Determination of Available and Digestible Phosphorus Release of Microtech Phytase in Nursery Pig Diets

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Summary

A total of 320 barrows (DNA 200 × 400; initially 23.6 ± 0.47 lb) were used in a 21-d growth trial to determine the available P (aP) and true total tract digestible P (TTTD P) release curves for Microtech phytase (Guangdong VTR Bio-Tech Co, Ltd. Zhuhai, Guangdong, China). At approximately 21 d of age, pigs were weaned and randomly allocated to pens and fed a common diet for 18 d and then fed a P depletion diet (0.11% aP and 0.20% STTD P) for 3 d. On d 21 post-weaning, considered d 0 of the study, pigs were blocked by average pen body weight (BW) and randomly allotted to one of eight dietary treatments with five pigs per pen and eight pens per treatment. Dietary treatments were derived from a single basal diet and phytase, monocalcium phosphate, limestone, and sand were added to create the treatment diets. Treatments included three diets with increasing aP (0.11, 0.19 and 0.27%) and STTD P (0.20, 0.27 and 0.35%) using monocalcium phosphate that were used to develop the standard curves for the determination of aP and TTTD P release, and five diets with 0.11% aP (0.20% STTD P) and increasing phytase (250, 500, 1,000, 1,500 and 2,000 FTU/kg) that were used to develop the aP and TTTD P release curves. Pigs were weighed weekly, and feed disappearance was recorded per pen for the calculation of ADG, ADFI and F/G. At d 14 of the trial, fecal samples were collected from three pigs per pen. Samples were oven dried, ground, pooled per pen and analyzed for dry matter (DM), N, P and TiO_2 for the calculation of apparent total tract digestibility (ATTD) of DM, N and P. At the conclusion of the experiment, one pig, closest to the mean weight of each pen, was euthanized for bone analysis. The right fibula, right tenth rib, and right third metacarpal were collected for the determination of bone density, bone ash weight, and percentage bone ash. Increasing aP (and STTD P) from inorganic P improved (quadratic, $P \leq 0.05$) final BW, ADG, and F/G. Increasing phytase increased (linear, $P < 0.02$) final BW and ADFI and improved (quadratic, $P \leq 0.05$) ADG and F/G. Dry matter digestibility decreased (quadratic, $P < 0.05$) with increasing aP (and STTD P) from inorganic P, while P digestibility increased (linear, $P < 0.01$) with increasing aP

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(and STTD P) from inorganic P and increasing phytase. Bone density, bone ash weight, and percentage bone ash increased (linear, $P < 0.01$) with increasing aP (STTD P) from inorganic P and increasing phytase. Results from average percentage bone ash of the three bones and P digestibility were used to determine the aP and TTTD P release values, respectively. The aP and STTD P release curves developed for Microtech phytase are: aP release, % = (0.868 × FTU/kg) ÷ (9,599.511 + FTU/kg) and TTTD P release, % = ($4.017e^{-5}$ × FTU) + 0.011, respectively.

Introduction

Microbial phytase is an enzyme used to release the phytate bound P and its complexes with nutrients in plant-based feed raw materials. This allows for more efficient digestion of P and other nutrients, thereby improving animal performance while reducing the P excretion. The use of phytase also allows for the reduction in the use of inorganic P, which reduces diet cost.

Several exogenous microbial phytase are commercially available and knowing the efficacy of the P release from the phytase is essential for its effective use in diet formulation. Undervalued P-release from phytase results in unnecessary cost in diet formulation and has a negative impact on the environment, whereas over estimation of P-release from plant-based sources may compromise animal growth. Microtech phytase is a new generation phytase developed by VTR Biotech (Guangdong, China) with little data available to describe its P release. The aim of this study was to determine the aP release of Microtech phytase in nursery pigs using monocalcium phosphate to develop a standard aP release curve, and the TTTD P release based on fecal P digestibility.

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 Segregate Early Weaning facility in Manhattan, KS. The facility has two identical barns that are completely enclosed and environmentally controlled. Pigs were placed in 4 × 4 ft pens that contain a four-hole dry self-feeder and a cup waterer for ad libitum access to feed and water.

