Journal of Animal Science, 2022, **100**, 1–13
https://doi.org/10.1093/jas/skac269
Advance access publication 18 August 2022
Non Ruminant Nutrition

Apparent digestibility of energy and nutrients and efficiency of microbial phytase is influenced by body weight of pigs

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Abstract

An experiment was conducted to test the hypothesis that regardless of pig body weight (**BW**), increasing dietary phytase results in increased phytate degradation and improved digestibility of minerals, amino acids (**AA**), and gross energy (**GE**). Eighteen pigs were equipped with a T-cannula in the distal ileum and allotted to a triplicated 6 × 3 Youden square design with six diets and three collection periods of 7 d, for a total of nine replicate pigs per diet. This design was repeated four times to simulate four production phases, and there was a 7-d resting period before each collection phase started (BW at start of collections: 29.3, 53.6, 85.1, and 114.4 kg for phases 1, 2, 3, and 4, respectively). Six corn-soybean meal diets were formulated by including 0, 250, 500, 1,000, 2,000, or 4,000 phytase units/kg feed (**FTU**). The six diets were used throughout the experiment. Samples of feces and ileal digesta were collected in each period. Results indicated that regardless of pig BW, increasing inclusion of phytase increased (quadratic; $P < 0.05$) apparent ileal digestibility (**AID**) of crude protein (**CP**) and most AA, increased apparent total tract digestibility (**ATTD**) of Ca, P, K, Mg (linear and quadratic; $P < 0.05$), and Na (linear; $P < 0.05$), but decreased (linear and quadratic; $P < 0.05$) AID and ATTD of GE. In all phases, ileal concentrations of inositol phosphate (**IP**) 6, IP5, IP4, and IP3 decreased (linear and quadratic; $P < 0.05$), whereas ileal inositol increased (linear and quadratic; $P < 0.05$) with increasing dietary phytase. However, as pig BW increased, AID of GE, CP, and AA increased (linear, $P < 0.05$), and the AID of a few AA (Met, Phe, Thr, Trp, Ala, Asp, Gly, and Ser) also increased quadratically ($P < 0.05$). The ATTD of GE, K, and Mg increased (linear and quadratic; $P < 0.05$), but ATTD of Ca and Na (linear; $P < 0.05$) and of P (linear and quadratic; $P < 0.05$) decreased as pig BW increased. Ileal IP6 and IP3 (linear and quadratic; $P < 0.05$) and ileal IP5 and IP4 (linear; $P < 0.05$) increased, whereas ileal inositol decreased (linear; $P < 0.05$) as pig BW increased. In conclusion, regardless of pig BW, increasing dietary phytase increased phytate degradation and inositol release in the small intestine, and consequently increased mineral and AA digestibility. Older pigs have reduced Ca, P, and Na digestibility, but increased K, Mg, AA, and GE digestibility compared with younger pigs. The efficiency of dietary phytase to degrade phytate appears to decrease as pigs get older.

Lay Summary

The influence of dietary phytase in pig nutrition is often investigated using pigs from 20 to 40 kg, but there are limited data to demonstrate that data obtained in young pigs can be extrapolated to pigs above 40 kg. Therefore, an experiment was conducted to determine effects of increasing phytase levels (0, 250, 500, 1,000, 2,000, and 4,000 phytase units) on phytate breakdown and nutrient digestibility of pigs throughout four productive phases (25 to 50 kg, 50 to 75 kg, 75 to 100 kg, and 100 to 125 kg). Results indicated that regardless of pig body weight, the digestibility of macro-minerals and most amino acids increased with increasing dietary phytase because of increased phytate breakdown.

Key words: body weight, energy, nutrient digestibility, phytase, phytate degradation, pigs

Abbreviations: AA, amino acids; ADFI, average daily feed intake; ADG, average daily gain; AEE, acid hydrolyzed ether extract; AID, apparent ileal digestibility; ATTD, apparent total tract digestibility; BW, body weight; CP, crude protein; FTU, phytase units per kilogram of feed; G:F, gain to feed ratio; GE, gross energy; IP, inositol phosphate

Introduction

Phytic acid (*myo*-inositol-hexakis dihydrogen phosphate) or its salt, phytate, is the main form of P storage in feed ingredients of plant origin and a potential P source for pigs. However, because pigs have limited phytase activity in the small intestine, use of microbial phytase at approximately 500 units per kilogram of feed (**FTU**) is common in commercial diets. Phytase releases some of the phytate-bound P in plant ingredients, reduces the need for feed phosphates in the diet, and consequently decreases the amount of P excreted in the manure (Adeola and Cowieson, 2011). At intestinal pH, phytate carries a strong negative charge that allows for chelation of cations such as Ca and binding of protein, and the quantity of phytase needed to release all chelated nutrients is greater than 500 FTU (Wilcock and Walk, 2016). Inclusion of more than 500 FTU of phytase, also known as super-dosing, may result in extra-phosphoric effects as phytate is degraded to lower inositol phosphate (**IP**) esters with less capacity for interaction with minerals and amino acids (**AA**; Bedford and Walk, 2016). Indeed, in broiler chickens, inclusion of 1,000 to 2,000 FTU of phytase results in increased mineral and AA

Received June 27, 2022 Accepted August 15, 2022.

digestibility (Brenes et al., 2003; Cowieson et al., 2017a). In pigs, phytase increases digestibility of Ca and other minerals (She et al., 2015, 2018), but AA digestibility has not consistently been improved by phytase (Zeng et al., 2016; Mesina et al., 2019).

In experiments with pigs, the effect on phytate degradation and nutrient digestibility of using up to 3,000 FTU of phytase has been reported (Liao et al., 2005; Zeng et al., 2016; Mesina et al., 2019), but effects of inclusion of greater levels of phytase is poorly researched. In most experiments, body weight (**BW**) of pigs have been between 20 and 40 kg, and to our knowledge, no data for the effect of super-dosing of phytase on phytate degradation and nutrient digestibility over time are available. As a consequence, it is not known if results of super-dosing phytase in 20- to 40-kg pigs can be extrapolated to the entire growing-finishing period. Therefore, the objective of this experiment was to test the hypothesis that increasing the inclusion of phytase from 0 to 4,000 FTU results in increased phytate degradation and improved apparent digestibility of minerals, AA, and gross energy (**GE**) in diets regardless of pig BW.

Materials and Methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for this experiment. Pigs used in the experiment were the offspring of Line 359 boars and Camborough females (Pig Improvement Company, Hendersonville, TN, USA).

