



Comparison of digestible and available phosphorus release values for a novel phytase determined with fecal phosphorus digestibility and bone mineralization in weaner pigs

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ABSTRACT

The objective of this study was to compare digestible and available P release from phytase through measuring fecal P digestibility and bone mineralization. A total of 336 weaner pigs (7.4 ± 0.9 kg) were used in a randomized complete block design. Three diets were established by including 2.5 (control), 6.0 or 9.5 g/kg monocalcium phosphate (MCP) to establish the standard response curves. The other 4 diets included a novel phytase at 500, 1000, 2000 or 3000 phytase units (FYT)/kg feed to the control. Each diet was fed to 6 pens of 8 pigs each for 21 days. All diets included 3 g/kg TiO_2 , and fecal samples were collected on d 13–15 of trial. At the end, one pig per pen was sacrificed for the collection of tibias. The results showed that increasing supplementation of MCP linearly ($P < 0.001$) improved growth performance (body weight gain, feed intake and gain:feed and bone mineralization (percent bone ash and weights of dry bone, bone ash, and bone Ca and P). These parameters responded to the supplementation of phytase both linearly and quadratically ($P < 0.01$). Supplemental phytase or MCP increased apparent total tract digestibility (ATTD) of Ca and P as well as the amount of ATTD P, but reduced the ATTD Ca-to-ATTD P ratio both linearly and quadratically ($P < 0.01$). Both MCP and phytase linearly ($P < 0.05$) increased percent bone ash P. The true total tract digestibility (TTTD) coefficient of P in MCP was estimated to be 0.94 by regressing the ATTD P against the dietary P concentration. The available P release increased from 1.58 to 2.21, 1.77–2.62, 1.17–1.82 and 1.25–1.97 g/kg feed when body weight gain, gain:feed, percent bone ash and bone P weight were used as the response variables, respectively, with increasing phytase dose from 500 to 3000 FYT/kg feed, whereas the digestible P release by phytase calculated from ATTD coefficient of P increased from 1.05 to 1.99 g/kg feed. By multiplying the TTTD coefficient of MCP (0.94), the available P release values based on bone P weight were transformed to their equivalent digestible values of 1.18–1.86 g/kg feed. In conclusion, the available P release from phytase depends on the response variables. Using the TTTD coefficient of P in MCP to convert the available P release values to digestible P values for phytase did not generate comparable results as measured from ATTD coefficient of P.

Abbreviations: ADG, average daily gain; ADFI, average daily feed intake; ATTD, apparent total tract digestibility or digestible; BW, body weight; DCP, dicalcium phosphate; DDGS, dried distillers grains with solubles; FYT, phytase unit; MCP, monocalcium phosphate; STTD, standardized total tract digestibility or digestible; TTTD, true total tract digestibility.

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1. Introduction

Phytase efficacy in a relative sense was measured by comparing the apparent total tract digestibility (ATTD) of P or the amount of digestible P in P-deficient diets with and without phytase (Kemmer et al., 1997; Kerr et al., 2010; Almeida et al., 2013). The control diet deficient in P serves as a baseline and determines the “room for improvement” for phytase. Phytase efficacy in terms of available P release was calculated also as a relative biological value for phytase by comparing with a standard mineral P source to achieve the same response of a sensitive indicator such as bone ash (Cromwell et al., 1993; Kornegay and Qian, 1996; Wensley et al., 2020a). In practical diet formulation, both digestible and available P release values for phytase are used to account for the contribution of phytase without due prudence, and adding to this confusion, different digestible P to available P conversion ratios have been used by different phytase producers (Dersjant-Li et al., 2015).

The underlying argument for this conversion of available P to digestible P is that the standard mineral P source such as monocalcium phosphate (MCP) has an assumed P bioavailability of 100% but an actual standardized total tract digestibility (STTD) of P between 80% and 90% (NRC, 1998, 2012). Such conversion has been challenged by relevant studies (Zhai et al., 2022). For example, the low-phytate corn was determined to contain at least 5 times as much available P in contrast to 3 times as much digestible P as normal corn (Spencer et al., 2000). Digestible and available P release values for phytase are essentially different in several aspects despite the fact that digestible P can be used for post-absorption net requirement at a high efficiency of approximately 95% (NRC, 2012). First, digestion is only a prerequisite of bioavailability (Sibbald, 1987). Second, digestible P is a point-of-time measurement whereas available P reflects a cumulative effect of phytase on bone mineralization. Thirdly, digestible P reflects the digestion and

Table 1

Ingredient and nutrient composition of the control diet containing 2.5 g/kg monocalcium phosphate (g/kg of feed, as-is basis)^a.