Animals and diets

A total of 320 barrows (DNA 200 × 400) were weaned at approximately 21 d of age. Weaned pigs were then fed a common phase 1 diet for 18 d. Subsequently, pigs were fed a phosphorus depletion diet (0.11% aP and 0.20% STTD P) for a 3-d period. On the fourth day after the start of the depletion feeding period, pens of pigs were blocked by body weight (BW; initially 23.6 ± 0.47) and randomly allotted to one of eight dietary treatments with five pigs per pen and eight pens per treatment. This was considered d 0 of the study.

Treatments included three diets with increasing aP (0.11, 0.19 and 0.27%) and STTD P (0.20, 0.27 and 0.35%) using monocalcium phosphate that were used to develop the standard curves for the determination of aP and TTTD P release and five diets with 0.11% aP (0.20% STTD P) and increasing phytase (250, 500, 1,000, 1,500 and 2,000 FTU/kg) that were used to develop the aP and STTD P release curves. Dietary treatments were derived from a single corn-soybean meal-canola meal-based diet and ingredients including phytase, monocalcium phosphate, limestone, and sand were added to create the treatment diets. Titanium dioxide at 0.5% was also added to the diets as an indigestible marker for the measurement of ATTD of DM, N and P. Prior to diet manufacturing, samples of corn, soybean meal, canola meal, limestone, monocalcium phosphate, vitamin premix and mineral premix were analyzed for Ca and P. Additionally, a sample of Microtech phytase was analyzed for phytase level using AOAC (2000) official method 2000.12[4] (Table 1). Results of the analyses were used in diet formulation.

All treatment diets were formulated to contain 1,078 kcal NE/lb, 1.24% SID Lys, 1.10:1 analyzed Ca:P ratio, and 0.32% phytate P (Table 2) and were manufactured in meal form at O. H. Kruse Feed Technology Innovation Center at Kansas State University. During bagging, diet samples were collected from every fourth bag, pooled, ground, and stored at -4°F. Samples were submitted in duplicate at K-State Research and Extension Soil Testing Laboratory, Manhattan, KS, for Ca, and P analyses, and at Eurofins Nutrition Analysis Center, Des Moines, IA, for phytase and phytic acid analyses. Moreover, diets were analyzed for DM, N, and TiO_2 at the KSU Swine Laboratory.

Measurements and sampling

Throughout the 21-d experiment, pig and feeder weights were measured every 7 d to determine ADG, ADFI and F/G. At d 14 of the trial, fecal samples were collected from three pigs per pen using rectal swabbing. Samples were oven dried, ground, pooled per pen, and analyzed for DM, N, P, and TiO_2 for the calculation of apparent total tract digestibility (ATTD) of DM, N, and P (Equation 1). Calculation of TTTD of P (Equation 2) was adapted NRC (2012)[5] with the total endogenous P losses estimated from the y-intercept of the regression of ATTD P (g/kg feed) against dietary P concentration (g/kg feed) in inorganic P diets.

$$\text{ATTD P, DM or N} = \left[1 - \left(\frac{\text{TiO}_2 \text{ in diet} \times \text{DM, N or P of feces}}{\text{TiO}_2 \text{ in feces} \times \text{DM, N or P of diet}}\right)\right] \times 100 \quad \text{Equation 1}$$

$$\text{TTTD P} = \text{ATTD P} + \left[\left(\frac{\text{total endogenous P loss}}{\text{P in diet}}\right) \times 100\right] \quad \text{Equation 2}$$

At the termination of the experiment, bones were collected from middle-weight pigs from each pen for determination of bone mineralization. The right fibula, tenth rib, and third metacarpal were collected, individually placed in plastic bags with 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 weighed while suspended in a vessel of water and the weights were 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

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

[5] Nutrient Requirements of Swine: Eleventh Revised Edition. 2012. National Academies Press, Washington, D.C.

24 h in a muffle furnace. This method was used to determine total bone ash weight and percentage ash relative to dried bone weight.

Calculations and statistical analysis

Growth performance, digestibility, and bone analyses data were analyzed as a randomized complete block design with pen as the experimental unit, treatment as fixed effect, and weight and barn blocks as random effects. The base model was evaluated using the MIXED procedure of SAS v.9.4 (SAS Institute, Inc., Cary, NC). Linear and quadratic polynomial contrasts were performed to determine the effects of increasing inorganic P and phytase level. Results were considered significant with P-values ≤ 0.05 and were considered marginally significant at $0.05 < P \leq 0.10$.