Animals and housing

Nineteen growing pigs were equipped with a T-cannula (6 cm length and 2.24 cm inner diameter) in the distal ileum. Three days after surgery, one pig was designated as the reserve pig and was not immediately used in the experiment, whereas the remaining 18 pigs (average BW: 24.0 ± 2.4 kg) were allotted to a triplicated 6 × 3 Youden square design with six diets and three collection periods of 7 d, for a total of nine replicate pigs per diet. This design was repeated four times during the growing period to simulate four production phases (24.0 to 46.8 kg, 46.8 to 76.1 kg, 76.1 to 106.3 kg, and 106.3 to 132.4 kg), and within each phase, there was a resting period of 7 d before sample collection started. Therefore, the experiment was conducted over 16 wk and pigs had an average BW of 29.3, 53.6, 85.1, and 114.4 kg at the start of collection for phases 1, 2, 3, and 4, respectively. Pigs were housed individually in 1.2 × 1.5 m pens that were equipped with a feeder and a nipple drinker in an environmentally controlled room. Pens had smooth sides and fully slatted tri-bar floors. Water was available at all times throughout the experiment. A spreadsheet program for making a balanced Latin square design (Kim and Stein, 2009) was used to allot pigs to experimental diets.

Diets and feeding

Pigs were allowed ad libitum access to feed throughout the experiment. Six diets based on corn and soybean meal (Table 1) were formulated based on estimated nutrient requirements for 50- to 75-kg pigs (NRC, 2012). Diets included 0, 250, 500, 1,000, 2,000, or 4,000 FTU of microbial phytase (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK), and no feed phosphate was included in the diets. Following recommendations from the manufacturer, provisions of total Ca and standardized total tract digestible P were reduced by 0.16 and 0.11%, respectively, compared with the requirement (NRC, 2012) to account for the expected release of Ca and P by phytase. All diets also contained 0.40% titanium dioxide as an indigestible marker. For the 7-d resting period, a common diet without phytase was formulated to meet estimated requirements for pigs from 50 to 75 kg (NRC, 2012). Therefore, a total of 7 diets were formulated (Table 2).

Table 1. Analyzed composition of ingredients

Item	Corn	Soybean meal	Calcium carbonate
Gross energy, kcal/kg	3,828	4,172	-
Dry matter, %	85.46	88.18	99.98
Ash, %	1.48	7.89	90.64
Crude protein, %	6.42	46.77	-
AEE[1], %	3.58	1.69	-
Ca, %	0.04	0.29	38.93
P, %	0.25	0.68	0.03
Phytate[2], %	0.63	1.54	-
Phytate-bound P, %	0.18	0.43	-
Non-phytate P[3], %	0.07	0.16	-

[1]AEE, acid hydrolyzed ether extract.
[2]Phytate was calculated by dividing the concentration of phytate-bound P by 0.282 (Tran and Sauvant, 2004).
[3]Non-phytate P was calculated as the difference between total P and phytate-bound P.

Sample collection

Ingredient samples were collected at the feed mill after diet mixing, and each diet sample was a mix of samples collected from 10 randomly chosen feed bags of 25 kg. Samples were later ground and sub-sampled for nutrient analysis. Pigs were weighed weekly and the amount of feed offered was recorded daily. Within each production phase, the initial 4 d of each collection period were considered an adaptation period to the diet. Feces samples were collected in the morning of day 5 via anal stimulation, and ileal digesta samples were collected for 8 h on d 6 and 7 (16 h in total). All samples were stored at −20 °C immediately after collection and no HCl was added to the collection bags (Lee et al., 2021). At the end of each collection period, pigs were deprived of feed overnight, and a new experimental diet was offered the following morning. At the conclusion of each phase, fecal and ileal digesta samples were thawed, mixed within animal and diet, and a sub-sample was lyophilized (Lagos and Stein, 2019) and finely ground.

Sample analysis

Ingredients and diets were analyzed for dry matter by oven drying at 135 °C for 2 h (Method 930.15; AOAC Int., 2019) and for ash at 600 °C for 2 h (Method 942.05; AOAC Int., 2019). Corn, soybean meal, limestone, diet, and fecal samples were analyzed for Ca and P by inductively coupled plasma-optical emission spectrometry (Method 985.01 A, B, and C; AOAC Int., 2019) after dry ash preparation (Method 942.05; AOAC Int., 2019) followed by wet digestion with nitric acid (Method 3050 B; US-EPA, 2000). Diets and feces

Table 2. Ingredient composition and analyzed values of experimental diets[1,2]

Item[3]	Phytase units (FTU)						Common diet
	0	250	500	1,000	2,000	4,000	
Ingredient, %							
Ground corn	67.69	67.685	67.68	67.67	67.65	67.61	67.35
Soybean meal, 48% CP	28.00	28.00	28.00	28.00	28.00	28.00	28.00
Soybean oil	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Calcium carbonate	0.86	0.86	0.86	0.86	0.86	0.86	1.00
Monocalcium phosphate	-	-	-	-	-	-	0.60
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Titanium dioxide	0.40	0.40	0.40	0.40	0.40	0.40	-
Vitamin mineral premix[4]	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Phytase concentrate[5]	-	0.005	0.01	0.02	0.04	0.08	-
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Analyzed values							
GE, kcal/kg	3,966	3,957	3,957	3,935	3,937	3,938	4,010
Dry matter, %	86.94	87.28	86.77	86.72	86.65	86.80	88.11
Ash, %	4.19	4.32	4.19	4.30	4.38	4.26	4.61
CP, %	16.39	16.54	16.39	16.33	16.26	16.30	16.31
AEE, %	3.37	3.63	3.62	3.65	3.41	3.65	3.82
Amino acids, %							
Arg	1.07	1.17	1.14	1.09	1.11	1.12	1.13
His	0.45	0.48	0.48	0.46	0.46	0.46	0.47
Ile	0.77	0.82	0.80	0.77	0.78	0.79	0.79
Leu	1.47	1.54	1.55	1.48	1.52	1.51	1.53
Lys	0.93	1.01	0.98	0.94	0.95	0.97	0.98
Met	0.27	0.26	0.27	0.27	0.27	0.27	0.28
Phe	0.85	0.91	0.90	0.86	0.88	0.89	0.89
Thr	0.62	0.67	0.67	0.64	0.65	0.65	0.67
Trp	0.20	0.20	0.20	0.21	0.21	0.20	0.21
Val	0.83	0.89	0.88	0.84	0.85	0.86	0.86
Ca, %	0.48	0.49	0.44	0.45	0.48	0.47	0.62
P, %	0.37	0.37	0.39	0.38	0.36	0.38	0.48
Phytate bound-P, %	0.26	0.22	0.24	0.24	0.18	0.18	0.24
Phytase activity, FTU	< 50	327	534	1,050	2,260	4,280	< 50
Phytate esters[6], nmol/g dry matter							
IP6	16,057	14,650	14,176	15,559	14,828	17,239	-
IP5	1,889	1,699	1,833	2,199	1,902	2,233	-
IP4	213	191	181	361	259	549	-

