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KANSAS STATE UNIVERSITY SWINE INDUSTRY DAY 2020

Determining the Phosphorus Release Curve for Smizyme TS G5 2,500 Phytase in Nursery Pigs[1]

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Summary

A total of 280 nursery pigs (DNA 241 × 600, initially 22.8 lb) were used in a 21-d growth trial to determine the available P (aP) release curve for Smizyme TS G5 2,500 (Origination, LLC., Maplewood, 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 7 dietary treatments with 5 pigs per pen and 8 pens per treatment. Seven 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 (0.11%, 0.19%, or 0.27% from monocalcium P) or increasing phytase (250, 500, 1,000, or 1,500 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[5] requirement estimates. Prior to beginning the 21-d study, all pigs were fed the negative control diet containing 0.11% 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,[6] and analyzed concentrations were 265, 470, 1,000, or 1,450 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 with one fibula defatted and the other not prior to ashing. Overall (d 0 to 21), pigs fed increasing aP from inorganic P had improved (linear, $P < 0.01$) performance across all response criteria measured. When using both the defatted and non-defatted bone mineral procedures, pigs fed increasing aP from inorganic P

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[5] NRC. 2012. Nutrient Requirements of Swine, 11th ed. Natl. Acad. Press, Washington D.C.

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

had increased (linear, $P < 0.01$) bone ash weights and percentage bone ash. Pigs fed increasing phytase had increased (linear, $P < 0.01$) bone ash weights and percentage bone ash (linear and quadratic, $P < 0.05$). The aP release increased (linear, $P < 0.01$) up to the highest phytase dose when using gain-to-feed ratio (G:F), bone ash weight, or defatted percentage bone ash as indicators of release. When using average daily gain and non-defatted percentage bone ash, aP release increased in a linear and quadratic fashion ($P < 0.01$). In conclusion, the magnitude of aP release curves depends on the response criteria measured, but Smizyme TS G5 2,500 appears to have a similar aP release to other commercially available phytase sources. When combining the release values for defatted bones in this experiment with a previous experiment,[7] the aP release 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) for ADG, G:F, and percentage bone ash, respectively.

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 of phosphorus (P) found in feedstuffs of plant origin. Pigs and other monogastric animals do not synthesize adequate levels of endogenous phytase to effectively cleave the phosphates from the phytate. 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 optimize growth and ensure normal bone formation. Consequently, adding phytase from an exogenous source has been shown to improve the hydrolysis of phytic acid, making P more available. This decreases the need for inorganic P, improves diet costs, and decreases the amount of P excreted in swine waste.

There are many commercially available phytase sources for producers to use; however, when a new phytase enters the marketplace, its analysis is required to determine its efficacy. Smizyme TS G5 2,500 (Origination, LLC., Maplewood, MN) is a newly available phytase source to the U.S. swine and poultry industries. In a recent trial,[7] Smizyme TS G5 2,500 improved average daily gain (ADG), feed-to-gain ratio (F/G), and percentage bone ash as phytase increased from 150 to 1,000 FTU/kg. However, this was the first study conducted feeding Smizyme TS G5 2,500 in nursery pig diets to investigate its impact on aP release. Additional research is needed to confirm this response and to determine the release at higher FTU. Therefore, the objective of this study was to evaluate the effects of increased Smizyme TS G5 2,500 on the growth performance and bone ash of 23- to 50-lb nursery pigs and 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.

[7] Wensley, M. W., J. C. Woodworth, J. M. DeRouchey, S. S. Dritz, M. D. Tokach, R. D. Goodband, and J. M. Faser. 2019. Determination of Efficacy of Smizyme TS G5 2,500 Phytase in Nursery Pigs. Kansas State University Swine Day Report. *Kansas Agricultural Experiment Station Research Reports*: Vol. 5: Iss. 8.

A total of 280 pigs (DNA 241 × 600, initially 22.8 lb) were used in a 21-d growth trial. Pigs were weaned at approximately 21 d of age, randomly allotted to pens based on initial weight, and fed a common starter diet. On d 21 post-weaning, considered d 0 of the study, pens were blocked by BW and randomly allotted to 1 of 7 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 (0.11%, 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. Prior to beginning the 21-d study, all pigs were fed the negative control diet containing 0.11% 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 an analyzed 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[5] requirement estimates. Ingredients containing Ca and P were analyzed prior to manufacturing diets to determine nutrient loading values used in formulation (Table 1). The phytase premix was also analyzed to determine its inclusion rate in the experimental diets and was found to contain 2,314,000 FTU/kg.

All dietary treatments were derived from seven, 1-ton batches of basal diet (Table 2). After manufacturing, each basal batch was bagged off into seven separate tons. For each experimental diet, a subset of bags from the 7, 1-ton batches of basal diet were 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 sample was ground 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.[6]

During the experiment, pig and feeder weights were measured every 7 d to determine ADG, average daily feed intake (ADFI), and F/G. After the 21-day study, 1 pig from each pen (weighing closest to the mean weight of pigs in the pen) was euthanized. The right and left fibula from each pig 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 were cleaned from the fibulas. Bones were then processed either with or without defatting. Using the non-defatted method, a total of 56 fibulas (one from each pig) 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 56 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 finally ashed at 600°C for 24 h. Both processing methods were used to determine total bone ash weight and percentage ash relative to dried bone weight. While there are limited data on the difference between the two analytical methods in nursery pigs, research shows that in

finishing pigs, defatting bones is more accurate in determining percent bone ash. Therefore, only the defatted bone data were used to develop the aP release curve for percent bone ash.