Available P release by phytase was calculated using a standard response curve that utilized the inorganic P diets. Standard response curves were developed using marginal aP intake [i.e., dietary aP% minus 0.11% (the aP in the basal diet) multiplied by ADFI] as predictor variable for each response criterion. 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 and barn blocks as random effects were 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. Formulated phytase levels were used to calculate all release values. To maintain consistent units of measure, gain-to-feed ratio was used to determine the aP release for feed efficiency. A model (Equation 3) 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 4.3.1, R Core Team, Vienna, Austria) to develop aP release curves for bone density, bone ash weight and percent bone ash.

$$\text{aP release,\%} = \frac{a \times \text{FTU}}{b + \text{FTU}} \qquad \text{Equation 3}$$

where a and -b are the horizontal and vertical asymptote, respectively.

The true total tract digestible P release from phytase was calculated using two approaches demonstrated by Zhai et al. (2023).[6] In the first approach that utilized the inorganic P diets, the aP release values based on percentage bone ash were transformed to its equivalent digestible P values by multiplying to the true total tract digestibility (TTTD) coefficient of P in monocalcium phosphate. The TTTD coefficient of P in monocalcium phosphate was represented by the slope of the linear regression of ATTD P against the formulated dietary P concentration. In the second approach, TTTD P release by phytase was calculated for each pen by subtracting the average of the TTTD P in control diet from the TTTD P in each diet added with phytase. Models were fitted to pen release values using first-degree polynomial with the formulated phytase level as

[6] Zhai, H., J. Zhang, Z. Wang, S. Wang, S. Prasad, K. Stamatopoulos, and S. Duval. 2023. Comparison of digestible and available phosphorus release values for a novel phytase determined with fecal phosphorus digestibility and bone mineralization in weaner pigs. Animal Feed Science and Technology. 297 (2023) 115580. doi: doi.org/10.1016/j.anifeedsci.2023.115580

fixed effect and weight and barn as random effects. The model parameters were estimated using the lme procedure of R (version 4.3.1, R Core Team, Vienna, Austria) to develop the release curve for STTD P.

Results and Discussion

Analyses of final diets for Ca and P were lower than formulated values but were consistent with increasing Ca and P for the inorganic P treatments (Table 2). Phytase activity of the complete diets increased across the phytase treatments with analyzed phytase concentrations of 268, 483, 1,060, 1,575, and 2,225 FTU/kg. Phytate P levels of the treatment diets ranged from 0.27 to 0.30%.

Pigs fed diets with increasing aP (and STTD P) from inorganic P had improved (quadratic, $P \leq 0.05$) final BW, ADG, and F/G (Table 3). Pigs fed diets with increasing phytase had increased (linear, $P < 0.02$) final BW and ADFI and increased (quadratic, $P \leq 0.05$) ADG and F/G.

Bone density, bone ash weight, and percentage bone ash of fibula, rib, metacarpal and their average increased (linear, $P < 0.01$) with increasing aP (and STTD P) from inorganic P and increasing phytase. As a result, the calculated percentage aP release from Microtech phytase based on different response criteria followed the same trend as the means previously listed (Table 4).

Percentage fecal DM, N, and P were not influenced by increasing aP (and STTD P) from inorganic P, whereas the increasing phytase decreased in fecal N (quadratic, $P = 0.015$) and fecal P (linear, $P = 0.009$) percentage. Dry matter digestibility decreased (quadratic, $P < 0.05$) with increasing aP (and STTD P) from inorganic P, while P digestibility increased (linear, $P < 0.01$) with increasing aP (and STTD P) from inorganic P and increasing phytase.

Figure 1 shows the aP release curves of Microtech phytase for bone density, bone ash weight, and percent bone ash for right fibula (a), right tenth rib (b), right third metacarpal (c), and the average of all three bones (d). Following the model presented in equation 3, the aP release based on average bone density, bone ash weight and percent bone ash of the three bones are: aP release, % = (0.519 × FTU) ÷ (5,210.321 + FTU); aP release, % = (0.392 × FTU) ÷ (3,124.916 + FTU); and aP release, % = (0.868 × FTU) ÷ (9,599.511 + FTU), respectively.

