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K-STATE Research and Extension

SWINE DAY 2022

KANSAS STATE UNIVERSITY SWINE INDUSTRY DAY 2022

Determining the Phosphorus Release Curve for Smizyme TS G5 2,500 Phytase from 500 to 2,500 FTU/kg in Nursery Pig Diets[1]

Katelyn N. Gaffield, Hadley R. Williams, Larissa L. Becker, Jason C. Woodworth, Joel M. DeRouchey, Mike D. Tokach, Robert D. Goodband, Jordan T. Gebhardt,[2] and Jill M. Faser[3]

Summary

A total of 320 pigs (DNA 241 × 600; initially 26.2 ± 0.48 lb BW) were used in a 21-d growth study to determine the available P (aP) release curve for Smizyme TS G5 2,500 (Barentz, Woodbury, MN). At approximately 19 d of age, pigs were weaned, randomly allotted to pens, and fed common starter diets. Pigs were blocked by average pen body weight (BW) and randomly allotted to 1 of 8 dietary treatments on d 18 post-weaning, considered d 0 of the study. Dietary treatments were derived from a single basal diet and ingredients including phytase, monocalcium P, limestone, and sand were added to create the treatment diets. Treatments included 3 diets containing increasing (0.11, 0.19, and 0.27%) inorganic P from monocalcium P, or 5 diets with increasing phytase (500, 1,000, 1,500, 2,000, or 2,500 FTU/kg) added to the diet containing 0.11% aP. All diets were corn-soybean meal-canola meal-based and were formulated to contain 1.24% SID Lys and an analyzed Ca:P ratio of 1.10:1. Prior to the beginning of the study, all pigs were fed a diet containing 0.11% aP for a 2-d period (d 16 to 18 post-weaning). At the conclusion of the study, 1 pig, closest to the mean weight of each pen, was euthanized and the right fibula, rib, and metacarpal were collected to determine bone ash, density, and total bone P. For the overall experimental period, pigs fed increasing inorganic P had improved (quadratic, $P \leq 0.053$) ADG, ADFI, F/G, and final BW. Pigs fed increasing phytase had improved (quadratic, $P \leq 0.004$) ADG, F/G, and final BW and increased (linear, $P = 0.019$) ADFI. For fibula, rib, and metacarpal characteristics, pigs fed increasing levels of aP from inorganic P had increased (linear, $P < 0.001$) bone ash weight, bone ash percentage, bone density, and bone P. Additionally, pigs fed increasing phytase had increased ($P < 0.05$) bone ash weight, bone ash percentage, bone density, and bone P in either a linear or quadratic manner depending upon bone. The available P release curve generated for Smizyme TS G5 2,500 for percentage bone ash is: aP = (0.219 × FTU) ÷ (993.238 + FTU).

[1] Appreciation is expressed to Barentz (Woodbury, MN) for partial financial support of this trial.

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Introduction

Typical corn-soybean meal diets contain phytate which acts as a major storage form of phosphorus in plant-based ingredients.[4] However, this form of phosphorus is largely unavailable to swine due to their lack of the enzyme, phytase. Therefore, it is common for most swine diets to contain an exogenous microbial phytase. This practice helps maximize economics by decreasing the use of expensive inorganic forms of phosphorus, reduces antinutritional properties of phytate, and minimizes phosphorus excretion.

There are multiple phytase sources currently available to producers. However, the efficacy of phytase varies based on multiple factors including phytase origin, activity in digestive tract, affinity to phytate, and resistance to degradation. Smizyme TS G5 2,500 has been shown to improve performance and increase bone ash as phytase increased from 150 to 1,000 FTU/kg.[5] Further, a trial was conducted to determine the release curve of Smizyme TS G5 2,500 up to 1,500 FTU/kg.[5] However, there is currently no research characterizing the response in growth performance, bone characteristics, or aP release above 1,500 FTU/kg. Therefore, the objective of this study was to evaluate the effects on growth performance and bone characteristics of 26- to 53-lb nursery pigs and to develop an aP release curve for Smizyme TS G5 2,500 included from 500 to 2,500 FTU/kg.

Procedures

The protocol used in this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee. The study was conducted at the Kansas State University Swine Teaching and Research Center in Manhattan, KS. Pigs were provided *ab libitum* access to feed and water with each pen containing a 4-hole, dry self-feeder and nipple waterer.

