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KANSAS STATE UNIVERSITY SWINE INDUSTRY DAY 2021

Determining the Phosphorus Release of GraINzyme Phytase in Nursery Pigs[1]

Larissa L. Becker, Madie R. Wensley, Joel M. DeRouchey, Jason C. Woodworth, Mike D. Tokach, Robert D. Goodband, Jordan T. Gebhardt,[2] R. Michael Raab,[3] and Philip A. Lessard[3]

Summary

A total of 360 pigs (200 × 400, DNA; initially 21.9 ± 0.42 lb) were used in a 21-d growth trial to determine the available P (aP) release curve for GraINzyme Phytase (Agrivida Inc., Woburn, MA). Pigs were weaned at approximately 21 d of age, randomly allotted to pens based on initial BW and fed common starter diets. From d 18 to 21 post-weaning, all pigs were fed a diet containing 0.11% aP. 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 9 pens per treatment. 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 levels of phytase (150, 250, 500, 1,000, or 1,500 FTU/kg). Diets were corn-soybean meal-based and contained 1.24% standardized ileal digestible (SID) Lys. On d 21 of the trial, 1 pig per pen (weighing closest to the mean pen BW) was humanely euthanized and the right fibula was collected to determine bone ash using the non-defatted processing method. Overall (d 0 to 21), pigs fed increasing aP from inorganic P or phytase had improved (linear, $P < 0.002$) ADG, ADFI, and F/G. Bone ash weight and percentage bone ash increased (linear, $P < 0.001$) with increasing inorganic P or added phytase. Based on these results, the release equations developed for GraINzyme for ADG, G:F, bone ash weight, and percentage bone ash are: aP = (0.255 × FTU) ÷ (1299.969 + FTU); aP = (0.233 × FTU) ÷ (1236.428 + FTU); aP = (45999.949 × FTU) ÷ (462529200 + FTU); and aP = (0.272 × FTU) ÷ (2576.581 + FTU), respectively.

Introduction

Most swine diets are formulated using ingredients which contain phytate-bound-phosphorus. Phytic acid, or phytate, is a six-fold dihydrogen phosphate ester of inositol that is the major storage form of phosphorus (P). Monogastrics do not naturally synthesize the enzyme phytase to cleave the phosphates from the phytic acid for absorption. Exogenous phytase can be added to swine diets to make P more available for animal use. The added phytase allows for reduced dietary inclusions of inorganic P, which results in reduced P excretion and lower feed cost.

[1] Appreciation is expressed to Agrivida Inc. (Woburn, MA) for partial financial support of this trial.

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There are several commercially available phytase sources for swine producers to utilize, and as new phytase products become available their efficacy must be determined in swine. While some phytase products have already undergone evaluation to determine their unique P release curve, other newer products have not been thoroughly tested.

GraINzyme Phytase (Agrivida, Inc., Woburn, MA) is a corn-expressed phytase that has been shown to be effective in poultry, and contains an engineered *Escherichia coli* phytase called Phy02. GraINzyme has been demonstrated to improve ADG, feed efficiency, bone mineralization, and bone strength when fed to young pigs.[4] However, there are limited data as to the aP release of GraINzyme for swine. Therefore, the objective of this study was to evaluate the effects of GraINzyme phytase on nursery pig growth performance and bone ash to develop an aP release curve.

Materials and Methods

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 Research Facility 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 360 barrows (200 × 400, DNA; initially 21.9 ± 0.42 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 after placement. From d 18 to 21 post-weaning, all pigs were fed a diet containing 0.11% aP. On d 21 post-placement, 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 9 pens per treatment. Treatments consisted of 3 diets with increasing P from monocalcium P formulated to provide 0.11, 0.19, or 0.27% aP; or 5 diets with increasing phytase (GraINzyme, Agrivida, Inc., Woburn, MA) 150, 250, 500, 1,000; or 1,500 FTU/kg added to the diet containing 0.11% aP. 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.

Diets were formulated to meet or exceed NRC[5] requirement estimates with the exception of Ca and P. Limestone and monocalcium phosphate were analyzed for Ca and P (K-State Research and Extension Soil Testing Laboratory, Manhattan, KS). Corn and soybean meal were also analyzed for Ca and P (Hubbard Feeds, Beloit, KS) to determine nutrient loading values used for formulation prior to the manufacturing of diets (Table 1). Other nutrient loading values for corn and soybean meal were based on NRC[5] estimates. The phytase was analyzed to determine inclusion rate in the experimental diets and was found to contain 9,738,000 FTU/kg.