For TTTD P release curve of Microtech phytase, two approaches were adapted from Zhai et al. (2023). The calculated digestible P release values are presented in Table 5. In both approaches, the TTTD P release increased (linear, $P < 0.001$) with increasing level of phytase. The first approach showed higher digestible P release values than the second approach. For the first approach, Figure 2 shows the regression of ATTD P against dietary P concentration of the inorganic P treatments. The regression equation was: ATTD P = (0.935 × total P) – 3.218 ($r^2 = 0.737$) with the slope representing the TTTD of P of monocalcium phosphate and the y-intercept representing the endogenous losses. The TTTD of P of monocalcium phosphate was used as multiplier to convert aP release to digestible P release (Approach 1: dP = aP × 0.935). For the second approach, Figure 3 shows the STTD P release curve of Microtech phytase as TTTD P = ($4.017e^{-5}$ × FTU) + 0.011.

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Table 1. Analyzed composition of ingredients used in diet formulation (as-fed basis).

Ingredient	Ca, %[1]	P, %[1]	Phytase, FTU/g[2]
Corn	0.10	0.31	---
Soybean meal	0.70	0.71	---
Canola meal	1.69	0.80	---
Limestone	39.99	0.01	---
Monocalcium phosphate	17.90	21.37	---
Vitamin premix	26.09	0.03	---
Mineral premix	18.67	< 0.01	---
Microtech phytase	---	---	10,758

[1] Ca and P levels of ingredients were analyzed at K-State Research and Extension Soil Testing Laboratory, Manhattan, KS.
[2] Analyzed at Eurofins Nutrition Analysis Center, Des Moines, IA.

Table 2. Composition, calculated analysis, and analyzed 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**	**2,000**
Basal mix[3]	98.88	98.88	98.88	98.88	98.88	98.88	98.88	98.88
Limestone	0.16	0.23	0.29	0.16	0.16	0.16	0.16	0.16
Monocalcium P	0.11	0.49	0.86	0.11	0.11	0.11	0.11	0.11
Sand[4]	0.85	0.43	---	0.85	0.85	0.84	0.84	0.83
Phytase[5]	---	---	---	0.0023	0.0046	0.0093	0.0139	0.0186
Total	100	100	100	100	100	100	100	100
Calculated analysis								
NE, kcal/lb	1,078	1,078	1,078	1,078	1,078	1,078	1,078	1,078
SID Lys, %	1.24	1.24	1.24	1.24	1.24	1.24	1.24	1.24
Ca, %	0.53	0.62	0.70	0.53	0.53	0.53	0.53	0.53
P, %	0.48	0.56	0.64	0.48	0.48	0.48	0.48	0.48
Ca:P ratio	1.10	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	0.32
Analyzed composition[6]								
Ca, %	0.46	0.54	0.64	0.46	0.46	0.53	0.47	0.49
P, %	0.38	0.46	0.56	0.40	0.42	0.42	0.44	0.45
Ca:P ratio	1.21	1.17	1.14	1.15	1.10	1.26	1.07	1.09
Phytate P, %	0.28	0.28	0.30	0.27	0.28	0.29	0.29	0.30
Phytase, FTU/g	---	---	---	268	483	1,060	1,575	2,225

[1] Diets were fed for 21 d starting at approximately 23.6 ± 0.47 lb BW.
[2] Microtech phytase, VTR Biotech, Guangdong, China.
[3] Basal mix contains: corn: 59.91%, soybean meal: 29.53%, canola meal: 7.5%, salt: 0.53%, 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%, and TiO_2: 0.50%.
[4] 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.
[5] Phytase was analyzed for phytase level and contained 10,758 FTU/g (Eurofins Scientific Inc., Des Moines, IA).
[6] Diet samples were collected from every fourth bag, pooled, ground, and stored at -4°F. Samples were submitted in duplicate at K-State Research and Extension Soil Testing Laboratory, Manhattan, KS, for Ca, and P analyses, and at Eurofins Nutrition Analysis Center, Des Moines, IA, for phytase and phytic acid analyses. Dry matter, N and TiO_2 were analyzed at KSU Swine Laboratory. Manhattan, KS.
[7] Analyzed at Eurofins Nutrition Analysis Center, Des Moines, IA.