A total of 320 pigs (DNA 241 × 600) were weaned at approximately 19 d of age. Following weaning, pigs were randomly allotted to pens and fed common starter diets. Pigs were blocked by BW (initially 26.2 ± 0.48 lb BW) and randomly allotted to 1 of 8 dietary treatments on d 18 post-weaning, considered d 0 of the study. There were 5 pigs per pen (3 barrows and 2 gilts or 2 barrows and 3 gilts) and 8 pens per treatment. Treatments included 3 diets containing increasing (0.11, 0.19, and 0.27%) inorganic P from monocalcium P, or 5 diets with increasing (500, 1,000, 1,500, 2,000, or 2,500 FTU/kg) phytase added to the diet containing 0.11% aP. Prior to the beginning of the study, all pigs were fed a diet containing 0.11% aP for a 2-d period (d 16 to 18 post-weaning). All diets were corn-soybean meal-canola meal-based and were formulated to contain 1.24% SID Lys and an analyzed Ca:P ratio of 1.10:1.

A single base diet was manufactured at Hubbard Feeds in Beloit, KS, with limestone, monocalcium phosphate, sand, and phytase additions added and mixed at the O.H. Kruse Feed Technology Innovation Center at Kansas State University, Manhattan, KS. The phytase premix was analyzed to determine inclusion rate and was found to contain 2,300,000 FTU/kg. Additionally, Ca and P concentrations were determined for ingre-

[4] Eeckhout, W., and M. De Paepe. 1994. Total phosphorus, phytate-phosphorus and phytase activity in plant feedstuffs. Animal Feed Science and Technology. 47:19-29. doi:10.1016/0377-8401(94)90156-2.
[5] Wensley, M. R., J. M. DeRouchey, J. C. Woodworth, M. D. Tokach, R. D. Goodband, S. S. Dritz, J. M. Fraser, and B. L. Guo. 2020. Determining the phosphorus release of Smizyme TS G5 2,500 phytase in diets for nursery pigs. Transl. Anim. Sci. 4(3). doi: 10.1093/tas/txaa058.

dients prior to manufacturing (Table 1). All diets contained 7.5% canola meal, and because of its high phytate P concentration, resulted in a diet with a calculated 0.30% phytate P. Dietary treatments were derived from eight, 1-ton batches of basal diet (Table 2). For each diet, a subset of basal diet was added to the mixer along with treatment-specific ingredients to produce the 8 final experimental diets. During bagging, complete diet samples were collected from every fourth bag, pooled, ground to reduce particle size, and stored at -4°F (-20°C). Samples were submitted for duplicate analysis of Ca and P (K-State Research and Extension Soil Testing Laboratory, Manhattan, KS) and two samples of each diet was submitted for complete phytase analysis (Barentz, Woodbury, MN) using the AOAC[6] official method 2000.12.

Throughout the 21-d experiment, pig and feeder weights were measured every 7 d to determine ADG, ADFI, and F/G. At the conclusion of the study, 1 pig, closest to the mean weight of each pen, was euthanized. The right fibula, rib, and metacarpal were collected, individually placed in plastic bags with identification, and stored at -4°F (-20°C) until bone analysis. For bone analyses, leftover extraneous soft tissue and cartilage caps were removed from each bone. For bone density, bones were submerged in ultra-purified water under vacuum for 4 h. Bones were then weighed while suspended in a vessel of water and the weights used to calculate bone density. For bone ash, bones were processed using the non-defatted method. Each fibula was dried at 221°F (105°C) for 7 d in a drying oven and subsequently ashed at 1,112°F (600°C) for 24 h in a muffle furnace. This method was used to determine total bone ash weight and percentage ash relative to dried bone weight. For bone P, a portion of the residual bone ash was placed in a tube with nitric acid and incubated at 140°F (60°C) for 6 h. The solution was then diluted using ultra-purified water and the P quantity was measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Data analysis

Data were analyzed as a randomized complete block design with pen as the experimental unit, treatment as a fixed effect, and weight block as a random effect. The base model was evaluated using the MIXED procedure of SAS v. 9.4 (SAS Institute, Inc., Cary, NC). Linear and quadratic contrasts were constructed within increasing inorganic P or phytase treatments. Results were considered significant with P-values ≤ 0.05 and were considered marginally significant with P-values ≤ 0.10.