Items	Control diet
Ingredients	
Corn	590.9
Soybean meal	340.5
Soybean oil	25.0
NaCl	4.5
NaHCO ₃	1.5
L-Lys-HCl	4.5
DL-Met	1.2
L-Thr	1.5
L-Val	1.0
Limestone	6.8
Monocalcium phosphate	2.5
Vitamin-mineral premix ^b	5.0
Rice hulls	9.1
Benzoic acid	3.0
TiO ₂	3.0
Total	1000.0
Calculated nutrients and energy ^c	
Net energy, MJ/kg	10.5
Metabolizable energy, MJ/kg	14.1
Crude protein	218
Total Ca	4.5
Total P	4.2
Phytate P	2.5
Standardized total tract digestible P	2.0
Standardized ileal digestible	
Lys	12.9
Met	3.9
Thr	7.7
Trp	2.1
Val	8.3

^a The rice hulls in control diet were replaced by monocalcium phosphate and phytase to create other experimental diets.

^b Premix supplied per kilogram of diet: vitamin A, 9750 IU; vitamin D₃, 3000 IU; vitamin E, 63 mg; vitamin K₃, 3.0 mg; vitamin B₁, 3.0 mg; vitamin B₂, 9.6 mg; vitamin B₆, 4.5 mg; vitamin B₁₂, 36 µg; D-biotin, 240 µg; D-calcium pantothenate, 30 mg; folic acid, 1.8 mg; niacin, 36 mg; Cu (tribasic copper chloride), 190 mg; I (potassium iodate), 0.6 mg; Fe (ferrous sulfate), 120 mg; Mn (manganese sulfate), 60 mg; Zn (zinc sulfate), 120 mg; Se (sodium selenite), 450 µg; choline (choline chloride), 300 mg; and Ca (calcium carbonate) 0.6 g.

^c The nutrients and energy were calculated by referring to the feed ingredient composition tables in NRC (2012).

absorption of P in a diet of specific ingredient composition whereas available P also considers the balance between supply and demand of P for animals. With the establishment of STTD P requirement by [NRC \(2012\)](#), it is necessary to explore the relationship between available and digestible P equivalence of supplemental phytase for pigs.

We hypothesized that the available and digestible P release values for phytase are different and their conversion using the measured true total tract digestibility (TTTD) coefficient of P in the standard P source, MCP, is not justified. The aim of the current study, therefore, was to measure and compare the digestible and available P release values by a novel phytase in weaner pigs as compared to MCP. The available P release from phytase was benchmarked to MCP using growth performance and bone mineralization as response variables. The digestible P release by phytase as well as the TTTD coefficient of P in MCP were calculated from measuring ATTD coefficient of P in experimental diets.

2. Materials and methods

This study was conducted at DSM (China) Animal Nutrition Research Center Co. Ltd. (Bazhou, P. R. China). The protocol of this study was approved by the Animal Welfare Committee of DSM (China) Animal Nutrition Research Center (AWCCAN), and the research complied with the guidelines in European Union council directive 2010/63/EU for animal experiments ([European Union, 2010](#)).

2.1. Animals and facilities

Three hundred and thirty-six barrows and gilts [PIC L1050 × L337; initial body weight 7.4 ± 0.9 kg (mean ± SD)] were used. The pigs were weaned at an age of approximately 21 d and transferred to a nursery house to adapt for 7 d. After the adaptation, the pigs were weighed individually, blocked by initial body weight of barrows and gilts separately, and then randomly assigned to 42 pens according to a randomized complete block design resulting in 42 pens of 8 pigs (4 barrows and 4 gilts per pen). The experimental diets were offered for a trial duration of 21 d. At the end of trial, the pigs weighed 16.8 ± 2.0 kg (mean ± SD). The nursery house was furnished with 80 pens (space/pen = 3.0×1.8 m²). Each pen had a plastic-coated wire floor and was equipped with two water nipples and one stainless-steel feeder. Feed and water were supplied *ad libitum*.

Room temperature and ventilation were controlled by a computer system to provide an optimal environment. The room temperature was 27 °C at the start and gradually reduced to 23 °C at the end. The relative humidity was recorded to range from 40% to 60%. The lighting was set as bright during the day and as dim during the night.

2.2. Experimental diets

The test phytase (HiPhorius™, DSM Nutritional Products, Switzerland) was encoded by a 6-phytase gene from *Citrobacter braackii* and expressed from a strain of *Aspergillus oryzae*.

