for difference observed in ADFI between treatments.

A marginally significant ($P < 0.10$) treatment effect on HCW was observed with pigs fed either the control or phytase only in the grower period having heavier HCW compared to pigs fed phytase throughout grower and finisher. No evidence for differences ($P > 0.10$) were observed for carcass yield, backfat, fat-free lean and loin depth characteristics.

Experiment 2

Overall (d 0 to 55), there was no evidence for difference observed in ADG or ADFI between treatments (Table 3-6). However, pigs fed the diet containing 1,500 FYT/kg assuming no nutrient release had increased ($P < 0.05$) G:F compared to pigs fed diets containing 1,500 FYT/kg assuming either CaP or CaPAA+full NE release, with others intermediate.

Discussion

Phytate is a known antinutritional factor that decreases P availability. It has previously been demonstrated that the enzyme phytase, when supplemented in swine diets, can effectively dephosphorylate the phytic acid present in cereal grains and oilseeds, improving the P digestibility (Selle et al., 2012). High levels of phytase have also been associated with the release

of minerals, AA, and energy (Coweison et al., 2011). Phytate, while generally unreactive in feedstuffs, takes on an electro-negative charge once exposed to the acidic conditions of the stomach (Adeola et al., 2011). The reduced nutrient digestibility and subsequent efficiency of monogastric animals fed diets high in phytic acid is a result of insoluble binary and tertiary complexes that form with proteins, minerals, and their associated digestive enzymes (Selle et al., 2012). The chelation of these nutrients to phytate depends on the isoelectric point of the protein or mineral in relation to the electro-negative charge and concentration of phytate in the diet (Selle et al., 2012). The formation of insoluble phytate complexes leads to hypersecretion of pepsin, HCl, and mucin, increasing nutrient flow into the lumen, therefore reducing the absorption of nutrients from the small intestine and increasing endogenous losses (Woyengo et al., 2013).

Several studies have investigated the extra-phosphoric potential of phytase; however, results have been inconsistent regarding its effects on the digestibility of AA. Improved apparent ileal digestibility (AID) of some AA in response to dietary phytase inclusion has been reported (Kemme et al., 1999; Adedokun et al., 2015). Zeng et al. (2016) observed that when supplementing diets with 20,000 FTU/kg, CP and AA utilization improved. Conversely, other studies that used more conventional levels of phytase (1,000 – 4,000 FTU/kg) did not observe the same results (Traylor et al., 2001; She et al., 2018). A meta-analysis conducted by Cowieson et al. (2017) indicated that the effect of phytase on AID was more evident when diets were intrinsically low in digestible AA. Furthermore, the AID of AA tended to be similar from 250 to 2,000 FYT/kg, indicating that phytase dose may have less application in swine diets when it comes to AA digestibility (Coweison et al., 2017; Zouaoui et al., 2018). Nonetheless, there is

evidence that nutrient release values credited to phytase are linked to the hydrolysis of phytate; therefore, phytases with similar P release values should have similar extra-phosphoric effects.

Limited research has been conducted regarding the effects of phytase on energy digestibility, but it appears the implication may be similar to that of AA. She et al. (2018) evaluated increasing levels of phytase on the apparent total tract digestibility (ATTD) of nutrients in growing pigs. When super-dosing phytase up to 4,000 FTU/kg in P deficient diets no phytase effect on ATTD of AA or gross energy was observed. This observation confirms earlier reports that the energy effects of phytase are limited to improved protein digestibility (Selle and Ravindran, 2008). Conversely, Adedokum et al. (2015) demonstrated that ATTD of AA and energy improved as phytase in the diet increased up to the highest dose (2,000 FTU/kg). Similarly, when calculating performance on a carcass basis, Holloway et al. (2018) reported improved energy efficiency in pigs fed graded levels of phytase from 1,000 to 2,500 FTU/kg in diets with less than optimal lysine and energy. These studies indicate that when diets are nutrient deficient, there is the potential for phytase to improve energy digestibility and subsequent utilization.

The supplementation of high levels of phytase have resulted in inconsistent results in growing and finishing pig growth performance (Flohr et al., 2014; Holloway et at., 2018; Santos et al., 2014). Even fewer studies have investigated the effects on carcass characteristics (Flohr et al., 2014; Holloway et at., 2018). Results from Exp. 1 suggest that when full nutrient release values were utilized, growing-finishing pigs fed diets containing 1,500 FYT of phytase until market had poorer performance compared to pigs fed diets without added phytase. Moreover, pigs that were withdrawn from the phytase-containing diets after the grower period and switched to the control diets without phytase until market were able to recover the loss in performance.

