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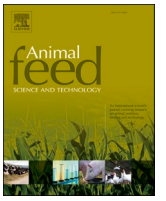

Influence of a novel phytase on Ca and P digestibility in diets fed to sows in late-gestation and lactation

Charmaine D. Espinosa [a], Leidy J. Torres-Mendoza [a], Jonathan R. Bergstrom [b], Hans H. Stein [a,*]

[a] Department of Animal Sciences, University of Illinois, Urbana 61801, USA
[b] DSM Nutritional Products Inc., Parsippany, NJ 07054, USA

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ABSTRACT

Two experiments were conducted to test the hypothesis that a novel phytase based on *Citrobacter braakii* increases Ca and P digestibility and influences concentrations of cytokines and bone biomarkers in the serum of gestating and lactating sows. In both experiments, a positive control (PC) diet that met Ca and P requirements was formulated, and a negative control (NC) diet was formulated to be deficient in Ca and P. Two additional diets were formulated by adding 1000 or 2000 phytase units (FYT)/kg of the novel phytase to the NC diet. An additional diet was formulated by adding 1000 FYT/kg of a commercial phytase to the NC diet, and a P-free diet was used to determine basal endogenous loss of P. In experiment 1, 72 gestating sows (day of gestation = 90) were allotted to the six diets and housed individually in metabolism crates. Feces and urine were quantitatively collected for 4 days after 5 day of adaptation. On day 90 and 105 of gestation, blood samples were collected from sows. Results indicated that sows fed diets containing phytase had greater ($P < 0.01$) P digestibility compared with sows fed diets without phytase, but no difference was observed for P digestibility among the three diets containing phytase. Carboxyterminal cross-linked telopeptide of type I collagen (CTX-I) was greater ($P < 0.05$) in sows fed the NC diet compared with sows fed the PC diet or diets containing phytase indicating increased bone tissue breakdown in sows fed the NC diet. In experiment 2, 66 lactating sows were allotted to the six diets on day 5 post-farrowing, and feces were collected for 4 days via grab-sampling after 5 days of adaptation. At the start and conclusion of the experiment, blood samples were collected from sows. Results indicated that sows fed diets containing phytase had greater ($P < 0.01$) digestibility of dry matter, Ca, and P compared with sows fed diets without phytase, but no difference was observed among the three diets containing phytase. Interleukin-1β in serum from sows fed 2000 FYT/kg of the novel phytase or the PC diet tended to be less ($P < 0.10$) compared with that of sows fed the NC diet. Sows fed the NC diet tended to have greater ($P < 0.10$) CTX-I compared with sows fed the PC diet or the diet containing the commercial phytase. In conclusion, phytase increased P digestibility in both gestating and lactating sows and reduced CTX-I in serum. When 2000 FYT/kg of the novel phytase or 1000 FYT/kg of the commercial phytase were added

Abbreviations: ATTD,, apparent total tract digestibility; BAP, bone-specific alkaline phosphatase; CTX-I, carboxyterminal cross-linked telopeptide of type I collagen; DM, dry matter; ELISA, enzyme-linked immunosorbent assay; FGF23, fibroblast growth factor 23; FYT, phytase units; IL, interleukin; NC, negative control; OC, osteocalcin; PC, positive control; STTD, standardized total tract digestibility.

* Correspondence to: University of Illinois Urbana-Champaign, Department of Animal Sciences, 1207 West Gregory Dr. Urbana, IL 61801, USA.
E-mail address: hstein@illinois.edu (H.H. Stein).

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to the diet, serum concentration of interleukin 6 was also reduced, which may be a result of decreased anti-nutritional effects associated with phytate.