There were 7 dietary treatments. The ingredient and nutrient composition of the control diet are presented in [Table 1](#). Three reference diets were established by including 2.5 (control diet), 6.0, or 9.5 g/kg MCP at the expense of rice hulls in the control diet, whereas the other 4 diets included 500, 1000, 2000, and 3000 phytase units (FYT)/kg feed. The ratio of total Ca to total P was maintained at 1.05 by adjusting the inclusion level of limestone. Titanium dioxide was included at 3 g/kg feed as an indigestible marker to enable the measurement of ATTD coefficient of Ca and P. The net energy and nutrients except for Ca and P of all experimental diets were above the recommendations in [NRC \(2012\)](#). The control diet was formulated to be short of 2 g/kg STTD P and 3.5 g/kg total Ca when compared to the recommendations for 7–11 kg pigs by [NRC \(2012\)](#). All diets were pelleted with a conditioning temperature at 75 °C.

2.3. Measurement and sampling

The pigs were weighed individually on d 0 and 21 of trial, and feed consumption was recorded per pen during the trial to calculate average daily gain (ADG), average daily feed intake (ADFI) and gain:feed (G:F).

Fresh and clean fecal samples were grabbed from each pen every 2 h from 0900 to 1700 h on d 13–15 of trial. All the pens were removed of feces and cleaned before collection on each collection day. A total of approximately 600 g of feces was collected per day per pen. All the fecal samples collected from each pen during the 3-d collection period were pooled and mixed to homogeneity with a hand-held blade mixer (TD-110, RuiBao Hardware Co. Ltd., Dongguan, P. R. China). A sub-sample of around 400 g for each pen was stored at – 20 °C until further processing.

The right tibia and a urinary sample from bladder (if adequate at sampling) were collected from the pig in each pen with BW closest to the pen average BW on d 21 of trial. The tibias were processed by referring to the non-defatting bone processing procedures described by [Wensley et al. \(2020b\)](#). In short, the bones were autoclaved at 120 °C for 30 min to facilitate the removal of muscular tissues and cartilaginous caps. The cleaned bones were left at room temperature for one day and then oven-dried at 105 °C for 7 d. Finally, the dried tibias were incinerated in a muffle oven for 72 h at 600 °C. All the bone and urine samples were stored at – 20 °C before processing.

2.4. Chemical analyses

The fecal samples were oven-dried to a constant weight and ground to pass through a 0.5-mm screen before analysis. The dietary

and fecal samples were dried at 105 °C in an oven for 4 h for dry matter determination (method 934.01; AOAC International, 2006). Titanium, Ca, and P were determined by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES; Optima TM 8000, PerkinElmer, Shelton, USA; method 985.01; AOAC International, 2006) after microwave digestion as described by Chevallier et al. (2015). Ten mL of each urine sample were dried at 60 °C before the microwave digestion. These aforementioned analyses were performed in duplicate. Phytase activity was measured in 5 replicates at BioPract (Berlin, Germany). One phytase unit was defined as the amount of enzyme that releases 1 µmol of inorganic phosphate from 50 mM phytate per minute at 37 °C and pH 5.5.

2.5. Calculations and statistical analyses

Apparent total tract digestibility coefficient of Ca and P were calculated using the following equation:

$$D = 1 - (T_i/T_o) \times (M_o/M_i);$$

where D is the ATTD coefficient of Ca or P; T_i and T_o are the titanium concentrations in diet and feces, respectively (% of DM); M_i and M_o are the concentrations of Ca or P in diet and feces (% of DM), respectively. The concentrations of ATTD Ca and P in diet (g/kg feed) were calculated by multiplying the concentrations of Ca and P in feed (g/kg feed) by their corresponding ATTD coefficients. Digestible Ca or P release by phytase was calculated by subtracting the average of the ATTD Ca or P in control diet from the ATTD Ca or P in each diet added with phytase.

The TTTD coefficient of P in MCP was represented by the slope of the linear regression of ATTD P against dietary P concentration as described in the following equation (Akinmusire and Adeola, 2009):

$$P_D = (D_{MCP} \times P_i) - EPL,$$

where P_i is the dietary P concentration (g/kg feed), P_D is the dietary concentration of ATTD P (g/kg feed) and D_{MCP} is the estimate of TTTD coefficient of P in MCP, EPL is the endogenous P losses (g/kg feed).

Available P release by phytase was calculated by referring to the method described by Wensley et al. (2020a). Standard response curves were developed using dietary P intake (g/d) as the predictor variable for ADG and using dietary P concentration (g/kg) for G:F, percent bone ash, or bone P weight. By substituting the response variable in each standard response equation with a measured value, a dietary P concentration equivalence was solved for each replicate pen fed a specific phytase dose. These equivalent values minus the P concentration in the control diet gave the available P release values for each phytase dose.

The study was in a randomized complete block design. Each pen or pig was an experimental unit. The REG procedure of SAS (SAS Inst. Inc., Cary, NC) was used for the regression analysis. The digestibility, performance, and bone mineralization data were analyzed by a MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with the model including the dietary treatment as a fixed effect, replicate (body weight blocks) as a random effect, and an error term. Polynomial orthogonal contrasts were constructed to test the linear and quadratic effects of supplementation of MCP or phytase. The least square means were presented, and the significance was defined at $P < 0.05$. A tendency was declared at $P < 0.10$.