Marginal intake of aP per day was calculated for each pen of pigs fed the inorganic P diets. The equation is as follows: dietary aP% minus 0.11% (the aP in the basal diet) multiplied by ADFI. This served as the predictor value to develop a standard curve for each response criterion. 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. Using the pen ADFI, this value was then converted to a marginal aP%.

A mixed model ANOVA with weight block as the random effect was used to evaluate aP release as a function of the calculated phytase dosage, assuming an intercept of no aP release for the 0.11% aP diet without phytase. Additionally, to evaluate the average aP release generated using data from all three bones, bone was added as a random effect

[6] AOAC. 2000. Official methods of analysis AOAC international. 17th ed. Gaithersburg, (MD): Association of Official Analytical Chemists.

to the model. Formulated phytase levels were used to calculate all release values. To maintain consistent units of measure, gain-to-feed ratios were used to determine the aP release for feed efficiency.
A model was fitted to pen release values using non-linear regression. The model parameters were estimated using the nls function from the stat package in R (version 3.5.1 (2018-07-02)) in order to develop aP release curves for G:F, bone ash weight, percent bone ash, bone density, and bone P.

Results and Discussion

Analysis of final diets for Ca, P, and phytase activity were similar to diet formulation (Table 3). Phytase activity of complete diets increased across the phytase treatments with analyzed phytase concentrations of 519, 1,046, 1,507, 2,036, and 2,499 FTU/kg.

From d 0 to 21, pigs had increased (quadratic, $P \leq 0.033$) ADG, ADFI, and final BW when fed increasing aP from inorganic P (Table 4). Additionally, pigs fed increasing inorganic P had improved (linear, $P < 0.001$ and quadratic, $P = 0.053$) feed efficiency. Similarly, pigs fed increasing phytase had improved (quadratic, $P \leq 0.004$) ADG, feed efficiency, and final BW. Furthermore, ADFI increased in a linear fashion ($P = 0.019$) for pigs fed increasing levels of phytase.

For fibula, rib, and metacarpal characteristics, pigs fed increasing levels of aP from inorganic P resulted in a linear increase ($P < 0.001$) for all bone properties with rib bone density also exhibiting an increase in a quadratic fashion (quadratic, $P = 0.002$). For fibula characteristics, pigs fed increasing phytase had increased bone ash weight (linear, $P < 0.001$), bone ash percentage (linear, $P < 0.001$ and quadratic, $P = 0.022$), bone density (linear, $P = 0.008$ and quadratic, $P = 0.032$), and bone P (linear, $P < 0.001$). Additionally, for rib characteristics, pigs fed increasing phytase resulted in increased bone ash weight (linear, $P < 0.001$ and quadratic, $P = 0.038$), bone ash percentage (linear, $P < 0.001$ and quadratic, $P = 0.005$), bone density (linear, $P < 0.001$ and quadratic, $P = 0.027$), and bone P (linear, $P < 0.001$). Furthermore, for metacarpal characteristics, pigs fed increasing phytase had increased bone ash weight (linear, $P < 0.001$), bone ash percentage (linear, $P < 0.001$ and quadratic, $P = 0.016$), bone density (linear, $P < 0.001$ and quadratic, $P = 0.086$), and bone P (linear, $P < 0.001$). The calculated percentage aP released from Smizyme TS G5 2,500 followed the same trends as the means previously listed. The calculated percentage of aP released would vary depending on the growth performance, different bone, and bone criteria measured (Table 5). The release values for each response criterion averaged across the three bones may provide the most robust estimate of aP release.

A previous study,[5] conducting two separate experiments, developed aP release values for Smizyme TS G5 2,500 up to 1,500 FTU. The calculated aP release values for bone ash percentage generated using data from all three bones from the current study are lower at both the 500 and 1,000 FTU/kg inclusion levels, but similar at the 1,500 FTU/kg inclusion level when compared to the previous study.

