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Determination of Efficacy of Smizyme TS G5 2,500 Phytase in Nursery Pigs

Abstract

A total of 320 nursery pigs (DNA; 241 × 600; initially 22.9 lb BW) were used in a 21-d growth trial to determine the available P (aP) release curve for Smizyme TS G5 2,500 (Origination, Inc., Saint Paul, MN). Pigs were weaned at approximately 21 d of age, randomly allotted to pens based on initial body weight (BW), and fed common starter diets. On d 21 post-weaning, considered d 0 of the study, pens were blocked by BW and randomly allotted to 1 of 8 dietary treatments with 5 pigs per pen and 8 pens per treatment. Eight 1-ton batches of basal diet were manufactured and subsequently divided to be the major portion of experimental diets. Dietary treatments were formulated to include increasing aP derived from either an inorganic P source (0.12%, 0.18%, or 0.24% from monocalcium P) or increasing phytase (150, 250, 500, 750, or 1,000 FTU/kg). Diets were corn-soybean meal-based and contained 1.24% standardized ileal digestible (SID) lysine with other amino acids set to meet or exceed NRC requirement estimates. Prior to beginning the 21-d study, all pigs were fed the negative control diet containing 0.12% aP for a 3-d period (d 18 to 21 post-weaning). Diets containing phytase were submitted for complete phytase analysis (Eurofins Scientific Inc., Des Moines, IA) using the AOAC official method 2000.12 and analyzed concentrations were 190, 310, 500, 790, or 850 FTU/kg. On d 21 of the experiment, the pig closest to the mean BW in each pen was euthanized and the right and left fibula were collected to determine bone ash. One fibula from each animal was processed with defatting, and the other fibula was processed without defatting. Overall (d 0 to 21), pigs fed increasing aP from inorganic P or phytase had improved (linear, $P < 0.01$) average daily gain (ADG), feed efficiency (F/G), and final BW. Additionally, pigs fed increasing aP from phytase had increased (linear, $P < 0.01$) average daily feed intake (ADFI). For the defatted bones, pigs fed increasing aP from inorganic P had increased (linear, $P < 0.01$) bone ash weights, resulting in increased (quadratic, $P < 0.01$) percentage bone ash, while those fed increasing levels of phytase had increased (linear, $P < 0.01$) bone ash weights and percentage bone ash. Similarly, using the non-defatted analytical method, pigs fed increasing aP from either inorganic P or phytase had increased (linear, $P < 0.01$) bone ash weights and percentage bone ash. The aP release increased (linear, $P < 0.01$) for all criteria up to the highest phytase dose when using ADG, F/G, or bone ash weight as the indicator of release, in contrast to using percentage bone ash of defatted bones which increased in a quadratic fashion ($P < 0.05$). In conclusion, the magnitude of aP release at different FTU inclusion rates depends on the response criteria, but Smizyme TS G5 2,500 appears to have a similar aP release to other commercially available phytase sources.

Keywords

nursery pig, bone ash, phosphorus, phytase

Cover Page Footnote

Appreciation is expressed to Origination, Inc. (Saint Paul, MN) for their support of this trial.

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K-STATE Research and Extension

SWINE DAY 2019

KANSAS STATE UNIVERSITY SWINE INDUSTRY DAY 2019

Determination of Efficacy of Smizyme TS G5 2,500 Phytase in Nursery Pigs[1]