3. Results

3.1. Experimental diets and the analyses

The total P in diets with 2.5 g/kg MCP were analyzed to be 4.6–4.7 g/kg, and the increments of 0.8 g/kg P in the reference diets agree with the graded inclusion of MCP (Table 2). The analyzed total Ca/P ratios ranged from 1.02 to 1.07 in comparison to the target of 1.05. The analyzed phytase activities are within + 12% of the targets of 500, 1000, 2000, and 3000 FYT/kg feed. The analyzed titanium concentrations are close to the intended values.

3.2. Growth performance and ATTD coefficient of Ca and P

Increasing supplementation of MCP improved final BW, ADG, ADFI, and G:F linearly ($P < 0.001$; Table 3), whereas a both linear and quadratic response was observed to the supplementation of phytase ($P < 0.001$).

Table 2

Analyzed nutrients of the experimental diets (as-is basis, g/kg)^a.

Items	Monocalcium phosphate, g/kg			Phytase, FYT/kg feed			
	2.5	6.0	9.5	500	1000	2000	3000
Dry matter	925	924	925	925	924	924	926
Ca	5.0	5.7	6.7	4.7	5.0	4.9	4.8
P	4.7	5.5	6.4	4.6	4.7	4.6	4.7
Ca/P ratio	1.07	1.05	1.05	1.02	1.06	1.07	1.03
Titanium	1.8	1.8	1.8	1.7	1.8	1.8	1.8
Phytase, FYT/kg	0	0	0	511	1009	2110	3333

^a Phytase activity was analyzed in 5 replicates and the others in duplicate.

Supplemental phytase increased ATTD coefficients of Ca and P as well as ATTD Ca and P ($P < 0.001$), but reduced the ATTD Ca:ATTD P ratio both linearly and quadratically ($P < 0.001$). Similar responses were observed for MCP with the exception that ATTD Ca increased only linearly ($P < 0.001$).

A regression equation was established as $y = 69.08x + 235.94$ ($r^2 = 0.57$) by regressing ADG against ATTD P in the experimental diets (Fig. 1). The regression equation was $y = -0.25x + 1.98$ ($r^2 = 0.83$) between ATTD Ca:ATTD P ratios and ATTD P in the experimental diets (Fig. 2).

3.3. True total tract digestibility of P in monocalcium phosphate

By regressing the ATTD P against the dietary P concentration, TTTD coefficient of P in MCP was estimated to be 0.94 (Fig. 3). The regression equation was $y = 0.94x - 2.52$ ($r^2 = 0.96$) with the slope representing the TTTD coefficient.

3.4. Bone mineralization and urinary concentrations of Ca and P

Graded supplementation of MCP linearly increased percent bone ash and weights of dry bone, bone ash, and bone Ca and P ($P < 0.001$; Table 4), whereas a both linear and quadratic response was observed for phytase ($P < 0.01$). Supplementation of MCP or phytase linearly increased percent bone ash P ($P < 0.05$). The Ca/P ratio in bone was reduced linearly ($P < 0.05$) by supplementation of MCP and both linearly and quadratically ($P < 0.05$) by phytase.

The urinary concentrations of Ca and P were plotted again the ATTD Ca/ATTD P ratios (Fig. 4). The urinary concentration of Ca increased linearly ($P < 0.01$) with increasing ATTD Ca/ATTD P ratios whereas a quadratic response ($P < 0.01$) of the urinary concentration of P was observed.

3.5. The available and digestible P release from phytase

The available P release increased from 1.58 to 2.21, 1.77–2.62, 1.17–1.82, and 1.25–1.97 g/kg feed when ADG, G:F, percent bone ash, and bone P weight were used as the response variables, respectively, with increasing phytase dose from 500 to 3000 FYT/kg feed (Linear effect: $P < 0.06$; Table 5). The digestible P release by phytase calculated from ATTD coefficient of P increased from 1.05 to 1.99 g/kg feed accordingly. The available P release values based on bone P weight were transformed to their equivalent digestible P values of 1.18–1.86 g/kg feed by multiplying the TTTD coefficient of 0.94 for P in MCP.

4. Discussion

4.1. Experimental diets, phytase, and monocalcium phosphate

In general, the experimental diets were considered acceptable for the research purpose of this study. The control diet and the diets supplemented with phytase were analyzed to contain almost identical P content. Increasing P concentrations in MCP diets reflected the incremental inclusion levels of MCP. The analyzed Ca to P ratios in the range of 1.02–1.07 centered on the target of 1.05. The concentrations of marker and analyzed phytase activities were in good agreement with the intended values. The recovered phytase activities in diets were slightly above the formulated values, which indicates the negligible effect of current pelleting conditions on this

Table 3

Growth performance and apparent total tract digestibility coefficients (ATTDc) of Ca and P of the pigs supplemented with monocalcium phosphate or phytase^a.