For Smizyme TS G5 2,500, this study has provided an aP release curve for phytase use in swine diets for nursery pigs weighing 26- to 53-lb at inclusion levels between 500 and 2,500 FTU/kg (Figures 1 to 4). The response criteria measured influenced the magnitude of aP release as FTU inclusion rates increased. Following are the aP (%) release

equations generated for Smizyme TS G5 2,500 for G:F, bone ash weight, percentage bone ash, bone density, and bone P: aP = (0.139 × FTU) ÷ (138.306 + FTU); aP = (0.323 × FTU) ÷ (2,480.166 + FTU); aP = (0.219 × FTU) ÷ (993.238 + FTU); aP = (0.228 × FTU) ÷ (1,086.047 + FTU); and aP = (0.565 × FTU) ÷ (6,889.378 + FTU), respectively.

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

Ingredient	Ca, %	P, %
Limestone[1]	31.88	0.01
Monocalcium P[1]	16.35	18.76
Canola meal[1]	0.60	1.01
Corn[2]	0.07	0.24
Soybean meal[2]	0.56	0.64

[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	60.90
Soybean meal	29.95
Canola meal	7.61
Sodium chloride	0.61
L-Lys-HCl	0.30
DL-Met	0.10
L-Thr	0.10
L-Val	0.01
Trace mineral premix	0.15
Vitamin premix	0.25
Total	100
Calculated analysis	
Standardized ileal digestible (SID) amino acids	
Lys, %	1.24
Ile:Lys	64
Leu:Lys	130
Met:Lys	33
Met and Cys:Lys	59
Thr:Lys	64
Trp:Lys	18.7
Val:Lys	71
His:Lys	43
Total Lys, %	1.44
ME, kcal/lb	1,500
NE, kcal/lb	1,095
SID Lys:NE, g/Mcal	5.14
CP, %	22.6
Ca, %	0.33
P, %	0.42
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**	**500**	**1,000**	**1,500**	**2,000**	**2,500**
Basal mix	98.55	98.55	98.55	98.55	98.55	98.55	98.55	98.55
Limestone	0.35	0.40	0.45	0.35	0.35	0.35	0.35	0.35
Monocalcium P	0.16	0.58	1.01	0.16	0.16	0.16	0.16	0.16
Sand[3]	0.95	0.48	0.00	0.92	0.90	0.88	0.86	0.84
Phytase[4]	----	----	----	0.0217	0.0435	0.0652	0.0870	0.1087
Total	100	100	100	100	100	100	100	100
Calculated analysis								
CP, %	22.3	22.3	22.3	22.3	22.3	22.3	22.3	22.3
Ca, %	0.48	0.57	0.66	0.48	0.48	0.48	0.48	0.48
P, %	0.44	0.52	0.60	0.44	0.44	0.44	0.44	0.44
Phytase, FTU/kg	----	----	----	500	1,000	1,500	2,000	2,500
Ca:P ratio	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Analyzed composition[5]								
Ca, %	0.52	0.63	0.74	0.50	0.48	0.54	0.56	0.51
P, %	0.45	0.56	0.65	0.44	0.45	0.46	0.46	0.45
Phytase, FTU/kg[6]	----	----	----	519	1,046	1,507	2,036	2,499

[1]Diets were fed for 21 d starting at approximately 26.2 ± 0.48 lb BW.
[2]Smizyme TS G5, Barentz, Rosemount, MN.
[3]Sand was used to equalize weight of hand-add batch including the addition of limestone, monocalcium P, and phytase when blended with the basal mix.
[4]Phytase was analyzed for phytase level and contained 2,300,000 FTU/kg (Eurofins Scientific Inc., Des Moines, IA).
[5]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 triplicate analysis of Ca and P (K-State Research and Extension Soil Testing Laboratory, Manhattan, KS).
[6]Two samples of each diet were submitted to Barentz (Woodbury, MN) for complete phytase analysis using the AOAC(2000) official method 2000.12.

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]