Table 3. Effects of increasing aP from monocalcium P or Microtech phytase on nursery pig growth performance and bone characteristics[1,2]

	Inorganic P, % aP[3]			Phytase, FTU/kg[4]						P =			
										Inorganic P		Phytase P	
Item	0.11	0.19	0.27	250	500	1,000	1,500	2,000	SEM	Linear	Quad	Linear	Quad
BW, lb													
d 0	23.6	23.6	23.6	23.6	23.6	23.5	23.5	23.6	0.47	0.801	0.781	0.775	0.759
d 21	43.1	46.0	46.5	44.6	45.7	46.0	46.4	47.2	0.68	< 0.001	0.050	< 0.001	0.063
d 0 to 21													
ADG, lb	0.89	1.02	1.04	0.96	1.00	1.02	1.04	1.07	0.021	< 0.001	0.043	< 0.001	0.042
ADFI, lb	1.64	1.69	1.67	1.65	1.71	1.68	1.71	1.73	0.028	0.325	0.331	0.012	0.671
F/G	1.85	1.66	1.61	1.72	1.72	1.65	1.64	1.62	0.021	< 0.001	0.012	< 0.001	0.001
Bone characteristics[5]													
Fibula													
Bone density, g/mL	1.15	1.20	1.21	1.16	1.19	1.18	1.19	1.21	0.014	0.004	0.217	0.001	0.840
Bone ash, g	0.640	0.806	0.865	0.666	0.747	0.775	0.873	0.872	0.0325	< 0.001	0.164	< 0.001	0.199
Bone ash, %	42.0	45.3	47.8	40.9	44.0	45.1	45.9	46.6	0.78	< 0.001	0.692	< 0.001	0.289
Rib													
Bone density, g/mL	1.17	1.22	1.24	1.18	1.21	1.21	1.22	1.23	0.009	< 0.001	0.290	< 0.001	0.291
Bone ash, g	0.780	1.001	1.182	0.759	0.938	0.990	1.036	1.149	0.0443	< 0.001	0.710	< 0.001	0.470
Bone ash, %	44.5	48.3	50.5	43.8	46.8	48.8	49.1	50.4	0.73	< 0.001	0.345	< 0.001	0.116
Metacarpal													
Bone density, g/mL	1.11	1.15	1.15	1.13	1.14	1.14	1.15	1.16	0.006	< 0.001	0.081	< 0.001	0.482
Bone ash, g	0.926	1.091	1.171	1.031	1.054	1.111	1.182	1.215	0.0382	< 0.001	0.300	< 0.001	0.181
Bone ash, %	31.9	34.9	36.3	32.1	34.5	35.1	35.6	37.1	0.84	0.001	0.446	< 0.001	0.468
Average													
Bone density, g/mL	1.15	1.19	1.20	1.16	1.18	1.18	1.18	1.20	0.008	< 0.001	0.103	< 0.001	0.463
Bone ash, g	0.782	0.963	1.073	0.818	0.913	0.959	1.030	1.079	0.0331	< 0.001	0.318	< 0.001	0.163
Bone ash, %	39.5	42.9	44.8	38.9	41.8	43.0	43.5	44.7	0.67	< 0.001	0.397	< 0.001	0.186
Digestibility													
Fecal dry matter, %	26.7	27.3	25.9	27.5	26.0	25.9	28.8	25.1	0.95	0.503	0.344	0.612	0.404
Fecal N, %	4.48	4.52	4.47	4.28	4.25	4.25	4.00	4.36	0.108	0.984	0.690	0.141	0.015
Fecal P, %	1.91	1.92	2.07	1.77	1.70	1.71	1.70	1.52	0.090	0.220	0.557	0.009	0.965
DM digestibility, %	84.14	82.36	83.49	82.78	81.91	82.53	83.81	82.03	0.612	0.427	0.043	0.279	0.493
N digestibility, %	79.94	77.39	79.24	79.45	80.71	79.07	81.51	78.15	0.911	0.584	0.049	0.473	0.199
P digestibility, %	27.82	32.97	44.31	32.03	33.63	34.39	42.69	44.04	3.268	< 0.001	0.412	< 0.001	0.924

[1] A total of 320 nursery pigs (DNA 200 × 400, initially 23.6 ± 0.47 lb) were used in a 21-d growth trial with five pigs per pen and eight replications per treatment.
[2] ADG = average daily gain; ADFI = average daily feed intake; F/G = feed to gain ratio.
[3] Inorganic P was added to the diet by increasing monocalcium P.
[4] Microtech phytase, VTR Biotech, Guagdong, China.
[5] One pig per pen (eight pens per treatment) was euthanized and the right fibula, right tenth rib, and right third 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 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.