1. Introduction

Microbial phytase is usually included in diets for pigs to increase P absorption and utilization by hydrolyzing phytic acid within the gastrointestinal tract of pigs (Pallauf et al., 1994). Dietary inclusion of phytase in low-P diets for weanling and growing-finishing pigs increases growth performance, bone ash, and mineral digestibility (Jongbloed et al., 2004; Rosenfelder-Kuon et al., 2020; Espinosa et al., 2021). However, the efficacy of phytase in sows may be different from growing pigs because it is possible that the effect of phytase is influenced by the physiological status of the animal (Kemme et al., 1997a). Indeed, the response to phytase in gestating sows is different from that in growing pigs (Lee et al., 2021). It is also possible that the impact of phytase on digestibility of Ca and P by gestating sows is dependent on gestation period (Lee et al., 2020). Kemme et al. (1997b) reported that addition of 500 phytase units/kg in diets for sows during mid-gestation (i.e., d 60 of gestation) did not influence Ca and P digestibility, but P digestibility increased upon phytase supplementation during the late gestation period (i.e., d 100 of gestation). Composition of diets and inclusion rate and source of phytase in diets may also influence phytase efficiency (Dias et al., 2010), and inclusion of more than 1000 phytase units (FYT) per kg diet may positively impact digestibility of Ca, P, and other minerals in sow diets and possibly have positive effects on sow immune status due to benefits from degradation of phytate. However, data demonstrating these effects are limited, but a new microbial phytase (i.e., HiPhorius; DSM Nutritional Products Inc., Parsippany, NJ, USA) may increase digestibility of Ca and P in diets for gestating and lactating sows to a greater degree than currently available phytases. Therefore, two experiments were conducted to test the hypothesis that addition of the novel phytase to diets for gestating or lactating sows increases apparent total tract digestibility (ATTD) of Ca and standardized total tract digestibility (STTD) of P, and influences concentrations of cytokines and bone biomarkers in the serum of gestating and lactating sows. The second objective was to test the hypothesis that the novel phytase increases Ca and P digestibility by sows to a greater degree than a commercial phytase.

2. Materials and methods

Protocols for two experiments were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois (Urbana, Illinois, USA). Sows used in both experiments were Camborough females (Pig Improvement Company, Hendersonville, TN, USA).

2.1. Animals and treatments

2.1.1. Experiment 1: Gestating sows

Seventy-two gestating sows (day of gestation = 90; initial body weight = 228.9 ± 24.7 kg; average parity = 2.7 ± 1.4) were allotted to six blocks of 12 sows using a randomized complete block design with sow breeding group being the blocking factor. Six diets were fed to the 12 sows in each block with two sows per diet in each block; thus, there were a total of 12 replicate sows for each treatment. A positive control (PC) diet was formulated based on corn, soybean meal, and 100 g/kg soybean hulls (Tables 1, 2, and 3). Vitamins and minerals were included in the PC diet to meet or exceed the requirements for gestating sows (NRC, 2012). A negative control diet (NC) was formulated to contain 1.5 g/kg Ca and 1.2 g/kg standardized total tract digestible P less than the requirement (NRC, 2012). Two additional diets were formulated by adding 5 g/kg or 10 g/kg of a phytase premix that contained 200,000 FYT/kg to the negative control diet to prepare diets that contained 1000 or 2000 FYT/kg diet from a novel microbial phytase (i.e., HiPhorius; DSM Nutritional

Table 1
Nutrient composition of ingredients used in experiment 1 and 2, as-fed basis.

	Experiment 1			Experiment 2	
Item	Corn	Soybean meal	Soybean hulls	Corn	Soybean meal
Dry matter, g/kg	866.4	896.9	911.5	867.3	903.9
Ash, g/kg	11.0	64.7	46.4	17.2	70.8
Crude protein, g/kg	76.2	469.7	114.3	74.8	452.2
Acid-hydrolyzed ether extract, g/kg	31.1	16.3	35.0	33.6	19.0
Soluble dietary fiber, g/kg	9.0	19.0	31.0	10.0	21.0
Insoluble dietary fiber, g/kg	101.0	172.0	625.0	96.0	153.0
Total dietary fiber, g/kg	110.0	191.0	656.0	106.0	174.0
Ca, g/kg	0.1	3.3	5.7	0.1	3.8
Total P, g/kg	2.4	6.0	1.6	2.4	5.9
Phytic acid, g/kg	6.3	14.4	1.9	7.3	15.6
Phytate-bound P[a], g/kg	1.8	4.1	0.5	2.1	4.4
Non-phytate P[b], g/kg	0.6	1.9	1.1	0.3	1.5

[a] Calculated as 282 g/kg of phytic acid (Tran and Sauvant, 2004).
[b] Calculated as total P – phytate-bound P.