[1]Diets were formulated to contain 2,526 kcal/kg of net energy and the following quantities of amino acids (expressed as standardized ileal digestible) Lys, 0.86%; Met, 0.27%; Thr, 0.59%; Trp, 0.20%.
[2]Diets were formulated to contain 0.43 and 0.59% Ca and 0.37 and 0.50% P, for the experimental diets and the common diet, respectively.
[3]AEE, acid hydrolyzed ether extract; CP, crude protein; GE, gross energy; IP, inositol phosphate.
[4]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as cholecalciferol, 2,210 IU; vitamin E as $_{DL}$-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin,6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate; Fe, 125 mg as ironsulfate; I, 1.26mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganoussulfate; Se, 0.30mg as sodium selenite and selenium yeast; and Zn, 125.1mg as zinc sulfate.
[5]The phytase concentrate contained 5,000 units of phytase/g (Quantum Blue, AB Vista, Marlborough, UK).
[6]The concentrations of IP3 and inositol in the diets were below detection limits.

were also analyzed for K, Mg, and Na via inductively coupled plasma-optical emission spectrometry. The concentration of AA was analyzed in diet and ileal digesta samples (Method 982.30 E [a, b, c]; AOAC Int., 2019) using a Hitachi AA Analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA). Corn, soybean meal, diets, and ileal digesta samples were also analyzed for N using the combustion procedure (method 990.03; AOAC Int., 2019) on a LECO FP628 (LECO Corp., Saint Joseph, MI, USA) and crude protein (**CP**) was then calculated as N × 6.25. Corn, soybean meal, diets, fecal, and ileal samples were analyzed for GE using an isoperibol bomb calorimeter (Model 6400, Parr

Instruments, Moline, IL, USA). Corn, soybean meal, and diets were also analyzed for acid hydrolyzed ether extract (**AEE**; Method 2003.06; AOAC Int., 2019) using an Ankom^{HCl} followed by an Ankom^{XT15} (Ankom Technology, Macedon, NY, USA). Diets, fecal, and ileal digesta samples were analyzed for IP esters and inositol using high-performance ion chromatography-based techniques as described by Walk et al. (2018). These samples were also analyzed for Ti following the procedure by Myers et al. (2004). Corn, soybean meal, and diets were also analyzed for phytate-bound P by wet chemistry using the Megazyme kit (Megazyme Inc., Chicago, IL, USA). Phytase activity was analyzed in diets by the enzyme-linked immunosorbent assay method using Quantiplate Kits for Quantum Blue (AB Vista, Plantation, FL, USA).

Calculations and statistical analyses

Average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain to feed ratio (**G:F**) were calculated for each productive phase. The percentage of phytate in corn and soybean meal was calculated by dividing the analyzed phytate-bound P by 0.282 (Tran and Sauvant, 2004) and non-phytate P was calculated by subtracting the amount of phytate-bound P from total P. The apparent ileal digestibility (**AID**) of GE, CP, AA, and IP6 and the apparent total tract digestibility (**ATTD**) of GE and macro minerals (Ca, P, K, Mg, and Na) in experimental diets were calculated as described by Stein et al. (2007):

$$\textit{Digestibility of nutrients, } \% = \left[1 - \left(\frac{\textit{nutrient in sample}}{\textit{nutrient in diet}}\right) \times \left(\frac{\textit{marker in diet}}{\textit{marker in sample}}\right)\right] \times 100$$

where digestibility is the AID or ATTD of nutrients or energy, nutrient in sample is the nutrient or energy concentration in ileal digesta or fecal samples and nutrient in diet is the concentration of nutrient or energy in the diet. Marker in diet and marker in sample are the concentrations of Ti in diets and ileal digesta or feces samples, respectively.

Normality of residuals and assumptions of the model were tested using the INFLUENCE, GPLOT, and UNIVARIATE procedures of SAS (SAS Inst. Inc., Cary, NC, USA). Data for growth performance, phytate degradation, and digestibility of minerals, phytate, AA, and GE were analyzed using the PROC MIXED procedure of SAS with pig as the experimental unit. The initial model included the main effects of phytase level and phase and the interaction between phytase level and phase, and the random effects of period within phase and pig. If the interaction was significant, contrast statements were used to determine linear and quadratic effects of phytase level within each phase. Coefficients for unevenly spaced linear contrasts were obtained using the PROC IML option of SAS. Likewise, if the effect of phase was significant, contrast statements were used to determine linear and quadratic effects of phase. However, if the interaction between phytase level and phase was not significant, only main effects were included in the final model, and contrast statements were used to determine linear and quadratic effects of phytase level and phase. Outliers were determined by plotting the residuals in a quantile-quantile plot against the normal distribution and identifying values that were beyond ± 3.0 standard deviations. Treatment means were calculated using the LSMEANS statement option in SAS. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

During the third phase, one pig was removed from the experiment by the end of the second collection period due to poor condition. This pig was replaced in phase 4 by the extra pig that was assigned to the experiment. The data for the removed pig from the first two periods of phase 3 were included in the statistical analysis. The new pig and the other 17 pigs consumed their assigned diets without apparent problems and no other health problems were observed.

During phase 1, there was a linear increase ($P < 0.05$) in ADG and G:F of pigs as phytase inclusion increased in the diets (Table 3). Likewise, during phase 2, ADG of pigs linearly increased ($P < 0.05$) with increasing levels of dietary phytase. A tendency ($P < 0.10$) for a linear increase in G:F of pigs as the inclusion of phytase in diets increased was also observed during phase 3. In phase 4, there was a tendency ($P < 0.10$) for a quadratic decrease in ADFI of pigs as the inclusion of dietary phytase increased.

There was no interaction between phytase inclusion level and phase for the AID of GE, CP, and AA in diets, therefore, the final model only included the main effects of phytase inclusion level and phase (Table 4). The AID of GE decreased (linear and quadratic; $P < 0.05$), whereas the AID of CP and all AA except His and Gly increased (quadratic; $P < 0.05$) as the inclusion of phytase in diets increased. The AID of Leu, Ala, and Cys also increased linearly ($P < 0.05$) and the AID of Thr and Val tended to increase linearly ($P < 0.10$) with increasing levels of phytase in diets. Likewise, the AID of Gly tended to increase (linear and quadratic; $P < 0.05$) with increasing dietary phytase, but there was a linear reduction ($P < 0.05$) in the AID of His as phytase inclusion in diets increased.

The AID of GE and CP increased (linear; $P < 0.05$) and tended to increase (quadratic; $P < 0.10$) with increasing BW of pigs. Similarly, there was an increase (linear and quadratic; $P < 0.05$) in the AID of Met, Phe, Thr, Trp, Ala, Asp, Gly, and Ser as pig BW increased and increasing BW resulted in a linear increase ($P < 0.05$) in the AID of all other AA.

The final model for the ATTD of GE and macro minerals only included the main effects of phytase inclusion level and phase because no interactions between main effects were observed (Table 5). The ATTD of GE decreased (linear and quadratic; $P < 0.05$), whereas the ATTD of Ca, P, K, and Mg increased (linear and quadratic; $P < 0.05$) with increasing dietary phytase. However, the positive effect ($P < 0.05$) of phytase level on the ATTD of Na in diets was only linear. The ATTD of GE, K, and Mg increased (linear and quadratic; $P < 0.05$) as BW of pigs increased. In contrast, there was a linear and quadratic reduction ($P < 0.05$) in the ATTD of P with increasing BW of pigs. The ATTD of Ca and Na also decreased (linear; $P < 0.05$) as pig BW increased.

