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Ty H. Kim
Kansas State University, Manhattan, thkim@k-state.edu

Katelyn N. Gaffield
Kansas State University, Manhattan, gaffield@k-state.edu

Mike D. Tokach
Kansas State University, Manhattan, mtokach@k-state.edu

See next page for additional authors

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K-STATE Research and Extension

SWINE DAY 2023

KANSAS STATE UNIVERSITY SWINE INDUSTRY DAY 2023

Determining the Phosphorus Release Curve for Sunphase HT Phytase from 250 to 2,000 FTU/kg in Nursery Pig Diets[1]

Ty H. Kim, Katelyn N. Gaffield, Mike D. Tokach, Joel M. DeRouchey, Jason C. Woodworth, Robert D. Goodband, Jordan T. Gebhardt,[2] Ying Zhou,[3] Xuerong Song,[3] Xiuyi Wu,[3] Mikhail Y. Golovko,[4] and Svetlana A. Golovko[4]

Summary

A total of 280 pigs (DNA 241 × 600; initially 22.9 ± 0.52 lb BW) were used in a 21-d growth study to determine the available P (aP) release curve for Sunphase HT phytase (Wuhan Sunhy Biology Co., Ltd.; Wuhan, P.R. China). 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 7 dietary treatments on d 21 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% aP) inorganic P from monocalcium P, or 4 diets with increasing phytase (250, 500, 1,000, or 2,000 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 3-d period (d 18 to 21 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 and density. For the overall experimental period, pigs fed increasing levels of aP from inorganic P had improved (linear, $P \leq 0.014$) ADG, F/G, and final BW. Similarly, pigs fed increasing phytase had increased (linear, $P \leq 0.011$) ADG and final BW as well as improved (quadratic, $P = 0.017$) F/G. For fibula bone ash weight and percentage bone ash, rib bone ash weight and bone density, and all metacarpal bone properties, pigs fed increasing levels of aP from inorganic P exhibited a linear improvement ($P \leq 0.019$), with a quadratic response ($P \leq 0.030$) for fibula bone density and rib percentage bone ash. Additionally, pigs fed increasing phytase had increased ($P < 0.05$) bone ash weight, percentage bone ash, and bone density in either a linear or quadratic fashion depending on the bone analyzed. The

[1] The authors appreciate Wuhan Sunhy Biology Co., Ltd. (Wuhan, P.R. China) for partial financial support of this trial.

[2] Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University.

[3] Wuhan Sunhy Biology Co., Ltd. (Wuhan, P.R. China).

[4] Department of Biomedical Sciences, University of North Dakota (Grand Forks, ND).

available P release curve generated for Sunphase HT for percentage bone ash combining values from right fibula, rib, and metacarpal is: aP = (0.360 × FTU) ÷ (2,330.250 + FTU).

Introduction

Most swine diets consist of plant-based ingredients containing phytate, which is a major storage form of phosphorus. However, phytate-bound phosphorus is largely unavailable for digestion and absorption to swine due to their inadequate production of the digestive enzyme, phytase. As a result, most swine diets are formulated with an exogenous microbial phytase, which makes plant-derived dietary P more available for utilization by swine. In turn, this enzyme decreases the use of inorganic forms of phosphorus, which lowers feed costs, reduces antinutritional properties of phytate, and minimizes the environmental impact by reducing phosphorus excretion.

As the feed industry develops new and next generation phytase sources, an evaluation of their efficacy is needed to properly formulate swine diets. One such new product, Sunphase HT, needs research to help determine the aP release curve to be used in diet formulation. Therefore, the objective of this study was to evaluate the effects on growth performance and bone properties of 23 to 47-lb nursery pigs and to develop an aP release curve for Sunphase HT included from 250 to 2,000 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. Each pen contained a 4-hole, dry self-feeder, and nipple waterer for *ad libitum* access to feed and water.