Items	Monocalcium phosphate, g/kg			Phytase, FYT/kg feed				SEM ^b	P-value			
	2.5	6.0	9.5	500	1000	2000	3000		L ^c	Q ^c	L ^d	Q ^d
Growth performance												
IBW (d 0), kg ^e	7.4	7.4	7.4	7.4	7.4	7.4	7.4	0.03	0.234	0.554	0.979	0.238
FBW (d 21), kg	14.9	16.0	16.8	16.9	17.7	17.6	17.7	0.17	< 0.001	0.450	< 0.001	< 0.001
ADG, g/d/pig	359	410	447	452	491	486	494	8.4	< 0.001	0.525	< 0.001	< 0.001
ADFI, g/d/pig	521	562	591	594	628	628	623	11.5	< 0.001	0.650	< 0.001	< 0.001
Gain:feed, g/kg	691	729	757	760	783	774	794	8.8	< 0.001	0.669	< 0.001	< 0.001
Digestibility												
ATTDc of P	0.389	0.503	0.535	0.626	0.720	0.759	0.819	0.01	< 0.001	< 0.001	< 0.001	< 0.001
ATTDc of Ca	0.562	0.628	0.641	0.713	0.779	0.757	0.801	0.01	< 0.001	0.005	< 0.001	< 0.001
Digested P, g/kg	1.83	2.74	3.39	2.88	3.37	3.48	3.82	0.03	< 0.001	0.005	< 0.001	< 0.001
Digested Ca, g/kg	2.82	3.58	4.28	3.33	3.86	3.70	3.86	0.04	< 0.001	0.473	< 0.001	< 0.001
Digested Ca/P ratio	1.55	1.31	1.26	1.16	1.15	1.06	1.01	0.01	< 0.001	< 0.001	< 0.001	< 0.001

^a Each least square mean represents 6 observations.

^b SEM: standard error of the mean.

^c Linear and quadratic effects of monocalcium phosphate.

^d Linear and quadratic effects of phytase including the treatment with 2.5 g/kg monocalcium phosphate.

^e IBW, initial body weight; FBW, final body weight; ADG, average daily gain; ADFI, average daily feed intake.

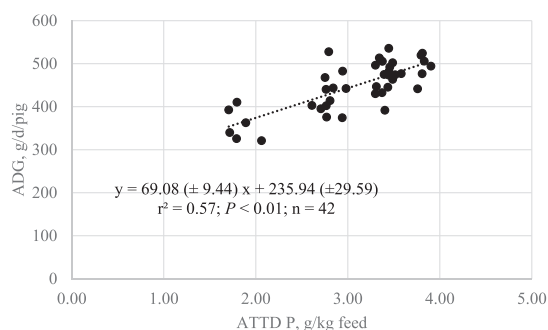


Fig. 1. Regression of average daily gain (ADG) of weaner pigs (7–17 kg) against apparent total tract digestible (ATTD) P (1.7–3.9 g/kg feed) in experimental diets (parameter estimates and their standard errors).

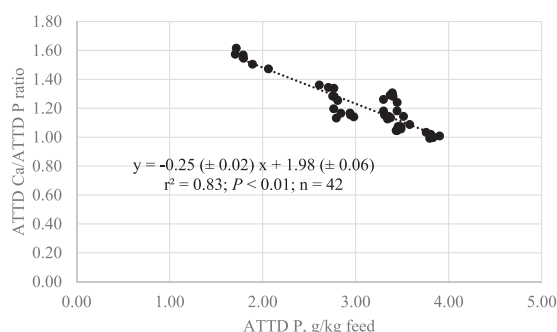


Fig. 2. Regression of apparent total tract digestible (ATTD) Ca/ATTD P ratios (1.0–1.6) against ATTD P (1.7–3.9 g/kg feed) in experimental diets (parameter estimates and their standard errors).