Regardless of phase, the AID of IP6 increased as increasing levels of phytase were added to the diets, but the increase was greater in phase 1 than in subsequent phases (interaction, $P < 0.05$; Table 6). Reductions of concentrations of IP6 and IP5 in ileal digesta were observed as levels of dietary phytase increased, but the magnitude of the responses diminished from phase 1 to phase 4 (interaction, $P < 0.05$; Table 7). Responses for IP4 and IP3 were similar to responses for IP6 and IP5 with the exception that in phase 4, concentrations obtained

Table 3. Growth performance of pigs fed diets containing 0, 250, 500, 1,000, 2,000, or 4,000 units of microbial phytase during phases 1, 2, 3, and 4[1]

Item, kg[2]	Phytase units (FTU)							*P*-value	
	0	250	500	1,000	2,000	4,000	SEM	Linear	Quadratic
Phase 1 (29.7–46.8 kg)									
ADG	0.759	0.786	0.757	0.758	0.876	0.894	0.065	0.020	0.766
ADFI	1.915	1.730	1.712	1.767	1.871	1.803	0.115	0.752	0.894
G:F	0.404	0.447	0.441	0.428	0.493	0.496	0.028	0.012	0.397
Phase 2 (53.6–76.1 kg)									
ADG	0.951	1.046	1.137	1.049	1.133	1.187	0.084	0.019	0.456
ADFI	2.391	2.568	2.580	2.426	2.634	2.564	0.155	0.147	0.260
G:F	0.391	0.420	0.452	0.432	0.428	0.446	0.021	0.222	0.539
Phase 3 (85.1–106.3 kg)									
ADG	1.060	0.956	0.930	1.098	1.006	1.121	0.105	0.313	0.757
ADFI	3.193	3.157	3.317	3.347	3.095	3.201	0.092	0.474	0.996
G:F	0.324	0.297	0.279	0.337	0.323	0.358	0.030	0.097	0.818
Phase 4 (114.4–132.4 kg)									
ADG	0.703	0.754	0.991	0.871	0.866	0.901	0.118	0.325	0.370
ADFI	3.516	3.520	3.531	3.450	3.382	3.664	0.106	0.303	0.050
G:F	0.199	0.216	0.277	0.247	0.253	0.247	0.032	0.376	0.188

[1]Data are least square means of 8 or 9 observations.
[2]ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed ratio.

with 4,000 FTU were not lower than those observed if no phytase was used in the diets. Ileal concentrations of inositol increased more with increasing dietary phytase in phase 1 than in subsequent phases (interaction, $P < 0.05$). In phase 1, concentrations of IP6 and IP5 in ileal digesta decreased (linear and quadratic; $P < 0.05$) as the inclusion of phytase increased from 0 to 4,000 FTU. There was also a decrease in the concentration of IP4 (linear; $P < 0.05$), but an increase and then a decrease in the concentration of IP3 (linear and quadratic; $P < 0.05$) in ileal digesta as phytase inclusion increased in the diet. The concentration of inositol in ileal digesta increased (linear and quadratic; $P < 0.05$) in phase 1 with increasing inclusion of dietary phytase. In phase 2, concentrations of IP6 and IP5 decreased (linear and quadratic; $P < 0.05$), whereas concentrations of IP4 and IP3 increased and then decreased (linear and quadratic; $P < 0.05$) in ileal digesta as phytase inclusion increased from 0 to 4,000 FTU. The concentration of inositol in ileal digesta in phase 2 increased (linear and quadratic; $P < 0.05$) as the inclusion of phytase increased. In phases 3 and 4, the concentration of IP6 decreased (linear and quadratic; $P < 0.05$) and the concentrations of IP5, IP4, and IP3 increased and then decreased (linear and quadratic; $P < 0.05$) in ileal digesta as the inclusion of dietary phytase increased. The ileal digesta concentration of inositol also increased (linear and quadratic; $P < 0.05$) in phases 3 and 4 with increasing inclusion levels of phytase.

Fecal concentrations of IP6 were reduced by increasing concentrations of dietary phytase, but the effect of low doses of phytase (250 and 500 FTU) on fecal IP6 concentration was greater in phases 3 and 4 (average IP6 disappearance vs. control = 56%) than in phases 1 and 2 (average IP6 disappearance vs. control = 18%; interaction, $P < 0.05$; Table 8). Fecal concentration of IP5 was not affected by dietary phytase in phase 1, but decreased with dietary phytase in phases 2, 3, and 4 (interaction, $P < 0.05$). In contrast, IP4 in feces increased with dietary phytase in phases 1, 3, and 4, but that was not the case in phase 2 (interaction, $P < 0.05$). In phase 1, the concentration of IP6 in feces linearly decreased ($P < 0.05$), whereas the concentration of fecal IP4 increased and then decreased (linear: $P < 0.05$ and quadratic: $P < 0.10$) as phytase inclusion increased. In phase 2, there was a reduction in fecal concentrations of IP6 (linear; $P < 0.05$) and IP5 (linear: $P < 0.05$; quadratic: $P < 0.10$) as the inclusion of phytase increased. However, phytase did not influence fecal concentrations of IP5 in phase 1 or IP4 in phase 2. In phase 3, the concentration of IP6 in fecal samples tended to decrease (linear and quadratic; $P < 0.10$) with increasing dietary phytase, and there was a reduction in the concentration of IP5 (linear: $P < 0.05$; quadratic: $P < 0.10$) and IP4 (linear; $P < 0.05$) in fecal samples as the inclusion of phytase increased. In phase 4, fecal concentrations of IP6 (quadratic; $P < 0.05$) and IP5 (linear and quadratic; $P < 0.05$) decreased, whereas fecal concentrations of IP4 increased (quadratic; $P < 0.05$) as phytase inclusion increased. Concentrations of IP3 and inositol in all fecal samples were below detection limits.

The AID of IP6 decreased (linear and quadratic; $P < 0.05$) with increasing BW of pigs (Table 9). There was an increase in concentrations of IP6 (linear and quadratic; $P < 0.05$), IP5 (linear; $P < 0.05$), IP4 (linear: $P < 0.05$ and quadratic: $P < 0.10$), and IP3 (linear and quadratic; $P < 0.05$) in ileal digesta as BW of pigs increased. However, the concentration of inositol in ileal digesta linearly decreased ($P < 0.05$) with increasing BW of pigs. In fecal samples, concentrations of IP6 (linear and quadratic; $P < 0.05$), IP5 (linear; $P < 0.05$), and IP4 (linear and quadratic; $P < 0.05$) also increased as pigs BW increased.