A total of 280 pigs (DNA 241 × 600) were weaned at approximately 21 d of age. Following weaning, pigs were randomly allotted to pens and fed common starter diets. On d 18 post-weaning, all pigs were fed a diet containing 0.11% aP for a 3-d period. Then, on d 21 post-weaning, considered d 0 of the study, pigs were blocked by BW (initially 22.9 ± 0.52 lb BW) and randomly allotted to 1 of 7 dietary treatments. 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 4 diets with increasing (250, 500, 1,000, and 2,000 FTU/kg) phytase added to the diet containing 0.11% aP. All diets were corn-soybean 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. Additions of limestone, monocalcium phosphate, sand, and phytase were added to the base diet and mixed at the Kansas State University Poultry Unit in Manhattan, KS. The phytase premix was analyzed to determine the inclusion rate and was found to contain 11,158,000 FTU/kg. Additionally, Ca and P concentrations were determined for monocalcium P and limestone prior to manufacturing (Table 1). The Ca and P concentrations for corn, soybean meal, and canola meal were based on historical analysis at the feed mill. All diets contained 7.5% canola meal, and due to its high phytate P concentration, resulted in a diet with a calculated 0.32% phytate P. Dietary treatments

were derived from eight, 1-ton pallets 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 7 final experimental diets. During bagging, complete diet samples were collected from every fourth bag, pooled, and stored at -4°F. Before analysis, samples were ground to reduce particle size. Two samples of each diet were submitted, one for analysis in duplicate and one for analysis in triplicate for Ca at the KSU Soils Lab, Manhattan, KS (AOAC 985.01, 2006),[5] and the average values were calculated. One sample of each diet was submitted for analysis in triplicate for P at Midwest Laboratories, Omaha, NE.[5] Additionally, one sample of each diet was submitted for complete phytase and phytic acid analysis (Eurofins Nutrition Analysis Center, Des Moines, IA) using the AOAC official method 2000.12 (AOAC, 2000)[6] and the method outlined in Analytical Biochemistry Vol. 77:536-539 (1977)[7] for the respective analyses.

Throughout the experiment, pig and feeder weights were collected every 7 d to determine ADG, ADFI, and F/G. At the conclusion of the 21-d study, blood was collected from the jugular vein of 1 pig, closest to the mean weight of each pen, using an EDTA anti-coagulant blood collection tube to determine plasma inositol concentrations. Blood samples were centrifuged at 39.2°F at 1,500 × g for 15 min. Plasma was frozen in separate aliquots for later analysis for plasma inositol, conducted at the University of North Dakota. For development of an internal standard, retained serum samples (10 µL) from the laboratory were mixed with 30 µL of 75% methanol containing 100 ng of myo-inositol-1,2,3,4,5,6-d_6 (Medical Isotopes, Pelham, NH) as an internal standard. After vortexing and centrifugation for 10 min at 2,000 g, 10 µL of supernatant was injected into the LC-MS system for quantification. After blood collection, the pig was euthanized and the right fibula, tenth rib, and metacarpal were collected, individually placed in plastic bags with permanent identification, and stored at -4°F 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 suspended in a vessel of water and weighed. The weights were then used to calculate bone density. For bone ash, bones were processed using the non-defatted method. Each bone was dried at 221°F for 7 d in a drying oven and subsequently ashed at 1,112°F for 24 h in a muffle furnace to determine total bone ash weight and ash percentage relative to dried bone weight.

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 GLIMMIX procedure of SAS version 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.

[5] AOAC. 2006. Official methods of analysis AOAC international. 18th ed. Arlington (VA): Association of Official Analytical Chemists.

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

[7] Ellis, R., E. R. Morris, and C. Philpot. 1977. Quantitative determination of phytate in the presence of high inorganic phosphate. Anal Biochem. 77:536–539. doi:10.1016/0003-2697(77)90269-X.