	Inorganic P, % aP[2]			Phytase, FTU/kg[3]						Inorganic P, *P* =		Phytase, *P* =	
Item	0.11	0.19	0.27	500	1,000	1,500	2,000	2,500	SEM	Linear	Quadratic	Linear	Quadratic
BW, lb													
d 0	26.2	26.2	26.2	26.2	26.1	26.1	26.1	26.1	0.48	0.824	0.867	0.797	0.883
d 21	47.8	52.7	53.2	53.3	52.1	52.2	53.5	53.4	0.83	< 0.001	0.009	< 0.001	0.004
d 0 to 21													
ADG, lb	1.02	1.26	1.29	1.29	1.24	1.24	1.27	1.30	0.032	< 0.001	0.005	< 0.001	0.002
ADFI, lb	1.70	1.90	1.87	1.93	1.85	1.83	1.88	1.91	0.045	0.007	0.033	0.019	0.190
F/G	1.67	1.51	1.45	1.50	1.49	1.48	1.48	1.47	0.022	< 0.001	0.053	< 0.001	< 0.001
Bone characteristics[4]													
Fibula													
Bone ash, g	0.611	0.749	0.982	0.739	0.808	0.902	0.974	1.002	0.043	< 0.001	0.381	< 0.001	0.224
Bone ash, %	42.5	45.3	49.1	45.0	46.0	48.5	48.5	48.2	0.83	< 0.001	0.637	< 0.001	0.022
Bone density, g/ml	1.17	1.17	1.22	1.19	1.20	1.21	1.21	1.20	0.011	0.001	0.119	0.008	0.032
P, g	0.107	0.151	0.178	0.130	0.129	0.178	0.173	0.184	0.010	< 0.001	0.505	< 0.001	0.406
Rib													
Bone ash, g	0.730	1.035	1.201	0.894	1.027	1.107	1.171	1.199	0.050	< 0.001	0.253	< 0.001	0.038
Bone ash, %	44.4	47.9	51.5	47.8	48.5	50.4	51.4	51.0	0.686	< 0.001	0.945	< 0.001	0.005
Bone density, g/ml	1.17	1.17	1.24	1.18	1.20	1.22	1.24	1.22	0.008	< 0.001	0.002	< 0.001	0.027
P, g	0.126	0.204	0.238	0.158	0.176	0.200	0.214	0.216	0.015	< 0.001	0.175	< 0.001	0.166
Metacarpal													
Bone ash, g	1.008	1.290	1.484	1.171	1.283	1.381	1.435	1.523	0.055	< 0.001	0.520	< 0.001	0.233
Bone ash, %	32.4	34.9	39.0	35.2	36.9	37.8	39.2	38.0	0.944	< 0.001	0.505	< 0.001	0.016
Bone density, g/mL	1.14	1.16	1.18	1.16	1.16	1.17	1.18	1.18	0.005	< 0.001	0.437	< 0.001	0.086
P, g	0.188	0.228	0.253	0.193	0.227	0.233	0.244	0.268	0.012	< 0.001	0.600	< 0.001	0.994

[1]A total of 320 nursery pigs (DNA 241 × 600, initially 26.2 ± 0.48 lb BW) were used in a 21-d growth trial with 5 pigs per pen and 8 replications per treatment.

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

[3]Smizyme TS G5, Barentz, Rosemount, MN.

[4]One pig per pen (8 pens per treatment) was euthanized and the right fibula, rib, and metacarpal were collected to determine bone density, bone ash weight, percentage bone ash, and bone P. After cleaning, bones were submerged in ultra-purified water under vacuum for 4 h. Weights were then collected, and bone density calculated. For bone ash, bones were placed in a drying oven at 221°F (105°C) for 7 d and then ashed in a muffle furnace at 1,112°F (600°C) for 24 h. For bone P, a portion of the residual bone ash was placed in a tube with nitric acid and incubated at 140°F (60°C) for 6 h. The solution was then diluted using ultra-purified water and P quantity measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