These findings suggest that the full nutrient release values attributed to the phytase were greater than what was actually released and consequently resulted in poorer performance.

In Exp. 1, nutrient release values were assigned not only to digestible P, but also to digestible Ca, NE, and AA. Based on the results observed, we hypothesized that the detrimental effects in performance of pigs supplemented with phytase may be due to an overestimation of the release values for NE or AA. Therefore, Exp. 2 was conducted with growing pigs, approximately 27 to 75 kg, to confirm this response. Pigs fed either the control diet with inorganic P from monocalcium P or any of the phytase-containing diets should have had similar performance, perhaps with the exception of pigs fed the diet formulated to contain 1,500 FYT/kg assuming no release values. Pigs fed full nutrient release values had the lowest G:F while pigs fed diets assuming Ca and P in addition to AA and half of the suggested NE release had G:F comparable to the control. Pigs fed diets assuming no release values were most efficient in converting feed into BW, indicating that high phytase levels added on top of nutritionally adequate diets provides additional nutrient release.

In summary, these studies provide valuable insight on the nutrient release values of phytase (Ronozyme HiPhos 2,500). These are the first studies, to our knowledge, that have been conducted to validate full nutrient release values associated with phytase in a commercial setting. In Exp. 1, growing-finishing pigs fed diets containing high levels of phytase with full nutrient release values until market had decreased ADG, G:F, and HCW compared with those not fed any phytase. Removing the phytase at the end of the grower period was sufficient for pigs to recover any loss in performance and to achieve similar overall growth rates and feed efficiency as the pigs fed diets without added phytase. A similar feed efficiency response was observed in Exp. 2 with growing pigs, confirming the hypothesis that full nutrient release values attributed to

phytase, especially for NE, were too aggressive and resulted in diets contributing less nutrients than needed to optimize performance.

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Table 3-1 Composition of Exp. 1 diets, Phases 1 and 2 (as-fed basis)[1,2]

	Phase 1		Phase 2	
Item Phytase, FYT/kg:	0	1,500	0	1,500
Ingredient, %				
Corn	60.92	63.54	68.59	71.23
Soybean meal	24.57	24.16	17.13	16.72
Corn DDGS[3]	10.00	10.00	10.00	10.00
Beef tallow	1.50	-	1.50	-
Monocalcium phosphate	0.90	0.15	0.75	-
Limestone	1.08	1.11	1.00	1.03
Sodium chloride	0.35	0.35	0.35	0.35
L-lysine HCl	0.37	0.35	0.39	0.37
DL-methionine	0.06	0.05	0.03	0.02
L-threonine	0.09	0.07	0.09	0.06
L-tryptophan	0.02	0.01	0.03	0.02
Phytase[4]	-	0.06	-	0.06
Vitamin and trace mineral premix[5]	0.15	0.15	0.15	0.15
Total	100.00	100.00	100.00	100.00
Calculated analysis				
SID[6] amino acids, %				
Lysine	1.10	1.10	0.93	0.93
Isoleucine:lysine	63	63	61	62
Leucine:lysine	142	143	149	150
Methionine:lysine	31	30	30	29
Methionine and cysteine:lysine	56	56	56	56
Threonine:lysine	62	62	62	62
Tryptophan:lysine	18.8	18.8	18.9	18.9
Valine:lysine	70	70	70	70
Total lysine, %	1.25	1.23	1.06	1.04
Net energy, kcal/kg	2,495	2,495	2,545	2,545
SID lysine:NE, g/Mcal	4.40	4.40	3.65	3.65
Crude protein, %	20.0	20.0	17.1	17.1
Calcium, %	0.72	0.60	0.62	0.51
STTD Ca[7], %	0.57	0.57	0.49	0.50
Phosphorus, %	0.62	0.46	0.55	0.39
STTD P[8], %	0.37	0.37	0.32	0.32
Available phosphorus, %	0.32	0.34	0.28	0.30
STTD Ca:STTD P	1.55	1.55	1.55	1.55
Chemical analysis[9]				
Dry matter	89.16	88.94	88.38	88.12
Crude protein	20.08	19.85	19.78	18.00
Calcium	0.73	0.42	0.63	0.63
Phosphorus	0.55	0.43	0.53	0.42

[1]Phase 1 diets were fed from d 0 to 29 (28 to 51 kg) and phase 2 diets were fed from d 29 to 57 (51 to 74 kg).