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 0.11% (the aP in the basal diet) multiplied by ADFI. A standard curve was then developed for each response criterion 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 the calculated phytase dosage, assuming an intercept of no aP release for the control diet without phytase. All release values were calculated using formulated phytase levels. Gain-to-feed ratio was used to determine the aP release for feed efficiency rather than F/G. This was to maintain consistent units of measure for developing aP release equations based on the metric system.

Non-linear regression was used to fit a model to pen release values, with the model parameters estimated using the nls function from the stats package in R (version 3.5.1 (2018-07-02)) in order to develop aP release curves for ADG, G:F, and percent bone ash.

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

Results and Discussion

Analysis of manufactured diets resulted in crude protein and P-values consistent with formulation (Table 3). Average values of analyzed Ca concentrations were approximately 2 and 4% lower than formulated values in the diets with no added phytase, and in phytase-containing diets, respectively. Phytase analysis of complete diets showed a stepwise increase in phytase.

From d 0 to 21, pigs fed increasing aP from either inorganic P (linear, $P < 0.001$; Table 4) or phytase (linear, $P < 0.001$ and quadratic, $P < 0.05$) had improved 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 (linear, $P < 0.01$) percentage bone ash, while those fed increasing phytase had increased (linear, $P < 0.01$ and quadratic, $P = 0.061$) bone ash weights and percentage bone ash (linear, $P < 0.01$ and quadratic, $P < 0.05$). 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, while those pigs fed increasing levels of phytase had increased (linear, $P < 0.01$ and quadratic, $P = 0.060$) bone ash weights and percentage bone ash (linear and quadratic, $P < 0.01$).

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 up to the highest phytase dose when using ADG (linear and quadratic, $P < 0.01$), G:F (linear, $P < 0.01$ and quadratic $P = 0.066$), or defatted and non-defatted bone ash weight (linear, $P < 0.01$ and quadratic $P = 0.059$) as the indicator of release. Similarly, when using the percentage bone ash of defatted and non-defatted bones, the calculated aP release increased linearly ($P < 0.01$) with a quadratic tendency ($P = 0.055$) or in a linear and quadratic fashion ($P < 0.01$), respectively.

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 250 and 1,500 FTU/kg. The results were similar to those observed in the previous trial[7]; however, by increasing phytase inclusion up to 1,500 FTU/kg, we were able to pick up a quadratic response, indicating diminishing marginal improvements as phytase dose increases beyond 1,000 FTU/kg. In conclusion, the magnitude of aP release at different FTU inclusion rates depends on the response criteria measured, but Smizyme TS G5 2,500 appears to have a similar aP release to other commercially available phytase sources. When combining the release values for defatted bones from Wensley[7] and the present experiment, 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 $aP = (0.165 \times FTU) \div (178.146 + FTU)$ for ADG, G:F, and percentage bone ash, respectively.

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	0.06	0.20
Soybean meal	0.60	0.64
Limestone	40.28	0.06
Monocalcium P	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. 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 cysteine: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, %	0.32
Phosphorus, %	0.35
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, %	0.11%	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	023	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 23 to 50 lb.
[2]Smizyme TS G5 2,500 (Origination, LLC., Maplewood, 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 2,314,000 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 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	0.11%	0.19%	0.27%	250	500	1,000	1,500	SEM[5]	Linear	Quadratic	Linear	Quadratic
BW, lb												
d 0	22.8	22.7	22.8	22.7	22.8	22.9	22.8	0.34	0.952	0.252	0.963	0.768
d 21	45.3	48.4	51.6	47.3	49.4	49.8	50.5	0.75	<0.001	0.881	<0.001	<0.001
d 0 to 21												
ADG, lb	1.07	1.22	1.37	1.17	1.27	1.28	1.32	0.024	<0.001	0.957	<0.001	<0.001
ADFI, lb	1.79	1.93	2.07	1.86	1.96	2.01	2.02	0.044	<0.001	0.979	<0.001	0.031
F/G	1.67	1.58	1.51	1.59	1.55	1.57	1.52	0.018	<0.001	0.649	<0.001	0.026
Defatted method[6]												
Bone ash, g	0.599	0.703	0.865	0.686	0.737	0.852	0.869	0.032	<0.001	0.449	<0.001	0.061
Bone ash, %	47.28	50.05	53.49	50.36	50.48	52.49	52.88	0.694	<0.001	0.696	<0.001	0.035
Non-defatted method[7]												
Bone ash, g	0.627	0.726	0.873	0.684	0.738	0.854	0.835	0.035	<0.001	0.561	<0.001	0.060
Bone ash, %	36.82	39.21	42.27	39.31	40.34	43.01	42.18	0.720	<0.001	0.677	0.002	0.002

[1]A total of 280 nursery pigs (DNA 241 × 600, initially 22.8 lb body weight) were used in a 21-day 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. BW = body weight. F/G = feed-to-gain 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. 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	250	500	1,000	1,500	SEM[4]	Linear	Quadratic
Performance							
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
Defatted method[5]							
Bone ash weight	0.060	0.094	0.165	0.173	0.023	<0.001	0.059
Percentage bone ash	0.088	0.091	0.143	0.152	0.024	<0.001	0.055
Non-defatted method[6]							
Bone ash weight	0.043	0.081	0.157	0.141	0.026	<0.001	0.059
Percentage bone ash	0.080	0.113	0.192	0.167	0.024	<0.001	0.005

[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 criterion 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 criterion.
[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. 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.
[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, they were then dried for 7 days at 105°C and then ashed in a muffle furnace at 600°C for 24 h.