Products Inc., Parsippany, NJ, USA) to the NC diet. The phytase premix was prepared by diluting a phytase concentrate that contained 20,000 FYT/g with ground corn. One FYT corresponds to the amount of enzyme that catalyzes the release of 1 uMole of P per minute from 5.1 mMole sodium phytate in pH 5.5 buffer at 37 °C. The novel phytase was encoded by a 6-phytase gene from *Citrobacter braakii* (ATCC51113) and is believed to have greater stability and wider pH and temperature ranges than current commercial phytases (Zhai et al., 2021). An additional diet was formulated by adding 5 g/kg of a phytase concentrate (200,000 FYT/kg) that was prepared by diluting a phytase concentrate (20,000 FYT/g) with ground corn to produce a diet that contained 1000 FYT/kg of a commercial microbial phytase (i.e., HiPhos; DSM Nutritional Products Inc., Parsippany, NJ, USA) to the NC diet, and a P-free diet was formulated to determine the basal endogenous loss of P.

2.1.2. Experiment 2: Lactating sows

Sixty-six lactating sows (initial body weight = 214.8 ± 19.3 kg; average parity = 2.8 ± 1.3) at day 5 post-farrowing were allotted to four blocks of 12 sows and one block of 18 sows using a randomized complete block design. Six diets were fed to sows with two or three sows per diet in each block; thus, there were a total of 11 replicate sows for each treatment. A PC diet was formulated to meet requirements (NRC, 2012) of all nutrients including vitamins and minerals (Tables 4 and 5). Five additional diets were prepared and formulated as explained for experiment 1 [i.e., NC diet, two diets containing the novel phytase (i.e., HiPhorius; DSM Nutritional Products Inc., Parsippany, NJ, USA) at 1000 or 2000 FYT/kg, one diet containing 1000 FYT/kg of the commercial phytase (i.e., HiPhos; DSM Nutritional Products Inc., Parsippany, NJ, USA), and P-free diet]. Corn and soybean meal were the main ingredients in all diets except in the P-free diet.

2.2. Experimental procedures

2.2.1. Experiment 1: Gestating sows

After a 24-h fast, blood samples were collected from the vena cava of sows on day 90 of gestation. After bleeding, sows were moved to metabolism crates (0.91 × 2.08 m) that were equipped with a self-feeder, a nipple drinker, and a fully slatted T-bar floor. A screen

Table 2
Ingredient composition of experimental diets in experiment 1, as-fed basis.

Ingredient, g/kg	PC[a]	NC[a]	NC + 1000 FYT[a]/kg novel phytase	NC + 2000 FYT/kg novel phytase	NC + 1000 FYT/kg commercial phytase	P-free
Ground corn	751.5	759.0	754.0	749.0	754.0	-
Soybean meal	110.0	110.0	110.0	110.0	110.0	-
Soybean hulls	100.0	100.0	100.0	100.0	100.0	-
Soybean oil	10.0	10.0	10.0	10.0	10.0	40.0
Cornstarch	-	-	-	-	-	481.1
Gelatin	-	-	-	-	-	200.0
Sucrose	-	-	-	-	-	200.0
Cellulose	-	-	-	-	-	50.0
Calcium carbonate	11.2	10.2	10.2	10.2	10.2	14.8
Monocalcium phosphate	10.7	4.2	4.2	4.2	4.2	-
L-Lys HCl, 780 g/kg Lys	0.8	0.8	0.8	0.8	0.8	-
DL-Met, 980 g/kg Met	-	-	-	-	-	1.6
L-Thr, 990 g/kg Thr	0.3	0.3	0.3	0.3	0.3	0.4
L-Trp, 990 g/kg Trp	-	-	-	-	-	0.9
L-His, 990 g/kg His	-	-	-	-	-	0.2
L-Ile, 990 g/kg Ile	-	-	-	-	-	0.3
L-Leu, 990 g/kg Leu	-	-	-	-	-	0.2
Potassium carbonate	-	-	-	-	-	4.0
Magnesium oxide	-	-	-	-	-	1.0
Sodium chloride	4.0	4.0	4.0	4.0	4.0	4.0
Novel phytase premix[b]	-	-	5.0	10.0	-	-
Commercial phytase premix[c]	-	-	-	-	5.0	-
Vitamin-mineral premix[d]	1.5	1.5	1.5	1.5	1.5	1.5

[a] FYT, phytase units; PC, positive control; NC, negative control.

[b] The novel phytase concentrate was mixed with ground corn to prepare a phytase premix that contained 200,000 FYT per kg (HiPhorius; DSM Nutritional Products Inc., Parsippany, NJ, USA). At 5.0 and 10.0 g/kg inclusion, the final diets were expected to contain 1000 and 2000 FYT/kg of microbial phytase, respectively.