Discussion

In the design of the experiment, it was decided to use the same diet throughout the growing-finishing phase to avoid potentially confounding effects of changing diets during the

Table 4. Effect of inclusion level of microbial phytase and phase on the apparent ileal digestibility of gross energy (GE), crude protein (CP) and amino acids (AA) in diets fed to growing pigs[1]

Item, %	Phytase units (FTU)							*P*-value		Phase[2]					*P*-value	
	0	250	500	1,000	2,000	4,000	SEM	Linear	Quad.	1	2	3	4	SEM	Linear	Quadratic
GE	73.0	72.6	72.3	73.7	73.4	70.2	0.54	< 0.001	< 0.001	69.5	72.3	73.7	74.7	0.52	< 0.001	0.064
CP	77.6	78.2	77.8	78.3	78.8	77.1	0.66	0.337	0.010	75.5	78.2	79.4	78.7	0.91	0.021	0.084
Indispensable AA																
Arg	89.5	90.5	90.2	90.3	90.6	89.7	0.24	0.503	< 0.001	89.1	90.1	90.5	90.8	0.26	0.001	0.183
His	83.6	85.0	84.5	84.2	83.4	82.1	0.38	< 0.001	0.138	81.8	84.1	83.6	85.6	0.40	< 0.001	0.673
Ile	82.3	84.1	83.5	83.7	84.0	82.8	0.35	0.505	< 0.001	82.4	83.6	83.1	84.5	0.33	< 0.001	0.723
Leu	82.7	84.1	83.9	84.2	84.3	82.6	0.37	0.037	< 0.001	82.1	83.4	84.1	85.0	0.33	< 0.001	0.388
Lys	80.9	83.0	82.5	82.8	83.2	82.5	0.44	0.108	< 0.001	80.2	82.6	82.6	84.5	0.47	< 0.001	0.572
Met	86.3	85.9	86.4	87.2	87.3	85.8	0.36	0.647	< 0.001	85.6	86.4	85.9	88.1	0.32	< 0.001	0.007
Phe	82.6	84.5	84.2	84.4	84.8	83.6	0.34	0.530	< 0.001	82.3	83.8	84.9	85.1	0.31	< 0.001	0.036
Thr	73.2	75.6	75.4	75.1	75.2	73.5	0.54	0.066	0.001	70.9	74.9	76.2	76.8	0.53	< 0.001	0.006
Trp	81.1	81.3	81.5	83.3	82.9	81.5	0.46	0.302	< 0.001	80.6	80.4	81.4	85.4	0.44	< 0.001	< 0.001
Val	78.7	80.8	80.3	80.2	80.4	78.9	0.42	0.062	0.001	78.2	79.5	80.7	81.2	0.37	< 0.001	0.184
Mean	82.3	83.9	83.6	83.7	83.9	82.6	0.36	0.256	< 0.001	81.6	83.2	83.7	84.8	0.34	< 0.001	0.339
Dispensable AA																
Ala	78.0	79.6	79.4	79.4	79.3	77.7	0.48	0.030	0.002	76.2	79.3	79.6	80.5	0.42	< 0.001	0.001
Asp	79.5	81.7	81.6	81.6	82.1	80.8	0.39	0.456	< 0.001	79.3	81.6	81.5	82.3	0.40	0.001	0.049
Cys	69.6	69.1	69.0	69.9	68.5	64.0	0.83	< 0.001	0.014	63.3	68.2	69.3	72.6	0.81	< 0.001	0.274
Glu	83.6	85.2	85.1	85.1	85.9	84.4	0.55	0.740	< 0.001	83.6	85.4	84.7	85.7	0.51	< 0.001	0.187
Gly	68.4	71.9	70.8	70.1	70.2	68.8	0.75	0.055	0.088	66.0	70.4	71.4	72.4	0.78	< 0.001	0.034
Ser	80.0	81.2	81.1	81.7	81.5	80.2	0.43	0.439	< 0.001	78.7	80.9	82.1	82.1	0.43	< 0.001	0.018
Tyr	83.1	84.5	84.0	84.2	84.7	83.3	0.36	0.468	< 0.001	82.5	83.7	84.1	85.5	0.38	< 0.001	0.715
Mean	79.8	81.6	81.4	81.3	81.8	80.3	0.47	0.492	< 0.001	79.0	81.4	81.4	82.3	0.44	< 0.001	0.056
All AA	81.0	82.7	82.5	82.5	82.8	81.4	0.40	0.364	< 0.001	80.3	82.3	82.5	83.5	0.38	< 0.001	0.127

[1]Data are least squares means of 34 to 36 observations for phytase and 52 to 54 observations for phase.
[2]Phase 1: 29.7 to 46.8 kg; Phase 2: 53.6 to 76.1 kg; Phase 3: 85.1 to 106.3 kg; Phase 4: 114.4 to 132.4 kg.

Table 5. Effect of inclusion level of microbial phytase and phase on the apparent total tract digestibility of gross energy (GE) and macro minerals in diets fed to growing pigs[1]

Item, %	Phytase units (FTU)							*P*-value		Phase[2]				*P*-value	
	0	250	500	1,000	2,000	4,000	SEM	Linear	Quad.	1	2	3	4	SEM	Linear	Quadratic
GE	84.8	83.7	84.5	84.4	85.0	83.0	0.44	0.003	0.003	82.1	83.5	86.0	85.4	0.45	< 0.001	0.018
Ca	48.6	64.8	70.3	71.9	73.9	75.9	1.46	< 0.001	< 0.001	76.8	71.4	66.1	55.9	1.60	< 0.001	0.111
P	23.1	45.7	57.4	68.1	77.1	82.2	1.56	< 0.001	< 0.001	63.6	58.8	61.5	51.8	1.30	< 0.001	0.046
K	82.8	82.1	84.4	83.8	86.4	84.2	0.96	0.028	0.001	78.6	83.0	87.6	86.6	1.23	0.001	0.049
Mg	16.9	19.8	23.9	26.5	31.0	26.8	2.03	< 0.001	< 0.001	14.5	25.2	30.6	26.3	2.06	0.002	0.005
Na	82.0	82.4	82.6	84.1	86.9	88.2	1.79	< 0.001	0.295	87.5	86.4	83.9	79.7	1.98	0.011	0.389

[1]Data are least squares means of 34 to 36 observations for phytase and 52 to 54 observations for phase.
[2]Phase 1: 29.7 to 46.8 kg; Phase 2: 53.6 to 76.1 kg; Phase 3: 85.1 to 106.3 kg; Phase 4: 114.4 to 132.4 kg.

experimental period. The advantage of this approach is that it was possible to compare results among different phases and thereby address the hypothesis that responses to phytase are not affected by BW of pigs. An alternative approach would have been to use different diets in the four phases, as is usually done in commercially fed pigs. This approach would have allowed us to analyze the phytase response within each phase, but we would not have been able to compare among phases, which was one of the objectives of the work. We therefore chose to use the same diet in the entire experimental period.