[2]Dietary treatments consisted of a control with no added phytase, or 2 treatments with 1,500 FYT fed either in the grower period (d 0 to 57) then switched to control diet until market, or phytase fed throughout the entire grower and finisher period (d 0 to market).

[3]DDGS = distiller's dried grains with solubles.

[4]Ronozyme HiPhos 2,500 phytase (DSM Nutritional Products, Inc., Parsippany, NJ) was included at 1,500 FYT/kg with assumed release values of 0.146% STTD P, 0.166% available P, 0.102% STTD Ca, 10.9 kcal/kg of ME, 8.6 kcal/kg of NE; and 0.0217, 0.0003, 0.0086, 0.0224, 0.0056, 0.0122, and 0.0163% digestible Lys, Met, Met + Cys, Thr, Trp, Ile, and Val, respectively.

[5]Provided per kg of diet: 110 ppm Zn, 110 ppm Fe, 33 ppm Mn, 17 ppm Cu, 0.33 ppm I, 0.30 ppm Se, 5290 IU vitamin A, 1322 IU vitamin D, 26 IU vitamin E, 2.6 mg vitamin K, 49.6 mg niacin, 16.5 mg pantothenic acid, 5.0 mg riboflavin, and 0.02 mg vitamin B12.

[6]Standardized ileal digestibility.

[7]Standardized total tract digestible calcium.

[8]Standardized total tract digestible phosphorus.

[9]Representative samples of treatment diets were taken from 6 feeders per dietary treatment 3 d after the beginning and 3 d before the end of the phase and stored at -4°F. After blending, subsamples were submitted to Ward Laboratories, Inc. (Kearney, NE) and were analyzed for dry matter, crude protein, calcium, and phosphorus.

Table 3-2 Composition of Exp. 1 diets, Phases 3 and 4 (as-fed basis)[1,2]

	Phase 3		Phase 4	
Item Phytase, FYT/kg:	0	1,500	0	1,500
Ingredient, %				
Corn	73.86	76.43	83.23	85.65
Soybean meal	12.06	11.50	12.89	12.64
Corn DDGS[3]	10.00	10.00	-	-
Beef tallow	1.45	-	1.45	-
Monocalcium phosphate	0.65	-	0.75	-
Limestone	0.95	1.04	0.73	0.77
Sodium chloride	0.35	0.35	0.35	0.35
L-lysine HCl	0.39	0.38	0.30	0.28
DL-methionine	0.01	-	0.02	0.01
L-threonine	0.10	0.08	0.10	0.08
L-tryptophan	0.03	0.03	0.03	0.02
Phytase[4]	-	0.06	-	0.06
Vitamin and trace mineral premix[5]	0.15	0.15	0.15	0.15
Total	100.00	100.00	100.00	100.00
Calculated analysis				
SID[6] amino acids, %				
Lysine	0.81	0.81	0.73	0.73
Isoleucine:lysine	59	60	60	62
Leucine:lysine	157	158	151	153
Methionine:lysine	29	28	30	29
Methionine and cysteine:lysine	57	57	58	58
Threonine:lysine	64	64	66	66
Tryptophan:lysine	18.9	18.7	19.5	19.5
Valine:lysine	70	70	70	70
Total lysine, %	0.93	0.91	0.83	0.81
Net energy, kcal/kg	2,576	2,576	2,603	2,603
SID lysine:NE, g/Mcal	3.14	3.14	2.80	2.80
Crude protein, %	15.1	15.0	13.4	13.4
Calcium, %	0.56	0.48	0.51	0.39
STTD Ca[7], %	0.44	0.48	0.40	0.41
Phosphorus, %	0.50	0.37	0.47	0.31
STTD P[8], %	0.29	0.31	0.26	0.27
Available phosphorus, %	0.25	0.29	0.21	0.23
STTD Ca:STTD P	1.55	1.55	1.55	1.55
Chemical analysis[9]				
Dry matter	88.36	88.30	88.14	88.26
Crude protein	14.60	15.13	13.78	14.00
Calcium	0.63	0.31	0.50	0.41
Phosphorus	0.45	0.34	0.46	0.31

[1]Phase 3 diets were fed from d 57 to 85 (74 to 99 kg) and phase 4 diets were fed from d 85 to 126 (99 to 135 kg).