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Summary

A total of 320 nursery pigs (DNA; 241 × 600; initially 22.9 lb BW) were used in a 21-d growth trial to determine the available P (aP) release curve for Smizyme TS G5 2,500 (Origination, Inc., Saint Paul, MN). Pigs were weaned at approximately 21 d of age, randomly allotted to pens based on initial body weight (BW), and fed common starter diets. On d 21 post-weaning, considered d 0 of the study, pens were blocked by BW and randomly allotted to 1 of 8 dietary treatments with 5 pigs per pen and 8 pens per treatment. Eight 1-ton batches of basal diet were manufactured and subsequently divided to be the major portion of experimental diets. Dietary treatments were formulated to include increasing aP derived from either an inorganic P source (0.12%, 0.18%, or 0.24% from monocalcium P) or increasing phytase (150, 250, 500, 750, or 1,000 FTU/kg). Diets were corn-soybean meal-based and contained 1.24% standardized ileal digestible (SID) lysine with other amino acids set to meet or exceed NRC[4] requirement estimates. Prior to beginning the 21-d study, all pigs were fed the negative control diet containing 0.12% aP for a 3-d period (d 18 to 21 post-weaning). Diets containing phytase were submitted for complete phytase analysis (Eurofins Scientific Inc., Des Moines, IA) using the AOAC official method 2000.12[5] and analyzed concentrations were 190, 310, 500, 790, or 850 FTU/kg. On d 21 of the experiment, the pig closest to the mean BW in each pen was euthanized and the right and left fibula were collected to determine bone ash. One fibula from each animal was processed with defatting, and the other fibula was processed without defatting. Overall (d 0 to 21), pigs fed increasing aP from inorganic P or phytase had improved (linear, $P < 0.01$) average daily gain (ADG), feed efficiency (F/G), and final BW. Additionally, pigs fed increasing aP from phytase had increased (linear, $P < 0.01$) average daily feed intake (ADFI). For the defatted bones, pigs fed increasing aP from inorganic P had increased (linear, $P < 0.01$) bone ash weights, resulting in increased (quadratic, $P < 0.01$) percentage bone ash, while those fed increasing levels of phytase had increased (linear, $P < 0.01$) bone ash weights

[1] Appreciation is expressed to Origination, Inc. (Saint Paul, MN) for their support of this trial.

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[4] National Research Council. 2012. Nutrient Requirements of Swine: Eleventh Revised Edition. Washington, DC: The National Academies Press. https://doi.org/10.17226/13298.

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

and percentage bone ash. Similarly, using the non-defatted analytical method, pigs fed increasing aP from either inorganic P or phytase had increased (linear, $P < 0.01$) bone ash weights and percentage bone ash. The aP release increased (linear, $P < 0.01$) for all criteria up to the highest phytase dose when using ADG, F/G, or bone ash weight as the indicator of release, in contrast to using percentage bone ash of defatted bones which increased in a quadratic fashion ($P < 0.05$). In conclusion, the magnitude of aP release at different FTU inclusion rates depends on the response criteria, but Smizyme TS G5 2,500 appears to have a similar aP release to other commercially available phytase sources.

Introduction

Phytase is a phosphatase enzyme that catalyzes the hydrolysis of phytic acid, the major storage form of P found in cereal grain and oilseeds. Phytic acid is indigestible to pigs and other monogastric animals because they do not naturally produce the digestive enzyme phytase. Thus, phytase is often added to swine diets to improve the digestibility of phytic acid P and increase P availability. Supplying enough available P (aP) in the diet is crucial to ensure normal body maintenance, growth, and bone formation. Furthermore, making P more available for utilization allows for reduced added P from inorganic sources, such as monocalcium phosphate. This makes it easier to meet the pig's requirement while subsequently reducing diet costs and the amount of P excreted in swine waste.

Phytase was first commercialized in 1991.[6] Nearly 30 years later, the role of phytase in swine diet formulation continues to gain attention. While there are many commercially available phytase sources for producers to use, new phytase sources must be analyzed to determine their efficacy. Therefore, the objective of this study was to evaluate the effects of a new phytase source, Smizyme TS G5 2,500 (Origination, Inc., Saint Paul, MN), on the growth performance and bone ash of 23- to 50-lb nursery pigs to develop an aP release curve.

Procedures

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 Swine Teaching and Research Center in Manhattan, KS. Each pen contained a 4-hole, dry self-feeder, and nipple waterer for *ad libitum* access to feed and water.

A total of 320 pigs (DNA; 241 × 600; initially 22.9 lb BW) were used in a 21-d growth trial. Pigs were weaned at approximately 21 d of age, randomly allotted to pens based on initial BW, and fed common starter diets. On d 21 post-weaning, considered d 0 of the study, pens were blocked by BW and randomly allotted to 1 of 8 dietary treatments with 5 pigs per pen (2 barrows and 3 gilts or 3 barrows and 2 gilts) and 8 pens per treatment. 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

[6] Lei, X. G., J. D. Weaver, E. Mullaney, A. H. Ullah, M. J. Azain. 2013. Phytase, a new life for an "old" enzyme. The Annual Review of Animal Bioscience 1:283-309. doi: 10.1146/annurev-animal-031412-103717.

FTU/kg) phytase added to the diet containing 0.12% aP. Prior to beginning the 21-d study, all pigs were fed the negative control diet containing 0.12% aP for a 3-d period (d 18 to 21 post-weaning). Diets were corn-soybean meal-based and contained 1.24% SID Lys. All diets were formulated to contain a Ca:P ratio of 1.10:1.