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

	Phytase, FTU/kg[2]						*P* =	
Item	**500**	**1,000**	**1,500**	**2,000**	**2,500**	**SEM[4]**	**Linear**	**Quadratic**
Performance								
ADG	0.133	0.108	0.108	0.123	0.140	0.0183	< 0.001	0.002
G:F	0.110	0.117	0.133	0.126	0.134	0.0187	< 0.001	< 0.001
Bone characteristics[3]								
Fibula								
Bone ash, g	0.060	0.093	0.135	0.163	0.170	0.0151	< 0.001	0.102
Bone ash, %	0.060	0.090	0.156	0.143	0.139	0.0215	< 0.001	0.026
Bone density, g/mL	0.080	0.119	0.166	0.157	0.120	0.0349	0.008	0.033
P, g	0.045	0.045	0.155	0.141	0.162	0.0178	< 0.001	0.242
Rib								
Bone ash, g	0.048	0.093	0.122	0.140	0.148	0.0154	< 0.001	0.020
Bone ash, %	0.076	0.096	0.140	0.158	0.148	0.0163	< 0.001	0.004
Bone density, g/mL	0.046	0.104	0.144	0.188	0.139	0.0201	< 0.001	0.041
P, g	0.036	0.061	0.098	0.114	0.116	0.0172	< 0.001	0.069
Metacarpal								
Bone ash, g	0.050	0.087	0.124	0.136	0.163	0.0153	< 0.001	0.125
Bone ash, %	0.074	0.119	0.139	0.169	0.141	0.0226	< 0.001	0.017
Bone density, g/mL	0.069	0.086	0.120	0.162	0.144	0.0170	< 0.001	0.071
P, g	0.009	0.089	0.108	0.129	0.180	0.0269	< 0.001	0.903
Average[4]								
Bone ash, g	0.052	0.091	0.126	0.145	0.160	0.0124	< 0.001	0.023
Bone ash, %	0.071	0.102	0.144	0.158	0.144	0.0174	< 0.001	0.004
Bone density, g/mL	0.064	0.103	0.144	0.171	0.134	0.0192	< 0.001	0.011
P, g	0.031	0.064	0.117	0.126	0.147	0.0139	< 0.001	0.176

[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] Smizyme TS G5, Barentz, Rosemount, MN.

[3] One pig per pen (8 pens per treatment) was euthanized and the right fibula, rib, and metacarpal were collected to determine bone density, bone ash weight, percentage bone ash, and bone P. After cleaning, bones were submerged in ultra-purified water under vacuum for 4 h. Weights were then collected, and bone density calculated. For bone ash, bones were placed in a drying oven at 221°F (105°C) for 7 d and then ashed in a muffle furnace at 1,112°F (600°C) for 24 h. For bone P, a portion of the residual bone ash was placed in a tube with nitric acid and incubated at 140°F (60°C) for 6 h. The solution was then diluted using ultra-purified water and P quantity measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

[4]Average aP release values generated using data from the right fibula, rib, and metacarpal.

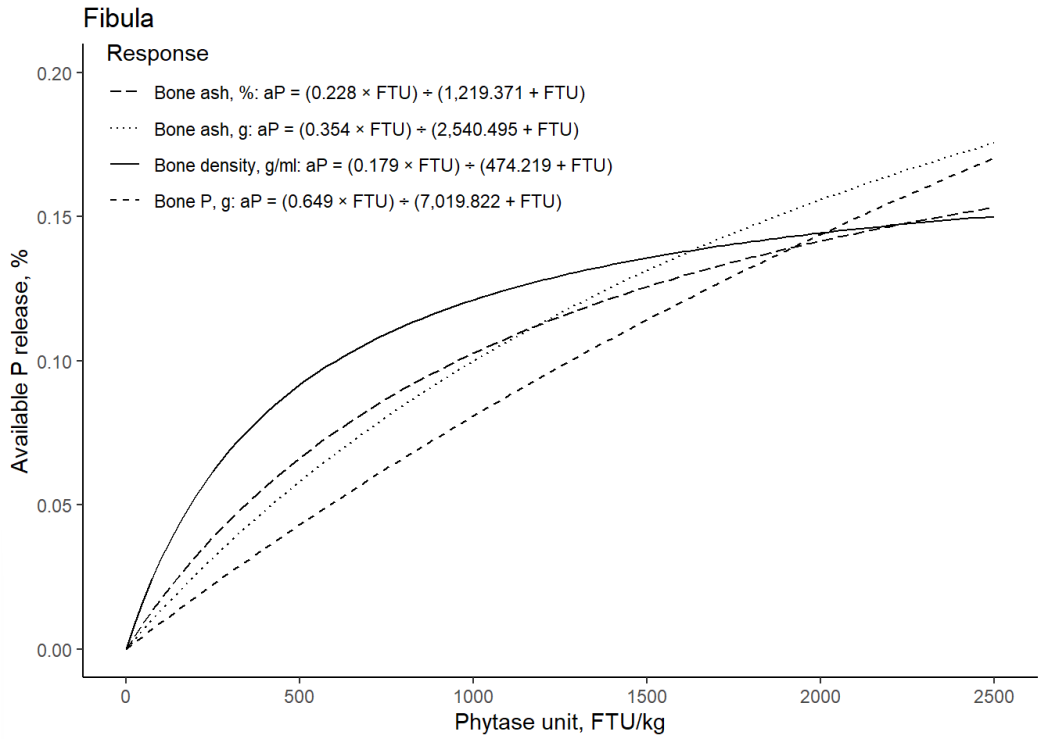

Figure 1. Available P release curves for right fibula characteristics including percentage bone ash, bone ash weight, bone density, and bone P generated using the release equations for Smizyme TS G5 2,500 from this experiment.