All dietary treatments were derived from five, 2-ton batches of a basal diet manufactured at Hubbard Feeds in Beloit, KS (Table 2). For each experimental diet, a subset of bags from each of the five batches was mixed along with treatment specific ingredients

[4] Broomhead, J. N., P. A. Lessard, R. M. Raab, and M. B. Lanahan. 2019. Effects of feeding corn-expressed phytase on the liver performance, bone characteristics, and phosphorus digestibility of nursery pigs. J. Anim. Sci. 97:1254-1261. doi:10.1093/jas/sky479.

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

(limestone, monocalcium P, sand, and phytase) to achieve the final experimental diets. These diets were manufactured at the Kansas State University O.H. Kruse Feed Technology Innovation Center in Manhattan, KS. Complete diet samples were taken using a grain probe during bagging from every fourth bag and pooled into one homogenized sample per dietary treatment then stored at -4°F (-20°C). Three sub-samples of each diet were submitted for analysis of Ca and P (KSU Soils Lab, Manhattan, KS). Five sub-samples of each diet were sent to Agrivida (Woburn, MA) for analysis of phytase activity using an adjusted extraction procedure that employed an optimized buffer and extraction process.[6] Reported nutrient concentrations are the average of the sub-sample analytical results for each respective nutrient.

During the trial, individual pig and feeder weights were measured every 7 d to determine ADG, ADFI, and F/G. After the 21-d study, 1 pig from each pen (weighing closest to the average BW of the pen) was humanely euthanized via penetrating captive bolt and transported to the Kansas State University College of Veterinary Medicine Diagnostic Lab for bone collection. The right fibula from each pig was collected to determine bone ash weight and percentage bone ash calculations. After removal, bones were individually placed in plastic bags with permanent identification and stored at -4°F (-20°C) until analysis. On the day of analysis, bones were autoclaved for one hour at 250°F (121°C). After cooling, any leftover extraneous soft tissue including cartilage cap was removed from the fibula. A total of 72 fibulas (one from each pig) were dried at 221°F (105°C) for 7 d in a drying oven, reweighed and then ashed in a muffle furnace at 1,112°F (600°C) for 24 h to determine total ash weight and percentage ash relative to dried bone weight. The data were used to develop the aP release curve for percentage 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 MIXED procedure of SAS OnDemand for Academics (SAS Institute, Inc., Cary, NC). Treatment was considered a fixed effect and weight block a random effect. Contrast coefficients were adjusted to account for unequal spacing in phytase treatments.

For pens of pigs fed the inorganic P diets, marginal intake of aP was calculated for each pen using the equation: dietary aP% - 0.11% (the aP in the basal diet) × 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 derived from the inorganic P diets was used to calculate aP release from each pen fed the different phytase doses 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 OnDemand for Academics (SAS Institute, Inc., Cary, NC), a mixed model ANOVA with weight block as random effect was performed to evaluate aP release as a function of the calculated phytase dose, assuming an intercept of no aP release for the control diet without phytase. All release values were calculated using formulated phytase levels. For feed efficiency, G:F was used to deter-

[6] Li, X., and R. M. Raab. 2016. Processes for increasing extraction of enzymes from animal feed and measure activity of the same. United States Patent Application US2016/033472.

mine the aP release rather than F/G 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), R Foundation for Statistical Computing, Vienna, Austria) in order to develop aP release curves for ADG, G:F, and percent bone ash. Results were considered significant with $P \leq 0.05$ and were considered marginally significant with $P \leq 0.10$.

Results and Discussion

Analysis of final diets were similar in Ca and P to those expected from diet formulation (Table 3). Phytase analysis of complete diets showed a stepwise increase in phytase activity, as expected, and were very similar to formulated values for each diet containing phytase. Analyzed phytase concentrations were 137, 223, 379, 919, and 1,623 FTU/kg.

From d 0 to 21, pigs fed increasing aP from inorganic P had improved (linear, $P \leq 0.002$) ADG, ADFI, F/G, and final BW (Table 4). In addition, pigs fed diets with increasing phytase had improved (linear, $P < 0.001$) growth performance across all response variables.