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[4] requirement estimates. Ingredients containing Ca and P were analyzed prior to the manufacturing of diets to determine nutrient loading values used for formulation (Table 1). The phytase premix was also analyzed to determine the inclusion rate in the experimental diets and contained 2,630,000 FTU/kg.

All dietary treatments were derived from eight, 1-ton batches of basal diet (Table 2). After manufacturing, each basal batch was bagged off into eight separate tons. For each experimental diet, a subset of bags (50 lb each) from the basal diet was added to the mixer along with treatment specific ingredients to achieve final experimental diets (Table 3). 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). 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.[5]

During the experiment, pig and feeder weights were measured every 7 d to determine ADG, ADFI, and F/G. After the 21-day study, 1 pig from each pen (weighing closest to mean weight of pigs in the pen) was euthanized. The right and left fibula from each animal were collected for bone ash and percentage bone ash calculations. After removal, bones were individually placed in plastic bags with permanent identification and stored at -20°C until analysis. On the day of analysis, bones were autoclaved for 1 hour at 121°C. After cooling, any leftover extraneous soft tissue including cartilage caps was cleaned from the fibulas. Bones were then processed either with or without defatting. Using the non-defatted method, a total of 64 fibulas (one from each animal) were dried at 105°C for 7 d in a drying oven and ashed in a muffle furnace at 600°C for 24 h. For the defatted method, a total of 64 fibulas (one from each animal) 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 finally ashed at 600°C for 24 h. Both processing methods were used to determine total ash weight and percentage ash relative to dried bone weight.

Data Analysis

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% (the aP in the basal diet) 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.

Using the GLIMMIX procedure of SAS version 9.4 (SAS Institute, Inc., Cary, NC), 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 phytase levels. Results were considered significant at $P \leq 0.05$.

Results and Discussion

Analysis of manufactured diets resulted in crude protein, Ca, and P values consistent with formulation (Table 3). Phytase analysis of complete diets showed a stepwise increase in phytase inclusion. Compared with formulated values, there was slightly more dietary phytase for the 150, 250, and 750 FTU/kg treatments and a slightly lower dietary phytase for the 1,000 FTU/kg treatment.

From d 0 to 21, pigs fed increasing aP from inorganic P had improved (linear, $P < 0.01$; Table 4) ADG, F/G, and final BW, with a tendency for increased ADFI (linear, $P = 0.08$). Additionally, pigs fed increasing aP from phytase had improved (linear, $P < 0.01$) performance across all response criteria measured.

For the defatted bones, pigs fed increasing aP from inorganic P had increased (linear, $P < 0.01$) bone ash weights, resulting in increased (quadratic, $P < 0.01$) percentage bone ash, while those fed increasing levels of phytase had increased (linear, $P < 0.01$) bone ash weights and percentage bone ash (linear, $P < 0.01$ and quadratic, $P = 0.066$). Similarly, using the non-defatted analytical method, pigs fed increasing aP from inorganic P had increased (linear, $P < 0.01$) bone ash weights and percentage bone ash (linear, $P < 0.01$ and quadratic, $P = 0.082$), while those pigs fed increasing levels of phytase had increased (linear, $P < 0.01$) bone ash weights and percentage bone ash.

The percent aP released from Smizyme TS G5 2,500 varied depending on the response criteria measured (Table 5). As the amount of phytase in the diet increased, the calculated aP release increased linearly ($P < 0.01$) up to the highest phytase dose when using ADG, F/G, or bone ash weight as the indicator of release, in contrast to using percentage bone ash of defatted bones which increased in a linear and quadratic fashion ($P < 0.05$).

This study has provided a range of aP release for Smizyme TS G5 2,500 phytase in nursery pigs weighing 23- to 50-lb when fed at levels between 150 and 1,000 FTU/kg. In conclusion, the magnitude of aP release at different FTU inclusion rates depends on the response criteria, but Smizyme TS G5 2,500 appears to have a similar aP release to other commercially available phytase sources.

Brand names appearing in this publication are for product identification purposes only. No endorsement is intended, nor is criticism implied of similar products not mentioned. Persons using such products assume responsibility for their use in accordance with current label directions of the manufacturer.