[2]Dietary treatments consisted of a control with no added phytase, or 2 treatments with 1,500 FYT fed either in the grower period (d 0 to 57) then switched to control diet until market, or phytase fed throughout the entire grower and finisher period (d 0 to market).

[3]DDGS = distiller's dried grains with solubles.

[4]Ronozyme HiPhos 2,500 phytase (DSM Nutritional Products, Inc., Parsippany, NJ) was included at 1,500 FYT/kg with assumed release values of 0.146% STTD P, 0.166% available P, 0.102% STTD Ca, 10.9 kcal/kg of ME, 8.6 kcal/kg of NE; and 0.0217, 0.0003, 0.0086, 0.0224, 0.0056, 0.0122, and 0.0163% digestible Lys, Met, Met + Cys, Thr, Trp, Ile, and Val, respectively.

[5]Provided per kg of diet: 110 ppm Zn, 110 ppm Fe, 33 ppm Mn, 17 ppm Cu, 0.33 ppm I, 0.30 ppm Se, 5290 IU vitamin A, 1322 IU vitamin D, 26 IU vitamin E, 2.6 mg vitamin K, 49.6 mg niacin, 16.5 mg pantothenic acid, 5.0 mg riboflavin, and 0.02 mg vitamin B12.

[6]Standardized ileal digestibility.

[7]Standardized total tract digestible calcium.

[8]Standardized total tract digestible phosphorus.

[9]Representative samples of treatment diets were taken from 6 feeders per dietary treatment 3 d after the beginning and 3 d before the end of the phase and stored at -4°F. After blending, subsamples were submitted to Ward Laboratories, Inc. (Kearney, NE) and were analyzed for dry matter, crude protein, calcium, and phosphorus.

Table 3-3 Composition of Exp. 2 diets, Phase 1 (as-fed basis)[1,2]

	No Phytase	1500 FYT/kg Phytase				
Ingredient, %	Control	CaP	CaPAA	CaPAA + half NE	CaPAA + full NE	None
Corn	63.00	64.13	64.48	64.88	65.61	62.88
Soybean meal	22.15	22.07	21.82	21.79	21.74	22.16
Corn DDGS[3]	10.00	10.00	10.00	10.00	10.00	10.00
Beef tallow	2.00	1.60	1.55	1.20	0.50	2.05
Monocalcium P	0.75	---	---	---	---	0.75
Limestone	1.08	1.13	1.13	1.10	1.13	1.08
Sodium chloride	0.35	0.35	0.35	0.35	0.35	0.35
L-Lysine-HCl	0.37	0.37	0.35	0.35	0.35	0.37
DL-Methionine	0.05	0.04	0.04	0.04	0.04	0.05
L-Threonine	0.09	0.09	0.07	0.07	0.07	0.09
L-Tryptophan	0.02	0.02	0.01	0.01	0.01	0.02
Vitamin and trace mineral premix [4]	0.15	0.15	0.15	0.15	0.15	0.15
Phytase[5]	---	0.06	0.06	0.06	0.06	0.06
Total	100	100	100	100	100	100
Calculated analysis						
SID[6] amino acids						
Lysine, %	1.04	1.04	1.04	1.04	1.04	1.04
Isoleucine:lysine	62	62	63	63	63	62
Leucine:lysine	145	145	145	145	145	144
Methionine:lysine	31	30	30	30	30	31
Methionine and cysteine:lysine	56	56	56	56	57	56
Threonine:lysine	62	62	62	62	62	62
Tryptophan:lysine	18.6	18.6	18.5	18.5	18.5	18.6
Valine:lysine	70	70	70	70	70	70
Histidine:lysine	42	42	42	42	42	42
Total lysine, %	1.18	1.18	1.16	1.16	1.16	1.18
Metabolizable energy, kcal/kg	3,366	3,368	3,366	3,368	3,368	3,366
Net energy, kcal/kg	2,534	2,534	2,534	2,534	2,534	2,534
SID lysine:NE, g/Mcal	4.09	4.09	4.10	4.09	4.10	4.09
Crude protein, %	19.0	19.1	19.0	19.0	19.0	19.0
Calcium, %	0.68	0.57	0.57	0.56	0.57	0.68
STTD Ca,[7] %	0.53	0.54	0.54	0.53	0.54	0.53
Phosphorus, %	0.57	0.41	0.41	0.41	0.41	0.57
STTD P,[8] %	0.33	0.34	0.33	0.33	0.34	0.33
Available phosphorus, %	0.29	0.29	0.29	0.29	0.29	0.29
STTD Ca:STTD P	1.60	1.60	1.60	1.60	1.60	1.60
Chemical analysis[9]						
Dry matter	88.74	88.11	88.94	87.89	88.52	87.98