For each pen of pigs fed the inorganic P diets, marginal intake of aP per day was calculated according to the following equation: dietary aP% minus 0.11% (the aP in the basal diet) multiplied by ADFI. Using the marginal aP release as the predictor variable, a standard curve was developed for each of the response criteria. The equation for the standard curve was used to calculate the 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. Additionally, to evaluate the average aP release generated using data from all three bones, treatment and bone were added as fixed effects and block and pen were added as random effects 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 fit to pen release values using non-linear regression. The model parameters were estimated using the nls function from the stat package in R (version 4.2.1 (2022-06-23)) in order to develop aP release curves for G:F, percentage bone ash, and bone density.

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 220, 400, 960, and 1,700 FTU/kg for the four treatments. Additionally, analysis of final diets for phytate % was similar across treatments, resulting in phytate P similar to diet formulation (Table 2).

From d 0 to 21, pigs fed increasing aP from inorganic P had increased final BW (linear, $P = 0.014$) due to increased ADG ($P = 0.009$; Table 4). Additionally, pigs fed increasing inorganic P had improved (linear, $P = 0.002$) feed efficiency. Pigs fed increasing phytase had increased (linear, $P = 0.007$) final BW driven by an improvement in ADG ($P = 0.011$). Furthermore, feed efficiency improved quadratically ($P = 0.017$) when pigs were fed increasing levels of phytase.

For bone characteristics, feeding increasing levels of aP from inorganic P resulted in a linear increase ($P \leq 0.019$) for all fibula, rib, and metacarpal bone properties, with fibula bone density and rib percentage bone ash also exhibiting an increase in a quadratic fashion ($P \leq 0.030$). Similarly, feeding increasing phytase resulted in a linear increase ($P < 0.001$) for all fibula bone characteristics, with fibula bone density also showing a quadratic response ($P = 0.005$). For all rib bone properties, increasing dietary phytase resulted in a linear increase ($P < 0.001$), with rib bone density also exhibiting a quadratic increase ($P = 0.047$) and rib percentage bone ash exhibiting a tendency for a quadratic response ($P = 0.080$). Furthermore, pigs fed increasing phytase had a linear increase ($P < 0.001$) in all metacarpal bone characteristics, with metacarpal bone ash weight and bone density also increasing in a quadratic fashion ($P \leq 0.037$).

Additionally, feeding increasing phytase resulted in a linear increase in plasma inositol concentration ($P = 0.045$).

The calculated percentage aP released from Sunphase HT phytase followed the same trends as the means listed above with calculated percent aP released varying depending on the growth performance, specific bone, and bone characteristic measured (Table 5). The available P release curve generated for Sunphase HT for percentage bone ash, combining values from right fibula, rib, and metacarpal is: aP = (0.360 × FTU) ÷ (2,330.250 + FTU). The release values for each response criteria averaged across the three bones may provide the most robust estimate of aP release.

For Sunphase HT, this study has provided an aP release curve for use in swine diets using data from nursery pigs weighing 23- to 47-lb at inclusion levels between 250 and 2,000 FTU/kg (Figure 1). The response criteria considered in this trial influenced the magnitude of aP release as FTU inclusion rates increased differently. Following are the aP (%) release equations generated for Sunphase HT for G:F, bone ash weight, percentage bone ash, and bone density: aP = (0.245 × FTU) ÷ (175.989 + FTU); aP = (0.344 × FTU) ÷ (1,156.954 + FTU); aP = (0.360 × FTU) ÷ (2,330.250 + FTU); and aP = (0.260 × FTU) ÷ (944.019 + FTU), respectively.

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

Ingredient	Ca, %	P, %
Limestone[1]	37.75	0.01
Monocalcium P[1]	18.57	23.09

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

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

Ingredient, %	
Corn	60.94
Soybean meal	29.93
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	63
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, %[2]	22.6
Ca, %	0.33
P, %	0.41
Available P, %	0.07
STTD P, %	0.17
Phytate P, %	0.32

[1]The basal batch was used as the major ingredient in each experimental diet.
[2]CP = crude protein.