The analyzed values for Ca, P, and phytate in corn, soybean meal, and limestone were close to those used in diet formulation (NRC, 2012), thus, the reason Ca concentration in diets was slightly greater than expected is likely the feed particle segregation that results in increased variability in analytical values for Ca compared with P (Jones et al., 2018). The observation that increasing dietary phytase resulted in improved growth performance of pigs during phases 1 and 2, but not in phases 3 and 4 is in agreement with Holloway et al. (2019) who reported that super-dosing of phytase provided smaller benefits in growing-finishing pigs than in nursery pigs. However, this observation may also be a consequence of diets containing Ca and P below or at the requirement in phases 1 and 2, but above the requirement in phases 3 and 4. The increased release of Ca and P from elevated levels of phytase may, therefore, have contributed to pigs absorbing Ca and P closer to the requirements in phases 1 and 2 as dietary phytase increased. In contrast, in phases 3 and 4, diets contained more Ca and P than required and the increased release of Ca and P from the greater levels of phytase did not provide additional benefits to the pigs (NRC, 2012). However, the experiment was not conducted as a performance experiment, and although growth performance parameters were analyzed because data for body weight and feed intake were recorded by phase, it is possible results are not representative of commercial pigs that are not cannulated.

The lack of an interaction between phytase inclusion level and phase for AA, mineral, and energy digestibility, indicates that the effect of phytase on nutrient digestibility is independent of pig BW. The quadratic increase in AID of most AA as the inclusion of dietary phytase increased is in agreement with Cowieson et al. (2017b) and Zouaoui et al. (2018) who reported a positive effect of microbial phytase on AA digestibility from a review of approximately 30 publications. The observation that phytase inclusion at 2,000 FTU resulted in a 2.0 percentage unit increase in AID of all AA is within the range of 1.7 to 2.8 percentage unit increase observed upon phytase supplementation (Selle and Ravindran, 2008; Cowieson et al., 2017b; Zouaoui et al., 2018). The linear reduction in the AID of His as dietary phytase increased was not expected, but a reduced median response in His digestibility to phytase compared with other AA, has been previously reported (Selle and Ravindran, 2008). Nevertheless, results from this experiment are in contrast with several experiments indicating a lack of phytase effect on AID of most or all AA (Liao et al., 2005; She et al., 2018; Mesina et al., 2019; Rosenfelder-Kuon et al., 2020a). The reason for this observation is likely the low number of replicates (i.e., six to eight) used in AA experiments, which results in a high standard error and a reduced capacity to detect statistical differences. There were between 34 and 36 replicate pigs per diet in this experiment, which resulted in smaller standard error of the means; therefore, a greater sample size may be necessary to validate effects of phytase on AID of AA.

Table 6. Apparent ileal digestibility (AID, %) of inositol phosphate (IP) 6 in diets containing 0, 250, 500, 1,000, 2,000, or 4,000 units of microbial phytase and fed to pigs during phases 1, 2, 3, and 4[1,2]

Phase[3]	Phytase units (FTU)							*P*-value	
	0	250	500	1,000	2,000	4,000	SEM	Linear	Quadratic
1	28.1	92.1	95.6	96.5	98.7	98.6	2.07	<0.001	<0.001
2	16.3	53.8	77.9	88.5	94.8	96.5	2.76	<0.001	<0.001
3	14.9	53.7	74.6	86.2	93.8	94.9	2.42	<0.001	<0.001
4	13.0	60.6	70.9	86.4	89.3	91.8	2.37	<0.001	<0.001

[1]Data are least square means of 8 or 9 observations.
[2]The AID of IP6 increased faster in response to phytase inclusion in phase 1 than in subsequent phases (interaction, $P < 0.05$).
[3]Phase 1: 29.7 to 46.8 kg; Phase 2: 53.6 to 76.1 kg; Phase 3: 85.1 to 106.3 kg; Phase 4: 114.4 to 132.4 kg.

Table 7. Concentrations of inositol phosphate (IP) esters and inositol (nmol/g dry matter) in ileal digesta samples from pigs fed diets containing 0, 250, 500, 1,000, 2,000, or 4,000 units of microbial phytase during phases 1, 2, 3, and 4[1,2]

Item	Phytase units (FTU)							*P*-value	
	0	250	500	1,000	2,000	4,000	SEM	Linear	Quadratic
Phase 1 (29.7–46.8 kg)									
IP6	36,863	6,279	2,309	2,157	665	720	1,989	<0.001	<0.001
IP5	6,107	1,144	474	424	95	80	360	<0.001	<0.001
IP4	2,806	5,176	3,262	1,007	1,133	151	821	<0.001	0.121
IP3	1,213	2,067	1,248	582	452	203	269	<0.001	0.046
Inositol	77	755	2,286	4,157	7,788	8,659	660	<0.001	<0.001
Phase 2 (53.6–76.1 kg)									
IP6	48,433	25,342	12,365	6,496	2,617	1,263	1,695	<0.001	<0.001
IP5	5,855	5,472	2,611	1,617	305	304	313	<0.001	<0.001
IP4	1,297	8,597	10,639	9,646	3,258	826	854	<0.001	<0.001
IP3	876	2,933	3,631	3,139	1,420	655	253	<0.001	<0.001
Inositol	57	357	908	2,209	5,146	7,245	477	<0.001	0.004
Phase 3 (85.1–106.3 kg)									
IP6	48,716	26,444	14,383	7,996	4,271	2,993	1,751	<0.001	<0.001
IP5	5,348	7,393	4,158	1,493	534	187	456	<0.001	<0.001
IP4	2,097	9,390	10,807	10,755	2,458	1,289	867	<0.001	0.003
IP3	747	3,033	3,710	3,975	1,442	793	169	<0.001	<0.001
Inositol	109	883	1,162	2,515	5,432	5,671	548	<0.001	<0.001
Phase 4 (114.4–132.4 kg)									
IP6	50,737	26,505	18,351	9,774	4,855	3,774	1,485	<0.001	<0.001
IP5	5,522	8,863	5,743	1,842	697	467	355	<0.001	<0.001
IP4	1,061	11,765	15,927	11,097	5,966	1,867	916	<0.001	<0.001
IP3	643	3,104	4,104	4,409	3,005	1,195	310	0.002	<0.001
Inositol	35	55	5	373	417	1,804	139	<0.001	0.007

[1]Data are least square means of 8 or 9 observations.
[2]The reduction in IP6, IP5, IP4, and IP3, and the increase in inositol in response to phytase inclusion was greater in phase 1 than in subsequent phases (interaction, $P < 0.05$).