Table 4. Calculated aP release values based on different response criteria[1]

	Phytase, FTU/kg[2]						*P* =	
Item	**250**	**500**	**1,000**	**1,500**	**2,000**	**SEM**	**Linear**	**Quadratic**
Performance								
ADG, lb	0.057	0.098	0.119	0.135	0.164	0.0202	< 0.001	0.015
G:F	0.067	0.069	0.121	0.119	0.137	0.0157	< 0.001	0.002
Bone characteristics[3]								
Fibula								
Bone density, g/mL	0.004	0.062	0.064	0.090	0.152	0.0428	0.002	0.899
Bone ash, g	0.005	0.067	0.084	0.151	0.147	0.0209	< 0.001	0.106
Bone ash, %	-0.034	0.043	0.082	0.102	0.119	0.0221	< 0.001	0.299
Rib								
Bone density, g/mL	0.008	0.062	0.084	0.100	0.136	0.0262	< 0.001	0.368
Bone ash, g	-0.009	0.057	0.082	0.098	0.139	0.0156	< 0.001	0.360
Bone ash, %	-0.029	0.042	0.108	0.117	0.148	0.0185	< 0.001	0.113
Metacarpal								
Bone density, g/mL	0.046	0.082	0.102	0.107	0.177	0.0225	< 0.001	0.366
Bone ash, g	0.061	0.076	0.111	0.155	0.172	0.0243	< 0.001	0.126
Bone ash, %	-0.006	0.082	0.105	0.125	0.178	0.0293	< 0.001	0.391
Average[4]								
Bone density, g/mL	0.016	0.068	0.082	0.098	0.154	0.0231	< 0.001	0.484
Bone ash, g	0.015	0.066	0.092	0.130	0.152	0.0151	< 0.001	0.075
Bone ash, %	-0.024	0.054	0.099	0.115	0.147	0.0175	< 0.001	0.145

[1] Available P release by phytase was calculated using a standard response curve that utilized the inorganic P diets. Standard response curves were developed using marginal aP intake [i.e., dietary aP% minus 0.11% (the aP in the basal diet) multiplied by ADFI] as the predictor variable for each response criterion. Marginal aP intake equivalence was solved for each replicate pen fed a specific phytase by substituting the response variable in each standard response equation with the measured value. Using the pen ADFI, aP intake was then converted to marginal aP release.
[2] Microtech phytase, VTR Biotech, Guagdong, China.
[3] One pig per pen (eight pens per treatment) was euthanized and the right fibula, right tenth rib, and right third 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 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.

Table 5. Calculated total tract digestible P release values based on two approaches demonstrated by Zhai et al (2023)

	Phytase, FTU/kg[1]						*P* =	
Item	250	500	1,000	1,500	2,000	SEM	Linear	Quadratic
Approach 1[2]								
TTTD P, %	-0.022	0.051	0.092	0.107	0.137	0.0163	< 0.001	0.144
Approach 2[3]								
TTTD P, %	0.022	0.038	0.038	0.081	0.090	0.0179	< 0.001	0.849

[1] Microtech phytase, VTR Biotech, Guagdong, China.
[2] The aP release values based on percent bone ash of the average of the three bones (Table 4) were transformed to its equivalent TTTD P values by multiplying to the TTTD coefficient of P in monocalcium phosphate of 0.935.
[3] TTTD P release by Microtech phytase was calculated for each pen by subtracting the average of the TTTD P in the control diet (no phytase) from the TTTD P in each phytase-containing diet.