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

	Inorganic P			Phytase[2]			
Ingredient, %	**0.11**	**0.19**	**0.27**	**250**	**500**	**1,000**	**2,000**
Basal mix	98.62	98.62	98.62	98.62	98.62	98.62	98.62
Limestone	0.37	0.43	0.49	0.37	0.37	0.37	0.37
Monocalcium P	0.19	0.54	0.89	0.19	0.19	0.19	0.19
Sand[3]	0.82	0.41	0.00	0.82	0.81	0.81	0.80
Phytase[4]	----	----	----	0.0022	0.0045	0.0090	0.0179
Total	100	100	100	100	100	100	100
Calculated analysis							
CP, %[5]	22.3	22.3	22.3	22.3	22.3	22.3	22.3
Ca, %	0.50	0.59	0.67	0.50	0.50	0.50	0.50
P, %	0.45	0.53	0.61	0.45	0.45	0.45	0.45
Phytase, FTU/kg	----	----	----	250	500	1,000	2,000
Ca:P ratio	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Phytate P, %	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Analyzed composition[6]							
Ca, %	0.51	0.57	0.76	0.50	0.47	0.53	0.53
P, %	0.48	0.54	0.65	0.48	0.47	0.47	0.49
Phytase, FTU/kg[7]	----	----	----	220	400	960	1,700
Phytic acid, %[7]	1.15	1.16	1.18	1.12	1.17	1.12	1.17

[1]Diets were fed for 21 d starting at approximately 22.9 ± 0.52 lb BW.
[2]Sunphase HT, Wuhan Sunhy Biology Co., Ltd. (Wuhan, P.R. China).
[3]Sand was used to equalize hand-added batches including the addition of limestone, monocalcium P, and phytase when blended with the basal mix.
[4]Phytase was analyzed and contained 11,158,000 FTU/kg (Wuhan Sunhy Biology Co., Ltd., Wuhan, P.R. China).
[5]CP = crude protein.
[6]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 until they were submitted for analysis of Ca (K-State Research and Extension Soil Testing Laboratory, Manhattan, KS) and P (Midwest Laboratories, Omaha, NE).
[7]One sample of each diet was submitted to Eurofins Nutrition Analysis Center (Des Moines, IA) for complete phytase and phytic acid analysis using the AOAC official method 2000.12 (AOAC, 2000) and the method outlined in Analytical Biochemistry Vol. 77:536-539 (1977) for the respective analyses.

Table 4. Effects of increasing aP from inorganic P or Sunphase HT 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**	**250**	**500**	**1,000**	**2,000**	**SEM**	**Linear**	**Quadratic**	**Linear**	**Quadratic**
BW, lb												
d 0	22.8	23.0	23.0	22.9	23.0	22.9	22.9	0.52	0.440	0.566	0.941	0.696
d 21	45.7	47.0	48.6	45.8	47.6	47.4	48.6	0.98	0.014	0.877	0.007	0.487
d 0 to 21												
ADG, lb	1.08	1.13	1.22	1.06	1.16	1.11	1.20	0.037	0.009	0.589	0.011	0.987
ADFI, lb	1.70	1.71	1.80	1.60	1.70	1.64	1.74	0.052	0.143	0.463	0.250	0.258
F/G	1.58	1.52	1.48	1.50	1.47	1.47	1.45	0.023	0.002	0.753	0.001	0.017
Bone characteristics[4]												
Fibula												
Bone ash, g	0.600	0.734	0.821	0.733	0.707	0.844	0.956	0.038	< 0.001	0.615	< 0.001	0.231
Bone ash, %	43.1	46.2	47.8	44.5	44.9	47.3	48.3	0.848	< 0.001	0.468	< 0.001	0.158
Bone density, g/mL	1.15	1.22	1.23	1.19	1.20	1.22	1.23	0.012	< 0.001	0.030	< 0.001	0.005
Rib												
Bone ash, g	0.753	0.913	1.168	0.945	0.922	1.102	1.318	0.061	< 0.001	0.530	< 0.001	0.408
Bone ash, %	46.4	50.8	51.0	48.5	48.9	51.2	53.1	0.692	< 0.001	0.016	< 0.001	0.080
Bone density, g/mL	1.20	1.22	1.24	1.20	1.22	1.26	1.25	0.011	0.019	0.932	< 0.001	0.047
Metacarpal												
Bone ash, g	0.872	1.073	1.277	1.053	1.160	1.182	1.359	0.039	< 0.001	0.972	< 0.001	0.018
Bone ash, %	32.5	34.7	37.7	32.5	34.5	35.7	36.1	0.828	< 0.001	0.723	< 0.001	0.119
Bone density, g/mL	1.13	1.16	1.18	1.15	1.16	1.17	1.18	0.006	< 0.001	0.338	< 0.001	0.037
Plasma inositol, ng/μL[5]	9.59	7.47	8.36	9.82	9.16	11.19	12.22	1.153	0.447	0.281	0.045	0.881