[c] The commercial phytase concentrate was mixed with ground corn to prepare a phytase premix that contained 200,000 FYT per kg (HiPhos; DSM Nutritional Products Inc., Parsippany, NJ, USA). At 5.0 g/kg inclusion, the final diet was expected to contain 1000 FYT/kg of microbial phytase.

[d] The vitamin-mineral premix provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,150 IU; vitamin D3 as cholecalciferol, 2210 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B12, 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 chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

and a urine pan were installed under the T-bar floor to allow for the total collection of feces and urine. Daily feed allotments were provided in 2 equal meals that were fed at 0800 and 1600 h. Daily feed allowance was 1.5 times the maintenance requirement for metabolizable energy (i.e., 100 kcal/kg body weight$^{0.75}$; NRC, 2012). Water was available at all times. The initial 5 days in the metabolism crates were considered the adaptation period to the diets. The adaptation period was followed by 4 days of fecal collection using the marker-to-marker procedure (Adeola, 2001). Fecal collection was initiated when the first marker (i.e., indigo carmine) appeared in the feces and ceased when the second marker (i.e., ferric oxide) appeared. Urine was collected in buckets placed under urine pans and 50 mL of 6 *N* HCl was added daily to each bucket. Buckets were emptied daily, the weight of the collected urine was recorded, and a subsample of the collected urine was stored at − 20 °C until analyzed. On day 105 (i.e., after feeding of experimental diets had concluded), sows were fasted for 24 h, and blood samples were collected from the vena cava of sows. Blood samples obtained on day 90 and day 105 were collected in vacutainers without anticoagulant and serum was obtained by centrifuging blood samples at 4,000g at 4 ºC for 13 min. Samples were stored at − 20 °C until analyzed.

2.2.2. Experiment 2: Lactating sows

Sows were housed individually in farrowing crates (2.1 × 1.5 m) with plastic coated slatted floors. Each crate was equipped with a stainless steel feeder and two nipple waterers. Sows were fed a common lactation diet until day 5 post-farrowing. However, from day 5 to day 8 post-farrowing, all sows were fed 4.5 kg/day of experimental diets, and from day 9 to day 14 post-farrowing, sows were fed 6 kg/day of experimental diets to standardize feed intake across treatments. The initial 5 days were considered the adaptation period to experimental diets. The adaptation period was followed by 4 days of fecal collection by grab-sampling. Fecal samples were stored at —20 ºC immediately after collection. Sows had free access to water throughout the experiment. Following a 12 h fasting period, blood samples were collected from the vena cava at the start (i.e., day 5 post-farrowing) and at the end of the experiment (i.e., day 14 post-farrowing). Serum from the collected blood samples was obtained and stored as explained for experiment 1.

2.3. Sample analyses

2.3.1. Experiment 1: Gestating sows

At the conclusion of the experiment, urine samples were thawed and mixed within animal and diet. Fecal samples were dried at 50 °C in a forced-air oven (Model 8; Metalab Equipment Corporation, Hicksville, Long Island, NY, USA). Fecal samples and diets were finely ground through a 1-mm screen (MM4; Schute Buffalo, NY, USA) prior to chemical analysis. Calcium and P in corn, soybean meal, soybean hulls, diets, feces, and urine samples were analyzed using inductively coupled plasma-optical emission spectrometry (Avio 200, PerkinElmer, Waltham, MA, USA). Diets were also analyzed for K, Mg, Na, Cu, Zn, Fe, and Mn. Sample preparation included dry ashing at 600 °C for 4 h (Method 942.05; AOAC Int, 2019) and wet digestion with nitric acid (Method 3050 B; U.S. U.S. U.S. Environmental Protection Agency, 2000). Ingredients were analyzed for phytate (Ellis et al., 1977), and diet samples were shipped to DSM for phytase activity analysis (DSM Nutritional Products Inc., Belvidere, NJ, USA) following the procedure of Zhai et al. (2021). Diets

Table 3
Analyzed nutrient composition of experimental diets in experiment 1, as-fed basis.