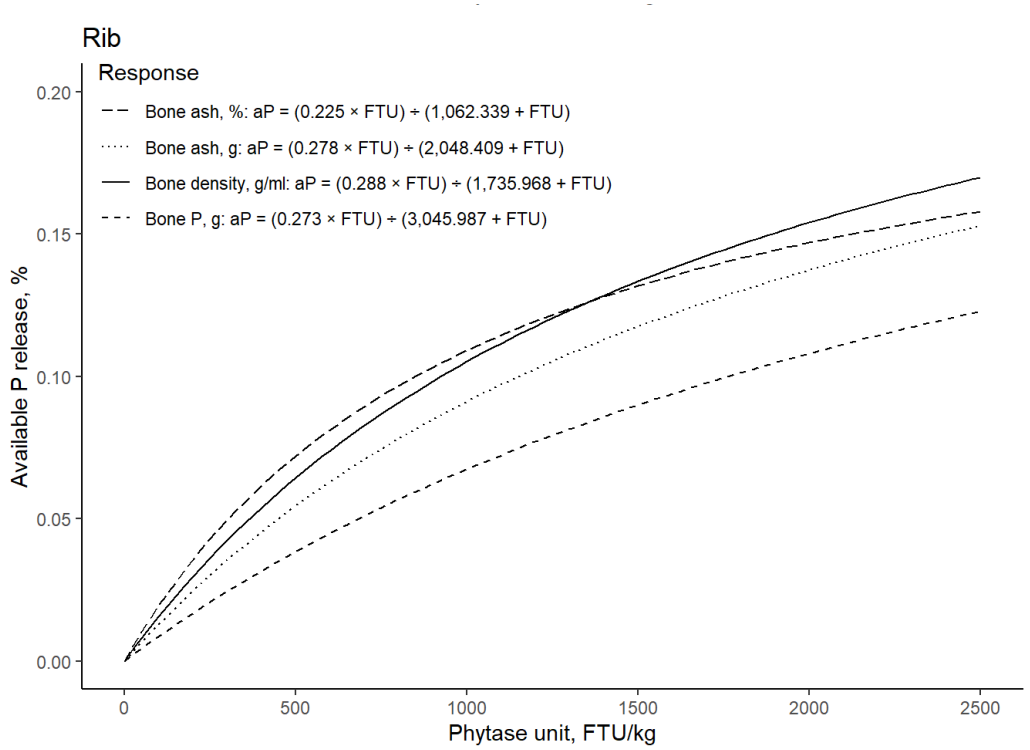

Figure 2. Available P release curves for right rib characteristics including percentage bone ash, bone ash weight, bone density, and bone P generated using the release equations for Smizyme TS G5 2,500 from this experiment.