[1]A total of 280 nursery pigs (DNA 241 × 600; initially 22.9 ± 0.52 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]Sunphase HT, Wuhan Sunhy Biology Co., Ltd. (Wuhan, P.R. China).
[4]One pig per pen (8 pens per treatment) was euthanized and the right fibula, tenth rib, and metacarpal were collected to determine bone density, bone ash weight, and percentage bone ash. After cleaning, bones were submerged in ultra-purified water under vacuum for 4 h. Weights were then collected, and bone density was calculated. For bone ash, bones were placed in a drying oven at 221°F for 7 d and then ashed in a muffle furnace at 1,112°F for 24 h.
[5] Prior to euthanizing, a blood sample was taken from the same pig in each pen to determine plasma inositol concentrations. Blood samples were centrifuged at 39.2°F at 1,500 × g for 15 min. Plasma was frozen in separate aliquots for later analysis for plasma inositol.

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

	Phytase, FTU/kg[2]					*P* =	
Item	**250**	**500**	**1,000**	**2,000**	**SEM[4]**	**Linear**	**Quadratic**
Performance							
ADG	-0.017	0.102	0.037	0.146	0.0433	0.017	0.945
G:F	0.139	0.195	0.194	0.231	0.0455	0.001	0.018
Bone characteristics[3]							
Fibula							
Bone ash, g	0.098	0.073	0.181	0.252	0.0263	< 0.001	0.140
Bone ash, %	0.040	0.053	0.155	0.176	0.0318	< 0.001	0.105
Bone density, g/mL	0.079	0.082	0.139	0.137	0.0245	< 0.001	0.003
Rib							
Bone ash, g	0.086	0.073	0.147	0.228	0.0202	< 0.001	0.272
Bone ash, %	0.047	0.062	0.156	0.225	0.0211	< 0.001	0.032
Bone density, g/mL	-0.032	0.074	0.290	0.238	0.0529	< 0.001	0.027
Metacarpal							
Bone ash, g	0.078	0.119	0.135	0.197	0.0170	< 0.001	0.012
Bone ash, %	0.006	0.067	0.117	0.120	0.0271	0.001	0.081
Bone density, g/mL	0.043	0.082	0.139	0.163	0.0262	< 0.001	0.031
Average[4]							
Bone ash, g	0.085	0.091	0.150	0.223	0.0204	< 0.001	0.076
Bone ash, %	0.020	0.054	0.130	0.159	0.0528	0.007	0.308
Bone density, g/mL	0.045	0.080	0.171	0.166	0.0268	< 0.001	0.004

[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] Sunphase HT, Wuhan Sunhy Biology Co., Ltd. (Wuhan, P.R. China).

[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, and percentage bone ash. After cleaning, bones were submerged in ultra-purified water under vacuum for 4 h. Weights were then collected, and bone density was calculated. For bone ash, bones were placed in a drying oven at 221°F for 7 d and then ashed in a muffle furnace at 1,112°F for 24 h.