Item	PC[a]	NC[a]	NC + 1000 FYT[a]/kg novel phytase[b]	NC + 2000 FYT/kg novel phytase	NC + 1000 FYT/kg commercial phytase[c]	P-free
Dry matter, g/kg	893.1	888.0	888.2	881.9	880.9	928.7
Ash, g/kg	46.0	36.5	38.6	40.7	35.0	25.0
Crude protein, g/kg	117.9	120.7	119.6	118.2	120.7	195.6
Acid-hydrolyzed ether extract, g/kg	38.5	40.9	37.5	39.9	40.5	19.7
Soluble dietary fiber, g/kg	14.0	14.0	13.0	14.0	16.0	-
Insoluble dietary fiber, g/kg	166.0	171.0	154.0	168.0	170.0	-
Total dietary fiber, g/kg	180.0	185.0	167.0	182.0	186.0	-
Phytic acid, g/kg[d]	6.50	6.55	6.57	6.49	6.52	-
Ca, g/kg	7.8	5.4	5.9	5.8	5.3	6.0
P, g/kg	4.8	3.8	3.8	3.7	3.7	0.2
K, g/kg	5.6	5.7	6.0	5.8	6.0	1.7
Mg, g/kg	1.2	1.3	1.3	1.3	1.3	0.7
Na, g/kg	1.7	1.7	1.8	1.7	1.7	1.6
Cu, mg/kg	38.15	34.79	35.55	37.28	31.34	26.32
Zn, mg/kg	158.41	157.25	157.69	163.38	155.93	124.29
Fe, mg/kg	245.11	229.58	260.76	260.60	263.33	184.78
Mn, mg/kg	78.01	73.90	74.32	72.29	73.49	72.11
Phytase activity, FYT/kg	235	274	1139	2394	1199	-

[a] FYT, phytase units; PC, positive control; NC, negative control.

[b] The novel phytase concentrate was mixed with ground corn to prepare a phytase premix that contained 200,000 FYT per kg (HiPhorius; DSM Nutritional Products Inc., Parsippany, NJ, USA). At 5.0 and 10.0 g/kg inclusion, the final diets were expected to contain 1000 and 2000 FYT/kg of microbial phytase, respectively.

[c] The commercial phytase concentrate was mixed with ground corn to prepare a phytase premix that contained 200,000 FYT per kg (HiPhos; DSM Nutritional Products Inc., Parsippany, NJ, USA). At 5.0 g/kg inclusion, the final diet was expected to contain 1000 FYT/kg of microbial phytase.

[d] Diet phytate values were calculated from analyzed phytate in ingredients.

Table 4
Ingredient composition of experimental diets in experiment 2, as-fed basis.

Ingredient, g/kg	PC[a]	NC[a]	NC + 1000 FYT[a]/kg novel phytase	NC + 2000 FYT/kg novel phytase	NC + 1000 FYT/kg commercial phytase	P-free
Ground corn	677.4	685.0	680.0	675.0	680.0	-
Soybean meal	255.0	255.0	255.0	255.0	255.0	-
Soybean oil	30.0	30.0	30.0	30.0	30.0	40.0
Cornstarch	-	-	-	-	-	403.7
Gelatin	-	-	-	-	-	260.0
Sucrose	-	-	-	-	-	200.0
Cellulose	-	-	-	-	-	50.0
Calcium carbonate	11.7	10.7	10.7	10.7	10.7	16.0
Monocalcium phosphate	12.4	5.8	5.8	5.8	5.8	-
L-Lys HCl, 780 g/kg Lys	0.5	0.5	0.5	0.5	0.5	-
DL-Met, 980 g/kg Met	-	-	-	-	-	2.0
L-Thr, 990 g/kg Thr	-	-	-	-	-	0.7
L-Trp, 990 g/kg Trp	-	-	-	-	-	1.4
L-His, 990 g/kg His	-	-	-	-	-	1.5
L-Ile, 990 g/kg Ile	-	-	-	-	-	1.6
L-Leu, 990 g/kg Leu	-	-	-	-	-	3.3
L-Val, 990 g/kg Val	-	-	-	-	-	1.8
Sodium chloride	4.0	4.0	4.0	4.0	4.0	4.0
Potassium carbonate	-	-	-	-	-	4.0
Magnesium oxide	-	-	-	-	-	1.0
Titanium dioxide	4.0	4.0	4.0	4.0	4.0	4.0
Novel phytase premix[b]	-	-	5.0	10.0	-	-
Commercial phytase premix[c]	-	-	-	-	5.0	-
Vitamin-mineral premix[d]	5.0	5.0	5.0	5.0	5.0	5.0