Crude protein	18.80	17.20	17.50	18.95	18.45	18.50
Calcium	0.61	0.62	0.62	0.60	0.61	0.66
Phosphorus	0.41	0.34	0.38	0.31	0.36	0.51

[1]Diets were fed for 26 d from approximately 29 to 51 kg.

[2]Dietary treatments consisted of a control with no phytase, or 5 diets with 1,500 phytase units assuming supplier-provided nutrient release values for Ca and P (CaP), Ca, P, and AA (CaPAA), Ca, P, AA, and half of the suggested net energy (CaPAA+halfNE), Ca, P, AA, and full NE (CaPAA+fullNE) and no nutrient release (None).

[3]DDGS = distiller's dried grains with solubles.

[4]Provided per kg of diet: 110 ppm Zn, 110 ppm Fe, 33 ppm Mn, 17 ppm Cu, 0.33 ppm I, 0.30 ppm Se, 5290 IU vitamin A, 1322 IU vitamin D, 26 IU vitamin E, 2.6 mg vitamin K, 49.6 mg niacin, 16.5 mg pantothenic acid, 5.0 mg riboflavin, and 0.02 mg vitamin B12.

[5]Ronozyme HiPhos 2,500 phytase (DSM Nutritional Products, Inc., Parsippany, NJ) was included at 1,500 FYT/kg with assumed release values of 0.146% STTD P, 0.166% available P, 0.102% STTD Ca, 10.9 kcal/kg of ME, 8.6 kcal/kg of NE; and 0.0217, 0.0003, 0.0086, 0.0224, 0.0056, 0.0122, and 0.0163% digestible Lys, Met, Met + Cys, Thr, Trp, Ile, and Val, respectively.

[6]Standardized ileal digestibility.

[7]Standardized total tract digestibility of calcium.

[8]Standardized total tract digestibility of phosphorus.

[9]Representative samples of treatment diets were taken from 6 feeders per dietary treatment 3 d after the beginning and 3 d before the end of the phase and stored at -4°F. After blending, subsamples were submitted to Ward Laboratories, Inc. (Kearney, NE) and were analyzed for dry matter, crude protein, calcium, and phosphorus.

Table 3-4 Composition of Exp. 2 diets, Phase 2 (as-fed basis)[1,2]

	No Phytase	1500 FYT/kg Phytase				
Item	Control	CaP	CaPAA	CaPAA + half NE	CaPAA + full NE	None
Ingredient, %						
Corn	69.54	70.79	71.09	71.55	72.23	69.42
Soybean meal	15.55	15.46	15.21	15.18	15.13	15.55
Corn DDGS[3]	10.00	10.00	10.00	10.00	10.00	10.00
Beef tallow	2.00	1.55	1.55	1.15	0.50	2.05
Monocalcium P	0.80	---	---	---	---	0.80
Limestone	1.10	1.13	1.13	1.11	1.13	1.10
Sodium chloride	0.35	0.35	0.35	0.35	0.35	0.35
L-Lysine-HCl	0.39	0.39	0.37	0.37	0.37	0.39
DL-Methionine	0.02	0.02	0.02	0.01	0.01	0.02
L-Threonine	0.09	0.09	0.07	0.06	0.06	0.09
L-Tryptophan	0.03	0.03	0.02	0.02	0.02	0.03
Vitamin and trace mineral premix[4]	0.15	0.15	0.15	0.15	0.15	0.15
Phytase[5]	---	0.06	0.06	0.06	0.06	0.06
Total	100	100	100	100	100	100
Calculated analysis						
SID[6] amino acids						
Lysine, %	0.89	0.89	0.89	0.89	0.89	0.89
Isoleucine:lysine	60	60	61	61	61	60
Leucine:lysine	151	152	152	152	152	151
Methionine:lysine	29	30	29	29	29	29
Methionine and cysteine:lysine	56	56	56	56	56	56
Threonine:lysine	62	62	62	62	62	62
Tryptophan:lysine	18.7	18.8	18.7	18.7	18.7	18.7
Valine:lysine	70	70	70	70	70	70
Histidine:lysine	42	42	42	42	42	42
Total lysine, %	1.02	1.02	0.99	1.00	1.00	1.02
Metabolizable energy, kcal/kg	3,370	3,373	3,373	3,370	3,375	3,370
Net energy, kcal/kg	2,572	2,572	2,572	2,572	2,572	2,572
SID lysine:NE, g/Mcal	3.45	3.45	3.45	3.45	3.45	3.45
Crude protein, %	16.4	16.5	16.3	16.4	16.4	16.4
Calcium, %	0.66	053	0.53	0.53	0.53	0.66
STTD Ca,[7] %	0.52	0.51	0.51	0.51	0.51	0.52
Phosphorus, %	0.55	0.38	0.38	0.38	0.38	0.55
STTD P,[8] %	0.32	0.32	0.32	0.32	0.32	0.32
Available phosphorus, %	0.29	0.28	0.28	0.28	0.28	0.29
Calcium:phosphorus	1.20	1.40	1.40	1.38	1.39	1.20
STTD Ca:STTD P	1.60	1.60	1.60	1.60	1.60	1.60