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

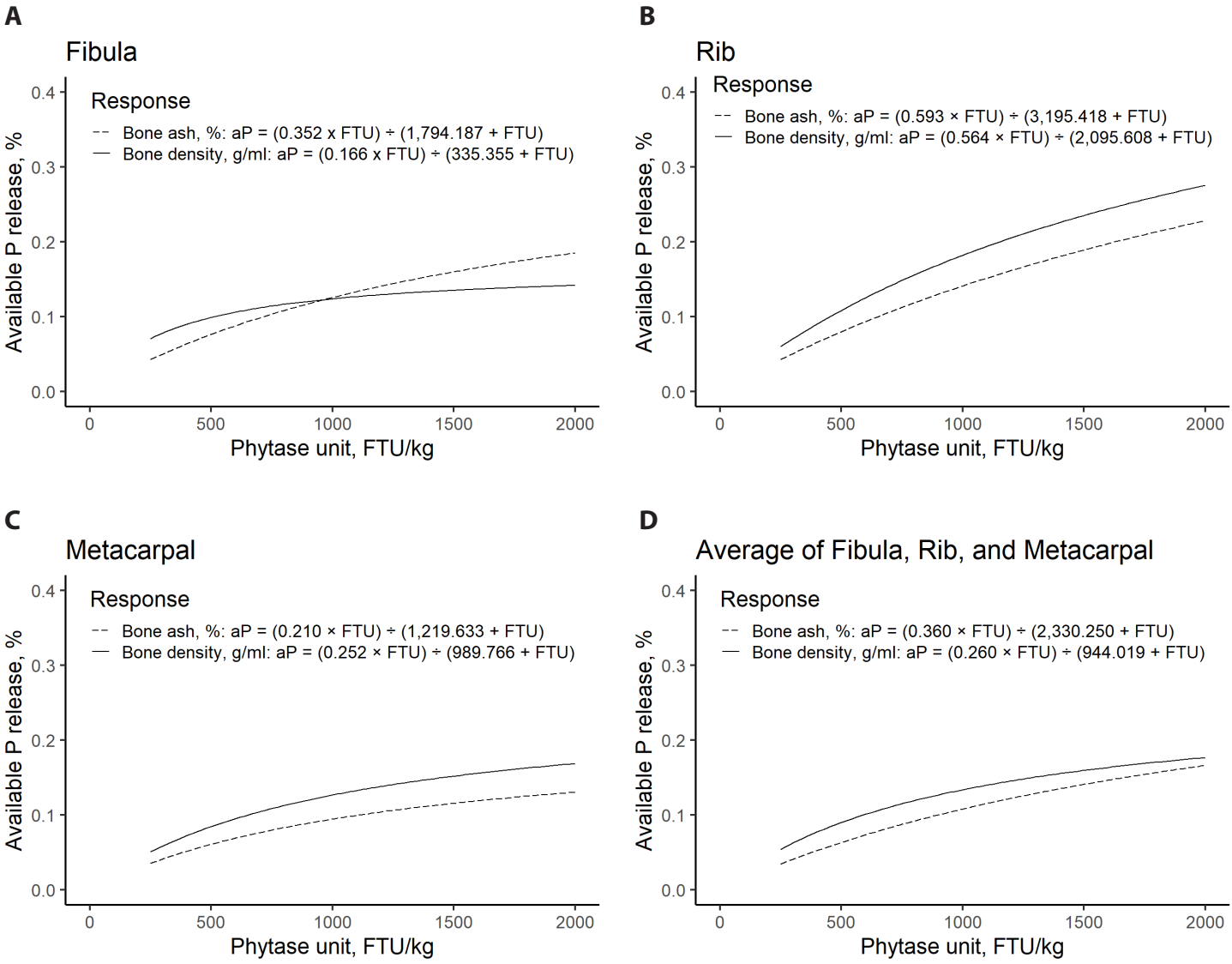

Figure 1. Available P release curves for A) right fibula, B) right rib, C) right metacarpal, and D) average of all three bones including percentage bone ash and bone density generated using the release equations for Sunphase HT from this experiment.