[a] FYT, phytase units; PC, positive control; NC, negative control.
[b] The novel phytase concentrate was mixed with ground corn to prepare a phytase premix that contained 200,000 FYT per kg (HiPhorius; DSM Nutritional Products Inc., Parsippany, NJ, USA). At 5.0 and 10.0 g/kg inclusion, the final diets were expected to contain 1000 and 2000 FYT/kg of microbial phytase, respectively.
[c] The commercial phytase concentrate was mixed with ground corn to prepare a phytase premix that contained 200,000 FYT per kg (HiPhos; DSM Nutritional Products Inc., Parsippany, NJ, USA). At 5.0 g/kg inclusion, the final diet was expected to contain 1000 FYT/kg of microbial phytase.
[d] The vitamin-mineral premix provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,150 IU; vitamin D3 as cholecalciferol, 2210 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B12, 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 chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

and ingredients were analyzed for dry matter (DM; Method 930.15; AOAC Int, 2019), and diets and ingredients were also analyzed for ash (Method 942.05; AOAC Int, 2019). Diet and ingredient samples were analyzed for acid hydrolyzed ether extract using 3 *N* HCl (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY, USA) followed by fat extraction (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY, USA; Method 2003.06; AOAC Int, 2019). Diets and ingredients were analyzed for nitrogen using the combustion procedure (Method 990.03; AOAC Int, 2019) on a LECO FP628 apparatus (LECO Corp., Saint Joseph, MI, USA). Crude protein was calculated as analyzed concentration of nitrogen multiplied by 6.25. Diet and ingredient samples were also analyzed for insoluble dietary fiber and soluble dietary fiber according to method 991.43 (AOAC Int, 2019) using the Ankom^{TDF} Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA). Total dietary fiber was calculated as the sum of insoluble dietary fiber and soluble dietary fiber.

Serum samples were analyzed for carboxyterminal cross-linked telopeptide of type I collagen (CTX-I) using a Pig Cross-Linked C-Telopeptide of Type I Collagen enzyme-linked immunosorbent assay (ELISA) kit (Abbexa Ltd., Cambridge, UK). Concentration of osteocalcin (OC) in serum samples was also analyzed using an N-MID® Osteocalcin ELISA 168 Kit (Immunodiagnostic Systems Ltd, The Boldons, UK), and bone-specific alkaline phosphatase (BAP) was analyzed using an Ostase® BAP ELISA Kit (Immunodiagnostic Systems Ltd, The Boldons, UK). Concentration of fibroblast growth factor 23 (FGF23) was analyzed using a Porcine FGF23 ELISA Kit (Antibodies-online, Limerick, PA, USA). Serum samples were also analyzed for the following cytokines: interferon-gamma, interleukin (IL)− 1 beta, IL-4, IL-6, IL-10, and tumor necrosis factor-alpha using a MILLIPLEX kit (EMD Millipore Corporation, Billercia, MA, USA) in a MagPix instrument with ProcartaPlex-Multiplex technology (R&D Systems, Inc., Minneapolis, MN, USA).

2.3.2. Experiment 2: Lactating sows

At the conclusion of the experiment, fecal samples were thawed and mixed within animal. Fecal samples were dried and ground as explained for experiment 1. Calcium and P in corn, soybean meal, diets, and feces were analyzed, and diets were also analyzed for phytase activity as explained for experiment 1. Diets were also analyzed for K, Mg, Na, Cu, Zn, Fe, and Mn. Phytate was analyzed in corn and soybean meal as explained for experiment 1. Diets, ingredients, and fecal samples were analyzed for DM. Ash was also

Table 5
Analyzed nutrient composition of experimental diets in experiment 2, as-fed basis.