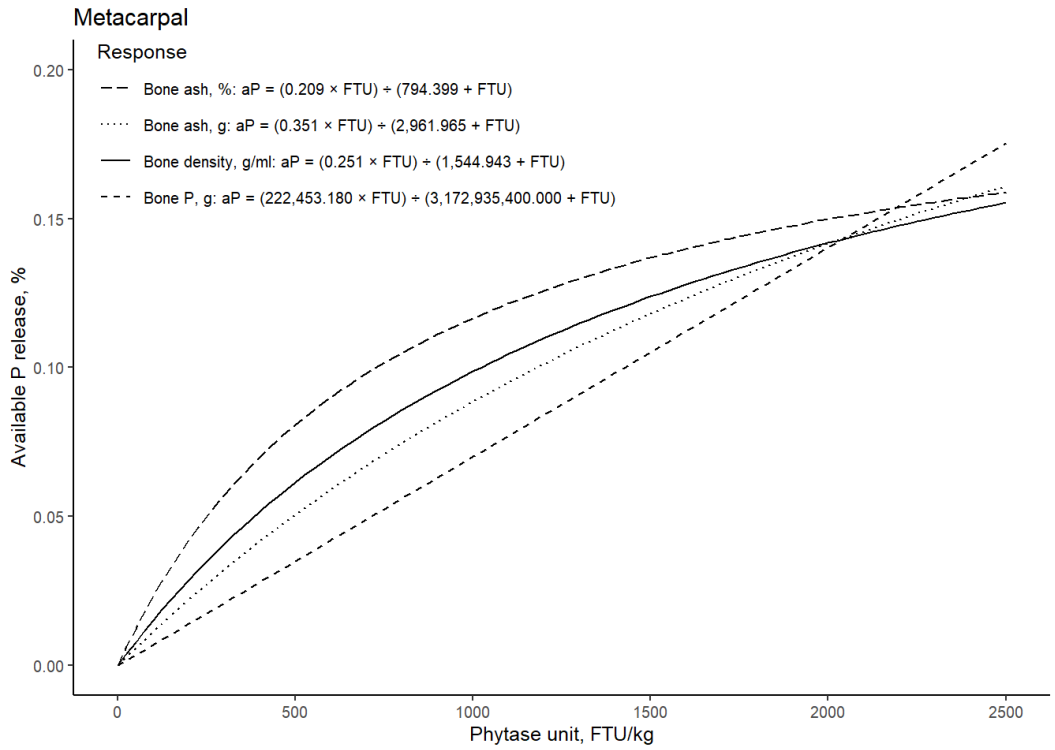

Figure 3. Available P release curves for right metacarpal characteristics including percentage bone ash, bone ash weight, bone density, and bone P generated using the release equations for Smizyme TS G5 2,500 from this experiment.

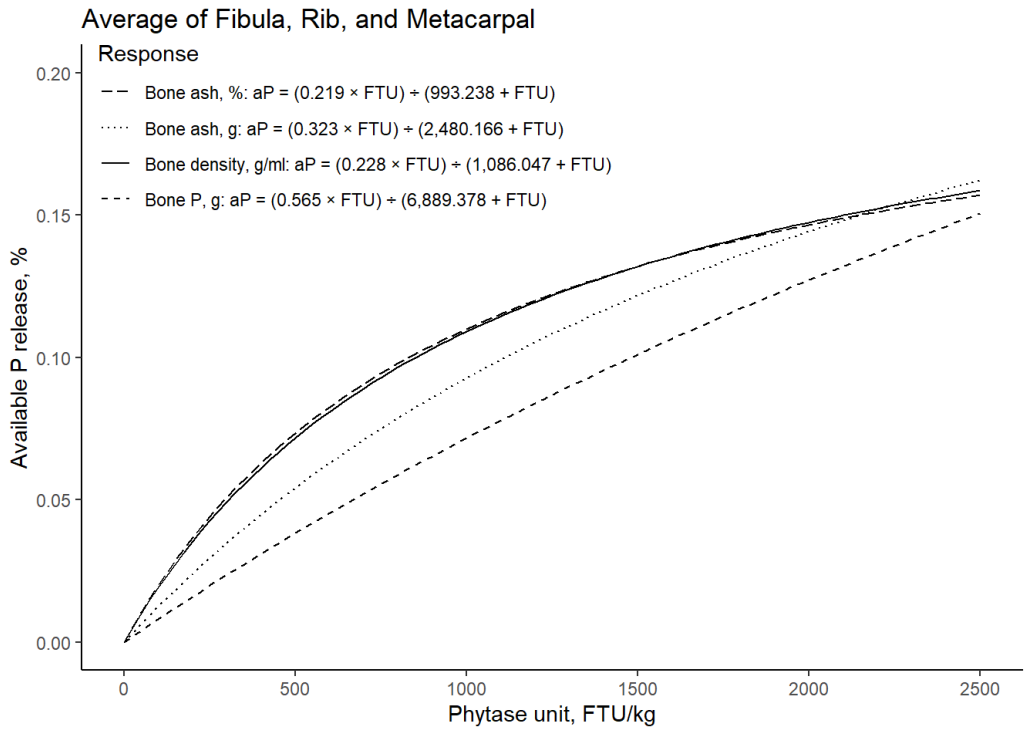

Figure 4. Available P release curves for bone characteristics averaged from the right fibula, rib, and metacarpal including percentage bone ash, bone ash weight, bone density, and bone P generated using the release equations for Smizyme TS G5 2,500 from this experiment.