Chemical analysis[9]						
Dry matter	88.75	88.60	89.74	88.87	88.53	88.16
Crude protein	16.25	16.35	15.55	15.50	16.20	17.70
Calcium	0.74	0.62	0.53	0.62	0.57	0.75
Phosphorus	0.46	0.31	0.36	0.32	0.34	0.43

[1]Diets were fed for 26 d from approximately 51 to 72 kg.

[2]Dietary treatments consisted of a control with no phytase, or 5 diets with 1,500 phytase units assuming supplier-provided nutrient release values for Ca and P (CaP), Ca, P, and AA (CaPAA), Ca, P, AA, and half of the suggested net energy (CaPAA+halfNE), Ca, P, AA, and full NE (CaPAA+fullNE) and no nutrient release (None).

[3]DDGS = distiller's dried grains with solubles.

[4]Provided per kg of diet: 110 ppm Zn, 110 ppm Fe, 33 ppm Mn, 17 ppm Cu, 0.33 ppm I, 0.30 ppm Se, 5290 IU vitamin A, 1322 IU vitamin D, 26 IU vitamin E, 2.6 mg vitamin K, 49.6 mg niacin, 16.5 mg pantothenic acid, 5.0 mg riboflavin, and 0.02 mg vitamin B12.

[5]Ronozyme HiPhos 2,500 phytase (DSM Nutritional Products, Inc., Parsippany, NJ) was included at 1,500 FYT/kg with assumed release values of 0.146% STTD P, 0.166% available P, 0.102% STTD Ca, 10.9 kcal/kg of ME, 8.6 kcal/kg of NE; and 0.0217, 0.0003, 0.0086, 0.0224, 0.0056, 0.0122, and 0.0163% digestible Lys, Met, Met + Cys, Thr, Trp, Ile, and Val, respectively

[6]Standardized ileal digestibility.

[7]Standardized total tract digestibility of calcium.

[8]Standardized total tract digestibility of phosphorus.

[9]Representative samples of treatment diets were taken from 6 feeders per dietary treatment 3 d after the beginning and 3 d before the end of the phase and stored at -4°F. After blending, subsamples were submitted to Ward Laboratories, Inc. (Kearney, NE) and were analyzed for dry matter, crude protein, calcium, and phosphorus.

Table 3-5 The effects of phytase feeding duration on growth performance and carcass characteristics of growing-finishing pigs, Exp. 1[1]

Item[4]	Treatment[2,3]			SEM	Probability, *P*=[6]
	Control	Phytase grower	Phytase grower and finisher		
Body weight, kg					
d 0	27.9	27.9	27.9	0.463	0.780
d 57	74.5[a]	73.2[b]	73.7[ab]	0.884	0.017
d 126	136.5[a]	135.9[a]	133.2[b]	1.238	0.030
Grower period (d 0 to 57)					
ADG, kg	0.82[a]	0.80[b]	0.81[ab]	0.008	0.011
ADFI, kg	1.69	1.70	1.70	0.021	0.570
G:F	0.49[a]	0.47[b]	0.47[b]	0.003	<0.001
Finisher period (d 57 to 126)					
ADG, kg	0.92[a]	0.94[a]	0.89[b]	0.010	0.004
ADFI, kg	2.73	2.76	2.71	0.027	0.575
G:F	0.34[a]	0.34[a]	0.33[b]	0.003	<0.001
Overall period (d 0 to 126)					
ADG, kg	0.87[a]	0.87[a]	0.85[b]	0.006	0.017
ADFI, kg	2.24	2.25	2.23	0.021	0.651
G:F	0.39[a]	0.39[a]	0.38[b]	0.003	<0.001
Carcass characteristics					
HCW, kg	99.9	100.2	97.9	1.063	0.097
Yield, %	72.63	72.83	72.27	0.337	0.406
Backfat, mm	15.98	16.66	16.46	-[5]	0.509
Fat-free lean, mm	57.63	57.30	57.36	-[5]	0.717
Loin depth, mm	70.98	71.69	71.23	-[5]	0.797