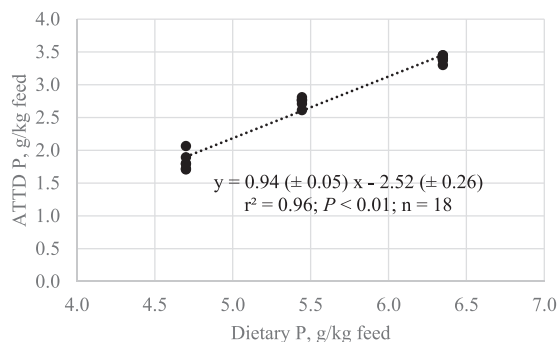


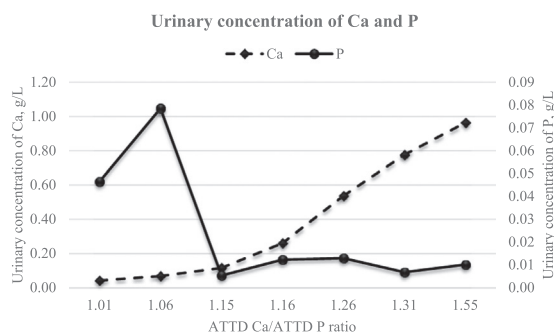
Fig. 3. Regression of apparent total tract digestible (ATTD) P against dietary P concentration in experimental diets supplemented with monocalcium phosphate to estimate the true total tract digestibility coefficient of P (the slope) in monocalcium phosphate (parameter estimates and their standard errors).

test phytase. The efficacy of this novel phytase used in the current study was validated in sows and nursery pigs (Thorsen et al., 2021; Zhai et al., 2021).

The TTTD of P of MCP was estimated to be 94% using the regression technique in the current study. The large variation in P digestibility was noted for feed phosphates despite its high P digestibility and consistent quality (Eeckhout and De Paepe, 1997; Poulsen, 2007). The ATTD of P in MCP ranged from 64% to 91% (Eeckhout and De Paepe, 1997) and from 79.5% to 88.5% (Stein et al., 2008), the absorption of P was determined to be 71–75% (Poulsen, 2007), and the TTTD of P was measured to be 89–95% (Petersen and Stein, 2006). This large variation could be related to the solubility and forms of feed phosphates, method of determination, and age of pigs (Eeckhout and De Paepe, 1997; Rodehutsord et al., 1999b; Poulsen, 2007). The ATTD of P in MCP was not affected by the dietary concentration of P (Stein et al., 2008). The age or BW of pigs appeared to be an influencing factor. Using a similar regression method as in the current study, the TTTD of P in MCP was estimated to be 67.5% in 15-kg pigs and 84.3% in 25-kg pigs, which indicated a substantial effect of pig's BW (Zhai and Adeola, 2013). Rodehutsord et al. (1999b) also reported an increase in ATTD of P with heavier pigs when dietary P mainly originated from MCP.

Table 4Bone mineralization of the pigs supplemented with monocalcium phosphate or phytase^a.

Items	Monocalcium phosphate, g/kg			Phytase, FYT/kg feed				SEM ^b	P-value			
	2.5	6.0	9.5	500	1000	2000	3000		L ^c	Q ^c	L ^d	Q ^d
Tibia dry weight, g	7.3	8.7	10.1	9.4	9.9	10.5	10.5	0.26	< 0.001	0.924	< 0.001	< 0.001
Tibia ash, %	43.7	46.0	50.1	47.7	47.4	50.6	50.2	0.78	< 0.001	0.340	< 0.001	0.008
Tibia Ca, % of ash	33.8	33.6	33.4	33.9	33.4	33.5	33.9	0.39	0.524	0.970	0.987	0.351
Tibia P, % of ash	16.9	17.0	17.2	17.2	17.4	17.4	17.5	0.11	0.042	0.423	0.003	0.067
Tibia ash, g	3.18	4.01	5.09	4.49	4.69	5.33	5.29	0.15	< 0.001	0.485	< 0.001	< 0.001
Tibia Ca, g	1.07	1.34	1.70	1.50	1.56	1.77	1.77	0.05	< 0.001	0.571	< 0.001	< 0.001
Tibia P, g	0.53	0.68	0.88	0.77	0.82	0.92	0.92	0.03	< 0.001	0.422	< 0.001	< 0.001
Tibia Ca/P ratio	2.00	1.98	1.94	1.96	1.91	1.93	1.93	0.02	0.012	0.467	0.011	0.006

^a Each least square mean represents 6 observations.^b SEM: standard error of the mean.^c Linear and quadratic effects of monocalcium phosphate.^d Linear and quadratic effects of phytase including the treatment with 2.5 g/kg monocalcium phosphate.**Fig. 4.** Urinary concentrations of Ca (linear effect: $P < 0.01$) and P (quadratic effect: $P < 0.01$) with increasing ratios of apparent total tract digestible (ATTD) Ca over ATTD P in experimental diets ($n = 36$).**Table 5**Comparison of available and digestible P release (g/kg feed) by phytase using different response variables^a.