The observed increase in AID of AA as BW of pigs increased is in contrast with data from Pedersen et al. (2016) who reported no differences in the standardized ileal digestibility of AA in soybean meal between 20- to 50-kg and pigs above 50-kg pigs fed semi-synthetic diets. We did not measure basal endogenous losses in this experiment, and therefore, we were not able to calculated standardized ileal digestibility of AA. It is possible that differences in basal endogenous losses between growing and finishing pigs is the reason we observed a different response for AID of AA than Pedersen et al. (2016) reported for standardized ileal digestibility of AA. However, gestating sows had increased AID of most AA in corn and soybean meal compared with growing pigs (Stein et al., 1999). Thus, the 3.2 percentage unit increase in the AID of indispensable AA in finishing pigs compared with growing pigs may indicate that in diets for finishing pigs, less soybean meal is needed, which may result in reduced production costs. However, it is not known if the reason for the increased AID of AA in finishing pigs is related to reduced endogenous secretion of AA or increased efficiency of AA absorption.

The observation that increasing dietary phytase resulted in reduced AID and ATTD of GE is in agreement with Mesina

Table 8. Concentrations of inositol phosphate (IP) esters (nmol/g dry matter) in fecal samples from pigs fed diets containing 0, 250, 500, 1,000, 2,000, or 4,000 units of microbial phytase during phases 1, 2, 3, and 4[1,2,3]

Item	Phytase units (FTU)							*P*-value	
	0	250	500	1,000	2,000	4,000	SEM	Linear	Quadratic
Phase 1 (29.7–46.8 kg)									
IP6	329	291	194	223	257	161	46.9	0.021	0.747
IP5	23	18	21	24	19	18	9.7	0.715	0.924
IP4	4	33	29	36	10	5	6.7	0.014	0.090
Phase 2 (53.6–76.1 kg)									
IP6	392	447	263	357	275	247	47.4	0.001	0.220
IP5	99	57	21	59	34	19	10.6	< 0.001	0.059
IP4	34	82	65	73	43	51	8.4	0.210	0.508
Phase 3 (85.1–106.3 kg)									
IP6	519	246	152	229	266	201	67.1	0.050	0.071
IP5	164	72	77	47	47	23	23.5	0.002	0.052
IP4	293	224	151	172	204	149	32.3	0.021	0.233
Phase 4 (114.4–132.4 kg)									
IP6	1,189	608	578	562	654	668	132.7	0.133	0.008
IP5	190	107	97	104	71	88	22.8	0.006	0.004
IP4	50	60	79	83	106	41	20.4	0.664	0.008

[1]Data are least square means of 8 or 9 observations.
[2]Concentrations of IP3 and inositol in fecal samples were below detection limits.
[3]The effect of low phytase doses of on fecal IP6 was more severe in phases 3 and 4 than in phases 1 and 2 (interaction, $P < 0.05$). Fecal IP5 decreased with increasing dietary phytase in phases 2, 3, and 4, but not in phase 1 (interaction, $P < 0.05$). Fecal IP4 increased with dietary phytase in phases 1, 3, and 4, but not in phase 2 (interaction, $P < 0.05$).

Table 9. Effect of phase on the apparent ileal digestibility (AID) of inositol phosphate (IP) 6 and concentrations of IP6, IP5, IP4, IP3 and inositol in ileal digesta and fecal samples from growing-finishing pigs[1]

Item	Phase[2]					*P*-value	
	1	2	3	4	SEM	Linear	Quadratic
AID of IP6, %	84.9	71.3	69.9	68.8	1.31	<0.001	<0.001
IP and inositol concentration, nmol/g dry matter							
Ileal digesta samples							
IP6	8,165	16,086	17,401	18,956	1,092	<0.001	0.007
IP5	1,361	2,708	3,181	3,825	230	<0.001	0.100
IP4	2,312	5,695	6,087	7,947	654	<0.001	0.062
IP3	984	2,100	2,268	2,728	131	<0.001	0.009
Inositol	3,938	2,613	2,629	717	291	<0.001	0.275
Fecal samples[3]							
IP6	239	333	268	710	50.2	<0.001	0.003
IP5	20	50	74	108	9.3	<0.001	0.743
IP4	20	58	196	70	8.9	<0.001	<0.001

[1]Data are least square means of 51 to 53 observations.
[2]Phase 1: 29.7 to 46.8 kg; Phase 2: 53.6 to 76.1 kg; Phase 3: 85.1 to 106.3 kg; Phase 4: 114.4 to 132.4 kg.
[3]Concentrations of IP3 and inositol in fecal samples were below detection limits.

et al. (2019), but in contrast with Velayudhan et al. (2015) and Arredondo et al. (2019) who reported an increase in ATTD of GE with increasing inclusion of phytase. Results of other experiments indicated that phytase supplementation did not influence the ATTD of GE (Liao et al., 2005; She et al., 2018; Rosenfelder-Kuon et al., 2020a). The ability of phytate to bind glucose, starch, and amylase is the reason phytase supplementation may result in increased energy digestibility (Woyengo and Nyachoti, 2013), but it is not clear why results for effects of phytase on AID and ATTD of GE are so inconsistent.

The increased ATTD of Ca and P upon phytase supplementation was anticipated as the positive correlation between phytase inclusion and Ca and P digestibility is well documented (Velayudhan et al., 2019; Rosenfelder-Kuon et al., 2020b). Likewise, the positive effect of phytase on the ATTD of K and Mg concurs with previous data from pigs (Velayudhan et al., 2015; She et al., 2018; Arredondo et al., 2019), and

is a result of phytate in plants being complexed with Ca, K, and Mg (Angel et al., 2002). As in this experiment, a linear increase in ATTD of Na by increasing dietary phytase has previously been observed (Arredondo et al., 2019), which is likely a result of reduced endogenous secretion of Na into the small intestine in response to reduced presence of phytate in the lumen of the small intestine as dietary phytase increased (Woyengo et al., 2009). However, an increased ATTD of Na in response to phytase has not always been observed (Velayudhan et al., 2015; She et al., 2018).

The observation that the ATTD of GE increased as BW of pigs increased concurs with data indicating that the concentration of digestible energy and the ATTD of GE in sows is greater than in growing pigs, regardless of the level of feed intake (Le Goff and Noblet, 2001; Lowell et al., 2015; Casas and Stein, 2017). Likewise, the observed increase in AID of GE with increasing BW of pigs supports data indicating that the greater ATTD of GE in sows than in growing pigs is not a result of a greater fermentation capacity in the hindgut of sows compared with growing pigs (Lowell et al., 2015; Casas and Stein, 2017). It is possible that older pigs have greater AID of starch than younger pigs due to increased prececal fermentation, but further research is needed to confirm this hypothesis. Nevertheless, data from this experiment indicates that BW of pigs influences the concentration of digestible energy in diets.