For bone characteristics, bone ash weight and percentage bone ash increased (linear, $P \leq 0.003$) for pigs fed either increasing inorganic P or phytase in the diet.

Percentage aP released from GraINzyme varied depending on the response criteria measured (Table 5). As the amount of phytase in the diet increased, the calculated aP release increased (linear, $P \leq 0.008$) for ADG, bone ash weight, and percentage bone ash. For G:F, there was both a linear ($P < 0.001$) and quadratic ($P < 0.045$) increase in aP release as the amount of phytase in the diet increased.

In conclusion, this study has provided an aP release curve that can be used for GraINzyme phytase in swine diets when included at levels between 150 and 1,500 FTU/kg (Table 6). The magnitude of aP release at different FTU inclusion rates depends on the response criteria measured. The release equations generated from this experiment for GraINzyme for ADG, G:F, bone ash weight, and percentage bone ash are: $aP = (0.255 \times FTU) \div (1299.969 + FTU)$; $aP = (0.233 \times FTU) \div (1236.428 + FTU)$; $aP = (45999.949 \times FTU) \div (462529200 + FTU)$; and $aP = (0.272 \times FTU) \div (2576.581 + FTU)$, 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)

Ingredient	Ca, %	P, %
Limestone[1]	38.03	0.10
Monocalcium P[1]	16.73	21.73
Corn[2]	0.03	0.26
Soybean meal[2]	0.38	0.68

[1]Ingredient samples were pooled and analysis was performed by the K-State Research and Extension Soil Testing Laboratory, Manhattan, KS.
[2]Ingredient samples were analyzed by Hubbard Feeds, Beloit, KS.

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

Item	
Ingredient, %	
Corn	64.35
Soybean meal	34.29
Sodium chloride	0.61
L-Lys-HCl	0.31
DL-Met	0.12
L-Thr	0.12
L-Val	0.01
Trace mineral premix	0.15
Vitamin premix	0.05
Total	100
Calculated analysis	
Standardized ileal digestible (SID) amino acids	
Lys, %	1.24
Ile:Lys	65
Leu:Lys	131
Met:Lys	34
Met and Cys:Lys	58
Thr:Lys	64
Trp:Lys	19.1
Val:Lys	71
His:Lys	42
Total lys, %	1.42
ME, kcal/lb	1,514
NE, kcal/lb	1,113
SID Lys:NE, g/Mcal	5.05
CP, %	22.1
Ca, %	0.18
P, %	0.40
Available P, %	0.08
STTD P, %	0.17

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

Table 3. Ingredient composition of experimental diets (as-fed basis)[1]

	Inorganic P			Phytase[2]				
Ingredient, %	**0.11**	**0.19**	**0.27**	**150**	**250**	**500**	**1,000**	**1,500**
Basal mix	98.18	98.18	98.18	98.18	98.18	98.18	98.18	98.18
Limestone	0.69	0.77	0.83	0.69	0.69	0.69	0.69	0.69
Monocalcium P	0.16	0.53	0.90	0.16	0.16	0.16	0.16	0.16
Sand	0.98	0.54	0.10	0.97	0.97	0.97	0.96	0.96
Phytase[3]	----	----	----	0.0015	0.0026	0.0051	0.0103	0.0154
Total	100	100	100	100	100	100	100	100
Calculated analysis								
CP, %	21.7	21.7	21.7	21.7	21.7	21.7	21.7	21.7
Ca, %	0.47	0.60	0.65	0.47	0.47	0.47	0.47	0.47
P, %	0.43	0.51	0.59	0.43	0.43	0.43	0.43	0.43
Phytase, FTU/kg	----	----	----	150	250	500	1,000	1,500
Ca:P ratio	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Analyzed composition[4]								
Ca, %	0.57	0.63	0.77	0.51	0.53	0.59	0.54	0.54
P, %	0.42	0.47	0.63	0.40	0.43	0.43	0.42	0.41
Phytase, FTU/kg[5]	----	----	----	137	223	379	919	1623

[1]Diets were fed for 21 d starting at approximately 21 ± 0.42 lb BW.
[2]GraINzyme (Agrivida Inc., Woburn, MA).
[3]Phytase was analyzed for phytase level and contained 9,738,000 FTU/kg (Agrivida Inc., Woburn, MA).
[4]Complete diet samples were taken during bagging of experimental diets from every fourth bag and pooled into one homogenized sample per dietary treatment. Samples were stored at -4°F (-20°C) until they were submitted for duplicate analysis of Ca and P (K-State Research and Extension Soil Testing Laboratory, Manhattan, KS).
[5]Five samples of each diet were submitted to Agrivida Inc. (Woburn, MA) for complete phytase analysis using an adjusted extraction procedure that employed an optimized buffer and extraction process.