Response variable	Phytase, FYT/kg feed				SEM ^b	P-value	
	500	1000	2000	3000		L ^c	Q ^c
Available P release, g/kg							
ADG ^d	1.58	2.10	1.99	2.21	0.12	0.007	0.277
Gain:feed	1.77	2.34	2.12	2.62	0.24	0.056	0.981
Percent bone ash	1.17	1.10	1.92	1.82	0.14	< 0.001	0.207
Bone P weight	1.25	1.48	1.98	1.97	0.14	< 0.001	0.130
Digestible P release, g/kg							
ATTD of P	1.05	1.54	1.65	1.99	0.03	< 0.001	0.002
Bone P weight ^e	1.18	1.39	1.86	1.85	0.13	< 0.001	0.130

^a Each least square mean represents 6 observations.^b SEM: standard error of the mean.^c Linear and quadratic effects of phytase.^d ADG, average daily gain; ATTD, apparent total tract digestibility.^e The digestible P release values were derived from multiplying the available P release values based on bone P weight by 0.94 as the true total tract digestibility coefficient of P in monocalcium phosphate estimated in Fig. 3.

4.2. Effects of phytase and MCP on growth performance and ATTD of Ca and P

The growth performance response to MCP or phytase was mainly determined by the available P supply, but the secondary effects of different digestible Ca/P ratios among the experimental diets should not be neglected. More ATTD P was measured with increasing supplementation of MCP or phytase, which had driven the improvement in growth performance. The dietary concentration of ATTD P was shown to account for 57% of the variation in body weight gain. The concentration of ATTD P in diet, however, was inversely correlated with the ATTD Ca:ATTD P ratios. Therefore, the improvement in growth performance in response to supplementation of MCP or phytase was subjected to the influence of varying digestible Ca/P ratios although the same total Ca/P ratio was maintained across the diets. In the literature, it has been shown that decreasing dietary Ca/P ratios would improve the growth performance of pigs

supplemented with phytase (Qian et al., 1996; Becker et al., 2020). This agrees with the finding by Lagos et al. (2021) that the ratios between STTD Ca and STTD P in diet shouldn't exceed certain limits to maximize growth performance of pigs. Moreover, the extra-phosphoric effects of phytase at superdoses (> 1500 FYT/kg) could be another confounding factor. The extra improvement in growth performance beyond the justification of P release by phytase could be partly attributed to the release of *myo*-inositol (Lu et al., 2019) because of its role in upregulating the pathways involved in protein accretion (Schmeisser et al., 2017).

The pigs appeared to be capable of digesting and absorbing more Ca than P as suggested by the linear increase in ATTD Ca with increasing inclusion rate of MCP in contrast to the linear and quadratic increase in ATTD P. This is supported by the findings by Stein et al. (2011) that ATTD of Ca was not affected by the dietary concentration of Ca in a range of 55–173% of the pig's Ca requirement and that Ca homeostasis is mainly regulated at kidney rather than at gut. On the contrary, the efficiency of P absorption could be downregulated when there is an excess of P supply in diet (Rodehutscord, 1999a). In addition, the seemingly early plateau in ATTD Ca at the dose of 1000 FYT phytase/kg feed questioned the involvement of Ca in pig's growth performance. Indeed, Ca and P requirements for soft tissue growth are independent (Crenshaw, 2001), and soft tissue development is dependent mainly on P (Lautrou et al., 2020). There was a close relationship between whole-body P mass and whole-body N mass or empty body weight (NRC, 2012; Bikker and Bloc, 2017).

4.3. Bone mineralization

Bone P weight appeared to be more sensitive than other bone parameters to added MCP or phytase. The continued improvement in percentage of bone ash P on top of the improvement in percentage of bone ash in the current study implied that the proportion of P in bone ash as well as the proportion of bone ash could be altered in certain degree by different P supply status in diet. This contrasted with the unaffected percentage of Ca in bone ash. These nuances in Ca and P composition of bone ash would otherwise not be revealed in percent bone ash. The bone parameters in weight than in percentage generally give greater response to supplementation of MCP or phytase because the size of bone adds another dimension of difference. For example, the concentration of Ca in bone ash did not change, but the amount of Ca accumulated in bone ash/bone increased drastically in response to either MCP or phytase supplementation in the current study. This agrees with the reports by Li et al. (2015) and Lee et al. (2021) that bone ash weight reflects bone mineralization better than bone ash percentage, and that the differences observed in bone ash weight are most likely a result of difference in the size of bones.

The Ca/P ratio in bone was shown to be affected by the supplementation of MCP or phytase, which indicates certain plasticity in bone's mineral composition. Sørensen et al. (2019) also found that the bone Ca/P ratio can be manipulated through different dietary P supply. In bone, Ca and P combine and deposit in approximately a 2.2:1 ratio to form a hydroxyapatite-like compound (Crenshaw, 2001). The lower bone Ca/P ratios observed in the current study pointed to the deficiency of Ca relative to P for maximal bone mineralization. The divergent ATTD Ca/ATTD P ratios for bone mineralization were accompanied by different urinary excretion of Ca and P. More urinary excretion of Ca, as suggested by the ascending urinary concentration of Ca in Fig. 4, resulted from increasing ATTD Ca:ATTD P ratios, whereas an elevation in urinary concentration/excretion of P occurred only at the two lowest ATTD Ca/ATTD P ratios. This indicates that a delicate balance between Ca and P is essential for their coordinated deposition in bone. Therefore, urinary concentrations/excretion of Ca and P could be used as a strong indicator for the homeostasis of Ca and P and the dietary balance between Ca and P.