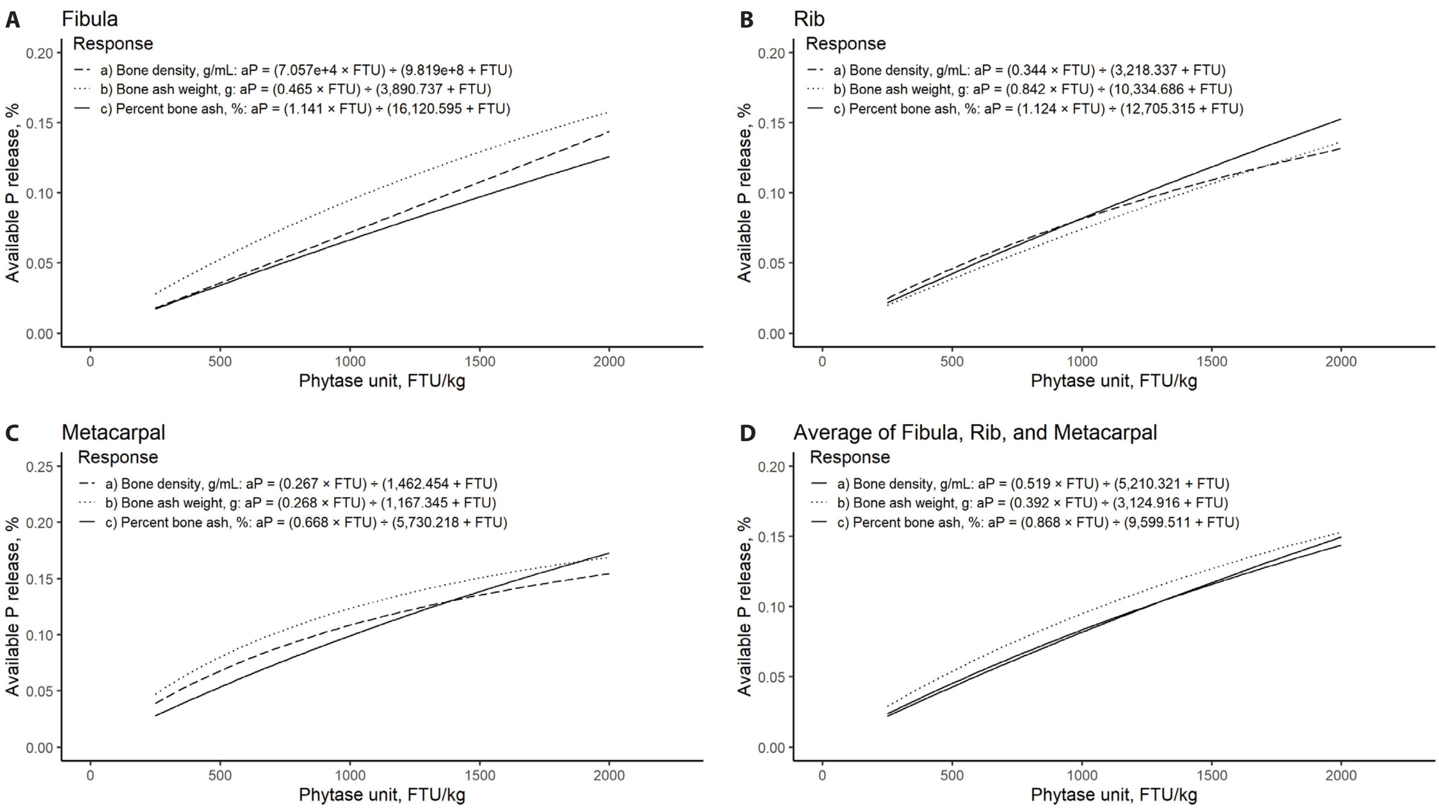

Figure 1. Available P release curves of Microtech phytase for bone ash weight, bone density and percent bone ash for right fibula (A), right tenth rib (B), right third metacarpal (C), and the average of all three bones (D)