Table 4. Effects of increasing aP from inorganic P or GraINzyme phytase on nursery pig growth performance and bone ash values[1]

	Inorganic P, % aP[2]			Phytase, FTU/kg[3]						Inorganic P, *P* =		Phytase, *P* =	
Item	0.11	0.19	0.27	150	250	500	1,000	1,500	SEM	Linear	Quadratic	Linear	Quadratic
BW, lb													
d 0	22.2	22.2	21.7	21.5	21.7	22.2	21.4	22.4	0.42	0.087	0.257	0.229	0.021
d 21	41.2	43.7	45.0	41.7	41.8	42.9	44.1	45.1	0.79	<0.001	0.334	<0.001	0.561
d 0 to 21													
ADG, lb	0.91	1.03	1.11	0.96	0.96	0.99	1.08	1.08	0.024	<0.001	0.527	<0.001	0.103
ADFI, lb	1.51	1.63	1.65	1.56	1.58	1.59	1.63	1.67	0.035	0.002	0.151	0.001	0.514
F/G	1.67	1.59	1.48	1.62	1.65	1.61	1.51	1.54	0.019	<0.001	0.552	<0.001	0.053
Bone characteristics[4]													
Bone ash, g	0.614	0.764	0.812	0.601	0.623	0.672	0.722	0.861	0.0284	<0.001	0.141	<0.001	0.162
Bone ash, %	39.7	43.8	45.4	39.0	41.7	43.0	41.3	44.2	0.01	0.001	0.364	0.003	0.665

[1]A total of 360 nursery pigs (200 × 400, DNA; initially 21.9 ± 0.42 lb BW) were used in a 21-d growth trial with 5 pigs per pen and 9 replications per treatment.

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

[3]GraINzyme, Agrivida, Woburn, MA.

[4]One pig per pen (9 pens per treatment) was euthanized and the right fibula was collected to determine bone ash weight and percentage bone ash. Fibulas were autoclaved for 1 h. After cleaning, bones were placed in a drying oven at 221°F (105°C) for 7 days and then ashed in a muffle furnace at 1,112°F (600°C) for 24 h.

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

	Phytase, FTU/kg[2]						*P* =	
Item	**150**	**250**	**500**	**1,000**	**1,500**	**SEM**	**Linear**	**Quadratic**
Performance								
ADG	0.039	0.035	0.058	0.128	0.129	0.0187	<0.001	0.078
G:F	0.044	0.018	0.052	0.138	0.110	0.0163	<0.001	0.045
Bone characteristics[3]								
Bone ash weight	-0.026	-0.008	0.032	0.074	0.182	0.0195	<0.001	0.138
Bone ash percent	-0.029	0.044	0.083	0.035	0.116	0.0339	0.008	0.721

[1]The marginal intake of available P (aP) per day was calculated for each pen using the equation: dietary aP% - 0.11% (the aP in the basal diet) × 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 criterion.

[2]GraINzyme, Agrivida, Woburn, MA.

[3]One pig per pen (9 pens per treatment) was euthanized and the right fibula was collected to determine bone ash weight and percentage bone ash. Fibulas were autoclaved for 1 hr. After cleaning, bones were placed in a drying oven at 221°F (105°C) for 7 days and then ashed in a muffle furnace at 1,112°F (600°C) for 24 h.

Table 6. Release equations generated from this experiment to determine available phosphorus (aP) for GraINzyme

ADG	aP = (0.255 × FTU) ÷ (1299.969 + FTU)
G:F	aP = (0.233 × FTU) ÷ (1236.428 + FTU)
Bone ash weight	aP = (45999.949 × FTU) ÷ (462529200 + FTU)
Percentage bone ash	aP = (0.272 × FTU) ÷ (2576.581 + FTU)