[1]A total of 1,215 pigs (PIC 359 × Camborough, initial BW of 27.9 kg) were used in a 126-d growth trial with 27 pigs per pen and 15 pens per treatment.

[2]Dietary treatments consisted of a control with no added phytase, or 2 treatments with 1,500 FYT fed either in the grower period (d 0 to 57) then switched to control diet until market, or phytase fed throughout the entire grower and finisher period (d 0 to market).

[3]Ronozyme HiPhos 2,500 phytase (DSM Nutritional Products, Inc., Parsippany, NJ) was included at 1,500 FYT/kg with assumed release values of 0.146% STTD P, 0.166% available P, 0.102% STTD Ca, 10.9 kcal/kg of ME, 8.6 kcal/kg of NE; and 0.0217, 0.0003, 0.0086, 0.0224, 0.0056, 0.0122, and 0.0163% digestible Lys, Met, Met + Cys, Thr, Trp, Ile, and Val, respectively.

[4]ADG = average daily gain; ADFI = average daily feed intake; G:F = gain-to-feed ratio; HCW = hot carcass weight.

[5]SEM for backfat were 0.392, 0.479, and 0.385; SEM for fat-free lean were 0.287, 0.352, and 0.283; and SEM for loin depth were 0.668, 0.813, and 0.652 for the control, phytase fed only in the grower phase, and phytase fed throughout the grower and finisher, respectively.

[6]Means with different superscripts within a row differ.

Table 3-6 Effects of different nutrient release values of Ronozyme HiPhos 2,500 phytase on pig growth performance, Exp. 2[1,2]

	No phytase[2]	1,500 FYT/kg Phytase[3]						
Item[4]	Control	CaP	CaPAA	CaPAA + half NE	CaPAA + full NE	None	SEM	Probability, *P* <
Body weight, kg								
d 0	28.5	28.5	28.5	28.5	28.5	28.5	0.61	0.999
d 55	72.4	72.9	72.5	71.9	72.2	72.6	0.95	0.786
Overall (d 0 to 55)								
ADG, kg	0.83	0.83	0.83	0.82	0.83	0.83	0.010	0.768
ADFI, kg	1.67	1.72	1.68	1.67	1.70	1.66	0.024	0.222
G:F	0.496[ab]	0.486[b]	0.495[ab]	0.491[ab]	0.484[b]	0.502[a]	0.0038	0.002

[1]A total of 2,268 mixed sex pigs (PIC 337 × 1050, initially 28.5 kg) were used in a 55-d growth study to determine the impact on performance when phytase is credited with additional nutrient release above P and Ca. There were 27 pigs per pen and 14 pens per treatment.

[2]Dietary treatments consisted of a control with no phytase, or 5 diets with 1,500 phytase units assuming supplier-provided nutrient release values for Ca and P (CaP), Ca, P, and AA (CaPAA), Ca, P, AA, and half of the suggested net energy (CaPAA+halfNE), Ca, P, AA, and full NE (CaPAA+fullNE) and no nutrient release (None).

[3]Ronozyme HiPhos 2,500 phytase (DSM Nutritional Products, Inc., Parsippany, NJ) was included at 1,500 FYT/kg with assumed release values of 0.146% STTD P, 0.166% available P, 0.102% STTD Ca, 10.9 kcal/kg of ME, 8.6 kcal/kg of NE; and 0.0217, 0.0003, 0.0086, 0.0224, 0.0056, 0.0122, and 0.0163% digestible Lys, Met, Met + Cys, Thr, Trp, Ile, and Val, respectively.

[4]ADG = average daily gain; ADFI = average daily feed intake; G:F = gain-to-feed ratio.