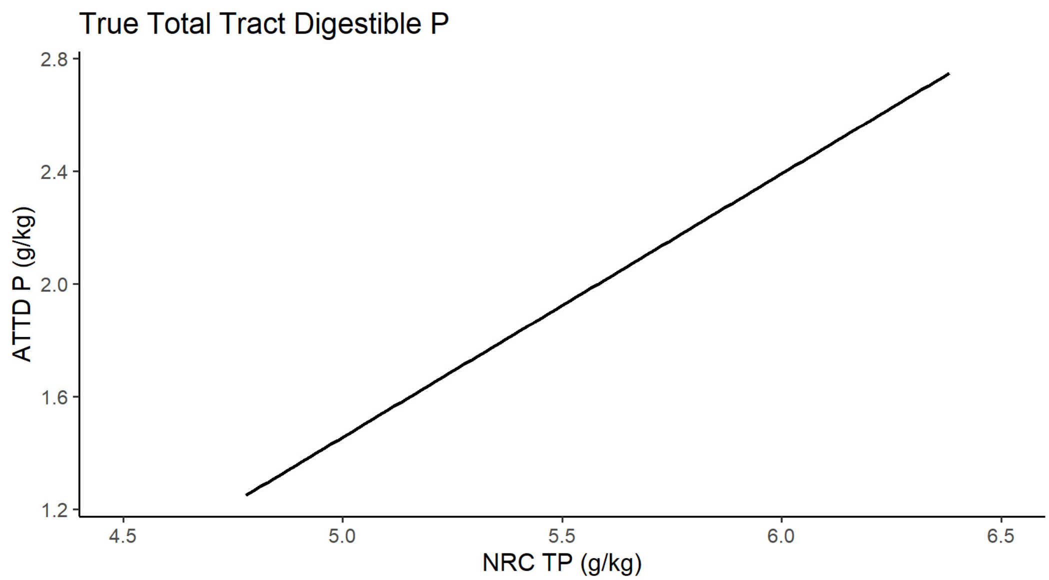

Figure 2. Regression of ATTD P (g/kg feed) against dietary P concentration (g/kg feed) in inorganic P diets to estimate the TTTD coefficient of P in MCP. The regression equation was: ATTD P = (0.935 × total P) – 3.218 (r^2 = 0.737) with the slope representing the TTTD of P of MCP and the y-intercept representing the endogenous losses. The TTTD of P of MCP was used as multiplier to convert aP release to digestible P release.

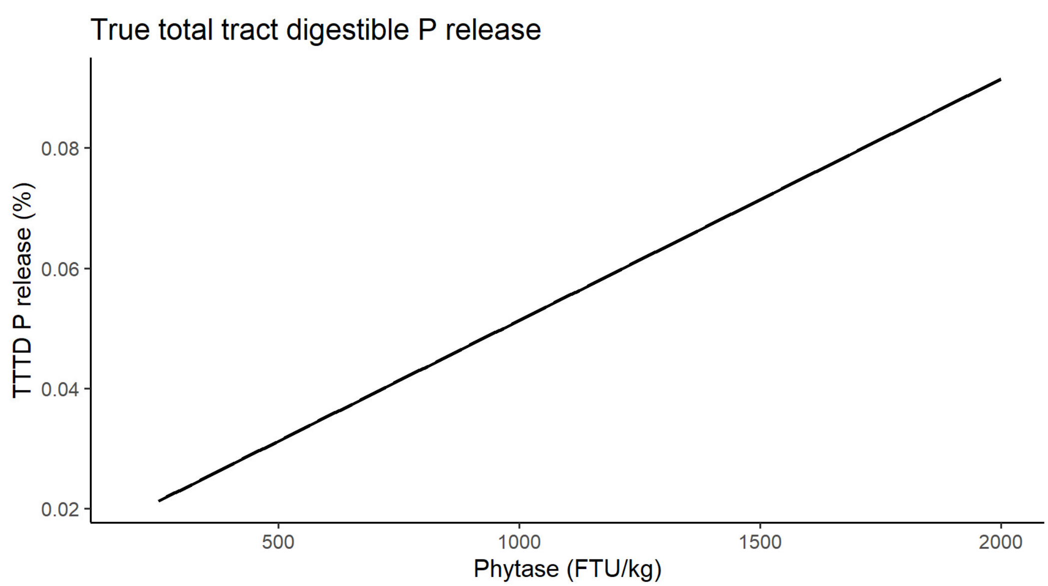

Figure 3. Standardized total tract digestible P release with supplemental Microtech phytase: TTTD P = ($4.017e^{-5}$ × FTU) + 0.011, r^2 = 0.236.