The reduced ATTD of Ca and P observed with increasing BW of pigs is in contrast with Kemme et al. (1997), but in agreement with Sulabo et al. (2004) who indicated a linear decrease in AID and ATTD of P as BW of pigs increased from 40 to 130 kg. This observation may be a consequence of providing Ca and P in excess of the requirement in phases 3 and 4, because the transcellular absorption of these minerals is activated by hormonal regulation as a result of low concentrations in plasma. As a consequence, high concentrations of Ca and P in the diets and in plasma results in negative feedback (Schröder et al., 1996; Crenshaw, 2001). However, high concentrations of Ca in diets also increases paracellular absorption of Ca (Lagos et al., 2019), which usually results in a lack of an effect of dietary Ca on the ATTD of Ca (Stein et al., 2011; González-Vega et al., 2014). Excess dietary Ca and P may also result in increased ATTD of Ca and P, respectively (González-Vega et al., 2013, 2016; Liu et al., 2018). It is possible that the digestibility of Ca and P decreases as pigs get older as indicated by the reduced digestibility of these two minerals in gestating sows compared with growing pigs (Kemme et al., 1997; Lee et al., 2018a, 2018b). To our knowledge, there are no available data about the effect of BW or physiological status on the ATTD of K, Mg, and Na. Therefore, more research is needed to validate the results observed in the present experiment and to elucidate the reason for the increased digestibility of K and Mg, but reduced digestibility of Na, with increasing BW of pigs.

The observation that regardless of phase, increasing dietary phytase resulted in increased AID of IP6, reduced concentrations of IP esters in ileal digesta, and increased inositol concentration in ileal digesta, indicates that regardless of BW, phytase degrades phytate and IP esters in the stomach and (or) small intestine of pigs. The decrease in IP6 and IP5, but increase and then decrease in IP4 and IP3 observed in phase 1 as phytase in diets increased is in agreement with data from pigs between 17 and 38 kg (Laird et al., 2018; Lu et al., 2019; Mesina et al., 2019; Rosenfelder-Kuon et al., 2020a). This observation reflects the progressive degradation of phytate into lower esters and inositol, and the further degradation of lower esters into inositol as inclusion of dietary phytase increased. Thus, the observed interaction between phytase inclusion level and phase for ileal degradation of phytate indicates that the efficiency of phytase to degrade phytate is dependent on BW of pigs. The efficiency of phytase appears to be reduced in older pigs compared with young pigs as indicated by the AID of IP6 that surpassed 90% upon supplementation of 250 FTU of phytase in phase 1, whereas for phases 2 to 4, more than 1,000 FTU were needed to degrade 90% of IP6.

A less efficient IP ester degradation by phytase was also observed in finishing pigs compared with growing pigs as indicated by the lack of a reduction in ileal IP5 at 250 FTU of phytase in phase 2, and a built-up of IP5 at the same phytase dosage in phases 3 and 4, compared with the control diet. The reduced phytase efficiency by increasing BW of pigs is also indicated by the increased accumulation of IP4 and IP3 in ileal digesta at 250 and 500 FTU of phytase in the finishing phases compared with the growing phases. The implication of this observation is that a greater amount of phytase is needed to decrease the concentration of ileal IP4 and IP3 in the later phases than in the earlier phases. Results for ileal inositol are aligned with the response of IP ester concentrations in ileal digesta to dietary phytase at each phase, and indicate that in older pigs, the amount of dietary phytase required to fully degrade phytate is greater than in young pigs. These data concur with results for growth performance from this experiment and from Holloway et al. (2019) indicating a smaller effect of phytase on growth performance of finishing pigs compared with growing pigs. Potential reasons for these differences may be that gastric pH and transit times differ between young and older growing pigs. Because the same diet was provided throughout the growing-finishing period, it is likely that there was an excess of Ca and P in the lumen of the older pigs compared with younger pigs due to the reduced requirement by older pigs. This increase in lumen concentrations of Ca and P may have contributed to a reduced efficacy of phytase in older pigs as has been demonstrated in poultry (Sommerfeld et al., 2017).

The lack of a clear pattern within and among phases for the response of fecal concentrations of IP6, IP5, and IP4 to phytase supplementation indicates that dietary phytase has a limited impact on phytate degradation in the hindgut. This observation and the reduced concentration of IP esters in feces compared with ileal digesta is in agreement with Mesina et al. (2019) and indicates that there is phytate degradation in the large intestine of pigs by phytase synthesized by hindgut microbes (Selle et al., 2010). This, however, has no impact on the nutritional status of pigs because there is no net absorption of Ca or P in the large intestine of pigs (González-Vega et al., 2014). Likewise, the absence of inositol in feces is likely due to microbial metabolism of inositol in the hindgut or absorption and metabolism for energy by the colonocytes.

The observation that regardless of phytase dosage, there was a reduced AID of IP6 and an increased concentration of IP esters in ileal digesta as pigs grow, concurs with the reduced ATTD of Ca and P observed with increasing BW of pigs. Therefore, it appears that as pigs get older, the digestibility of Ca and P decreases and the efficiency of phytase

to degrade phytate and release Ca and P also decreases. This hypothesis is supported by data from gestating sows indicating that inclusion of phytase at 500 FTU did not improve the digestibility of Ca or P (Lee et al., 2019). Excess dietary Ca may reduce phytase efficiency in pigs an poultry (Tamim et al., 2004; Sommerfeld et al., 2017) because formation of complexes between Ca and phytate results in difficulties for phytase to access phytate (Selle et al., 2009).

The reduced concentration of inositol in ileal digesta of pigs as BW increased may be a result of the reduced phytase efficiency in degrading phytate as pig BW increased, but it is also possible that older pigs have increased inositol absorption compared with young pigs. Likewise, the increased concentration of IP6 and IP5 in feces with increasing BW of pigs may indicate a reduced capacity for phytate degradation in the hindgut as pigs grow, or it may be the result of a greater amount of IP esters entering the hindgut from the small intestine. To our knowledge, no data for the effect of phytase supplementation on phytate degradation at different production phases are available, but research is needed to elucidate the reason BW of pigs influences the efficiency of phytase to degrade phytate. However, because the same diet was used throughout the growing-finishing period, an experiment in which the diets are adapted to the pig requirements in all phases need to be conducted to confirm results for pigs fed diets similar to those used in commercial production.

Conclusions

Increasing inclusion of phytase resulted in increased AA and macro-mineral digestibility, but reduced energy digestibility, regardless of BW of pigs. However, the efficiency of phytase to degrade phytate and phytate esters into inositol in the small intestine of pigs decreased as pig BW increased. Considerable phytate degradation takes place in the large intestine of pigs presumably due to phytase synthesized by hindgut microbes, but independently of dietary phytase. Older pigs had increased energy, AA, K, and Mg digestibility and reduced Ca, P, and Na digestibility compared with younger pigs. Finishing pigs also had increased concentrations of IP esters in ileal digesta and feces, but reduced inositol concentration in ileal digesta compared with growing pigs. Therefore, more research needs to be conducted to elucidate the influence of BW on nutrient digestibility and phytase efficiency to degrade phytate.

Acknowledgment

The financial support from AB Vista, Marlborough, United Kingdom, is greatly appreciated.

Conflict of Interest Statement

M.R.B. is an employee at AB Vista, Marlborough, United Kingdom, a global supplier of phytase to the animal feed industry. L.V.L. and H.H.S. have no conflicts of interest.

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