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

Ingredient	Ca, %	P, %
Corn	0.07	0.24
Soybean meal	0.56	0.64
Limestone	40.24	0.03
Monocalcium P	17.90	20.23

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

Item	
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/lb	1,511
Net energy (NE), kcal/lb	1,111
SID lysine:NE, g/Mcal	5.06
Crude protein, %	22.0
Calcium, %	0.31
Phosphorus, %	0.37
Available phosphorus, %	0.07
STTD P, %[2]	0.16

[1]The basal batch was used as the major ingredient in each experimental diet.
[2]STTD P = Standardized total tract digestible phosphorus.

Table 3. Composition of experimental diets (as-fed basis)[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 23- to 50-lb.
[2]Smizyme TS G5 2,500 (Origination, Inc., Saint Paul, MN).
[3]Sand was used to equalize the addition 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 4. Effects of increasing aP from inorganic P or Smizyme TS G5 2,500 phytase on nursery pig growth performance and bone ash values[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, lb													
d 0	23.0	23.1	22.9	22.7	22.9	23.0	22.9	22.9	0.37	0.548	0.284	0.693	0.671
d 21	47.2	50.1	50.9	48.3	49.0	50.5	51.1	52.0	0.89	<0.001	0.162	<0.001	0.259
d 0 to 21													
ADG, lb	1.15	1.29	1.33	1.21	1.24	1.31	1.34	1.39	0.032	<0.001	0.198	<0.001	0.273
ADFI, lb	1.95	2.08	2.07	1.91	2.01	2.04	2.05	2.09	0.054	0.080	0.204	0.007	0.725
F/G	1.69	1.61	1.55	1.58	1.62	1.56	1.53	1.51	0.025	<0.001	0.744	<0.001	0.179
Defatted method[6]													
Bone ash, g	0.581	0.659	0.810	0.683	0.708	0.754	0.738	0.920	0.031	<0.001	0.344	<0.001	0.683
Bone ash, %	45.08	44.63	51.82	48.66	49.19	49.99	51.54	52.17	0.890	<0.001	0.001	<0.001	0.066
Non-defatted method[7]													
Bone ash, g	0.624	0.687	0.855	0.686	0.697	0.786	0.771	0.916	0.027	<0.001	0.127	<0.001	0.696
Bone ash, %	37.96	38.56	43.59	39.76	39.71	41.01	41.32	42.27	1.020	<0.001	0.082	0.002	0.496

[1]A total of 320 nursery pigs (DNA; 241 × 600; initially 22.9 lb body weight (BW)) were used in a 21-day growth study to determine the available P (aP) release curve for Smizyme TS G5 2,500 phytase. There were 5 pigs per pen and 8 pens per treatment.

[2]ADG = average daily gain; ADFI = average daily feed intake; F/G = feed efficiency.

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

[4]Smizyme TS G5 2,500 (Origination, Inc., Saint Paul, 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. Fibulas were autoclaved for 1 h. After cleaning, bones were placed in Soxhlet extractors containing petroleum ether for 7 days as a means of removing water and fat. They were then dried at 105°C for 24 h, and then ashed in a muffle furnace at 600°C for 24 h.

[7]One pig per pen (8 pens per treatment) was euthanized and fibulas were collected to determine bone ash weight and percentage bone ash. Fibulas were autoclaved for 1 h. After cleaning, they were then dried for 7 days at 105°C and then ashed in a muffle furnace at 600°C for 24 h.

Table 5. Calculated aP release values based on different response criteria[1,2]

	Phytase, FTU/kg[3]						Probability, $P <$	
Item	150	250	500	750	1,000	SEM[4]	Linear	Quadratic
Performance								
ADG	0.031	0.052	0.094	0.109	0.139	0.019	<0.001	0.184
F/G	0.102	0.060	0.116	0.148	0.164	0.022	<0.001	0.092
Defatted method[5]								
Bone ash, g	0.066	0.078	0.104	0.096	0.191	0.017	<0.001	0.847
Bone ash, %	0.095	0.102	0.114	0.142	0.149	0.014	<0.001	0.028
Non-defatted method[6]								
Bone ash, g	0.046	0.050	0.100	0.089	0.166	0.014	<0.001	0.860
Bone ash, %	0.061	0.057	0.085	0.091	0.108	0.020	0.003	0.407

[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; F/G = feed efficiency.
[3]Smizyme TS G5 2,500 (Origination, Inc., Saint Paul, 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. Fibulas were autoclaved for 1 h. After cleaning, bones were placed in Soxhlet extractors containing petroleum ether for 7 days as a means of removing water and fat. They were then dried at 105°C for 24 h, and then ashed in a muffle furnace at 600°C for 24 h.
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