4.4. Available and digestible P release by phytase

The available P release values for phytase depend on the selected response variables which have different correlation with dietary P supply and different susceptibility to varying Ca supply. In the current study, the available P release values based on body weight gain and G:F appeared higher than those based on percent bone ash and bone P weight. The growth performance response could be explained by the dependence of soft tissue growth on P and the extra-phosphoric effect of phytase beyond P nutrition (Lu et al., 2019; Lautrou et al., 2020). On the contrary, bone ash accumulation is driven mainly by Ca (Lautrou et al., 2020), and maximum bone mineralization requires more Ca than for maximum growth. Conventionally the same total Ca to total P ratio was maintained for both the reference diets and the test diets with phytase. As shown in the current study, however, the vain attempt to maintain the same total Ca/total P ratios in experimental diets resulted in declining ATTD Ca:ATTD P ratios with increasing phytase dose. This Ca/P imbalance was manifested by increased excretion of Ca and decreased excretion of P through urine when the ATTD Ca:ATTD P ratio increased whilst the opposite was observed when the ratio declined. These adjustments in urinary excretion of Ca and P indicate the pig's effort to restore the systemic balance between Ca and P when fed a diet with an unbalanced Ca/P ratio.

The available P release from phytase benchmarked to MCP as well as the TTTD of P in MCP were measured in the current study. This enabled us to convert the available P release (equivalent to P in the form of MCP) from phytase to digestible P release using the measured TTTD coefficient of 0.94 for P in MCP, and thus to do a methodological comparison between the measurements based on bone P weight and ATTD of P. As a consequence, there was no clear pattern in the comparative results, which questioned the validity of conversion between the 2 approaches of generating P matrix values using a coefficient. In pigs, digested P can be used for post-absorption net requirement at an efficiency of approximately 95% (NRC, 2012), and it is expected that the ratio of bioavailability of P between two P ingredients should be similar to the ratio of their digestibility of P. In other words, the digestibility of P in an ingredient could be calculated by multiplying its relative bioavailability of P with the digestibility of P in the standard P source, but such conversion was not justified by relevant studies. Baker et al. (2013) determined the STTD of P in dried distillers grains with solubles (DDGS) and dicalcium phosphate (DCP) to be 63.1% and 93.1% indicating a percentage of 67.8% of STTD of P in DDGS

relative to that in DCP, but the relative bioavailability of P in DDGS was measured to be 86–88% of that in DCP. The discrepancy between P digestibility and P relative bioavailability was also reported in broilers (Munoz et al., 2020). Essentially the fecal P digestibility is a measurement at a specific time point, whereas bone ash reflects a cumulative effect of MCP or phytase over time. Moreover, bone mineralization reflects not only the amount of digested and absorbed P, but also the coordinated deposition of Ca and P after their absorption. The different post-absorptive Ca/P ratios in the experimental diets could have impacted the proportion of absorbed P for bone deposition. Even though the available P release reflects a direct link between phytase efficacy and bone development (a practical utility), the digestible P release is laudable for its ease of measurement and connection with the ingredient/diet properties. Considering the growing body of research to define STTD Ca and P requirements to fulfil different production goals such as body weight gain *versus* bone mineralization, the determination of digestible P release as well as digestible Ca release from phytase is warranted.

5. Conclusion

In this study with weaner pigs, we observed that the measurement of available P release from phytase depends to a great extent on the selected response variables. Fixing the same total Ca/total P ratio in the experimental diets resulted in different ATTD Ca/ATTD P ratios, which led to different urinary excretion of Ca and P. Using the TTD coefficient of P in the standard P source to convert the available P release values to digestible values for phytase did not generate consistent results as those measured with ATTD coefficient of P. Therefore, these methodological differences should be noted when generating and applying the P matrix values of phytase.

CRedit authorship contribution statement

H. Zhai, K. Stamatopoulos: Conceptualization, Investigation, Writing – original draft. **J. Zhang, Z. Wang, S. Wang:** Investigation, Validation, Formal analysis. **S. Prasad, K. Stamatopoulos, S. Duval:** Writing – review & editing.

Disclosures

The authors are employees of DSM Nutritional Products.

Conflict of Interest

The authors declare that they have no competing interests.

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