Item	PC[a]	NC[a]	NC + 1000 FYT[a]/kg novel phytase[b]	NC + 2000 FYT/kg novel phytase	NC + 1000 FYT/kg commercial phytase[c]	P-free
Dry matter, g/kg	899.8	900.9	899.3	897.3	901.2	946.6
Gross energy, MJ/kg	16.6	16.7	16.7	16.8	16.7	17.3
Ash, g/kg	51.0	47.5	46.6	45.0	43.8	33.1
Crude protein, g/kg	162.8	164.1	165.2	161.8	165.2	269.0
Acid-hydrolyzed ether extract, g/kg	58.4	61.6	59.1	67.1	67.4	32.4
Soluble dietary fiber, g/kg	17.0	17.0	15.0	17.0	14.0	-
Insoluble dietary fiber, g/kg	108.0	109.0	111.0	117.0	111.0	-
Total dietary fiber, g/kg	125.0	126.0	126.0	134.0	125.0	-
Phytic acid, g/kg[d]	8.92	8.98	8.94	8.91	8.94	-
Ca, g/kg	7.5	6.1	6.0	6.4	6.3	6.5
P, g/kg	5.9	4.8	4.9	4.7	4.4	-
K, g/kg	7.7	8.0	7.9	8.0	7.4	1.8
Mg, g/kg	1.5	1.4	1.4	1.3	1.4	0.7
Na, g/kg	1.9	1.8	1.7	1.5	1.6	1.2
Cu, mg/kg	40.25	36.57	33.64	36.14	35.09	22.61
Zn, mg/kg	141.52	148.57	146.01	152.69	156.29	111.01
Fe, mg/kg	138.55	130.99	139.91	137.76	134.93	94.10
Mn, mg/kg	72.20	75.93	76.29	76.10	78.73	70.91
Phytase activity, FYT/kg	71	70	992	1994	1410	-

[a] FYT, phytase units; PC, positive control; NC, negative control.
[b] The novel phytase concentrate was mixed with ground corn to prepare a phytase premix that contained 200,000 FYT per kg (HiPhorius; DSM Nutritional Products Inc., Parsippany, NJ, USA). At 5.0 and 10.0 g/kg inclusion, the final diets were expected to contain 1000 and 2000 FYT/kg of microbial phytase, respectively.
[c] The commercial phytase concentrate was mixed with ground corn to prepare a phytase premix that contained 200,000 FYT per kg (HiPhos; DSM Nutritional Products Inc., Parsippany, NJ, USA). At 5.0 g/kg inclusion, the final diet was expected to contain 1000 FYT/kg of microbial phytase.
[d] Diet phytate values were calculated from analyzed phytate in ingredients.

analyzed in diets and ingredients (Method 942.05; AOAC Int, 2019), and diets and fecal samples were analyzed for gross energy using bomb calorimetry (Model 6400; Parr Instruments, Moline, IL, USA). Diet and ingredient samples were analyzed for nitrogen (for crude protein calculation), acid hydrolyzed ether extract, and for total dietary fiber as explained for experiment 1. Serum samples were analyzed for CTX-I, OC, BAP, and cytokines as explained for experiment 1.

2.4. Calculation and statistical analyses

2.4.1. Experiment 1: Gestating sows

The ATTD of Ca and P in experimental diets were calculated (Almeida and Stein, 2010). Values for the STTD of P in experimental diets were calculated by correcting the ATTD values for the basal endogenous loss of P, which was calculated from sows fed the P-free diet (NRC, 2012).

Data were analyzed using the MIXED Procedure of SAS (SAS Institute Inc, 2016) with sow as the experimental unit. Homogeneity of the variances among treatments was confirmed using the UNIVARIATE procedure. Outliers were identified as values that deviated from the 1st or 3rd quartiles by more than 1.5 times the interquartile range. During data analysis, the following outliers were identified: Three from the PC diet, 2 from the NC diet, one from NC with 2000 FYT/kg of novel phytase, and one from NC with commercial phytase. The fixed effect was diet, and block and replicate within block were considered random effects. Data for cytokines and bone biomarkers in the serum obtained on day 90 were used as covariate to analyze blood data from day 105. Treatment means were calculated and separated using the LSMEANS statement and the PDIFF option of PROC MIXED, respectively. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

2.4.2. Experiment 2: Lactating sows

The ATTD of DM, gross energy, Ca, and P in experimental diets were calculated (Almeida and Stein, 2010; NRC, 2012). Values for the STTD of P in experimental diets were also calculated (NRC, 2012). Data were statistically analyzed as explained for experiment 1.

During data analysis, two outliers were identified from the diet with NC and 2000 FYT/kg of novel phytase and one from NC with commercial phytase. Data for cytokines and bone biomarkers in the serum obtained from sows on day 5 post-farrowing were used as covariate to analyze blood data from day 14 post-farrowing.

3. Results

3.1. Gestating sows

Diets were formulated based on expected ingredient compositions (NRC, 2012) and analyses indicated that the intended

concentrations of Ca and P were present in all diets, and concentrations of other nutrients were not affected by dietary treatment. Diets also contained the intended quantities of phytase. The analyzed concentrations of Ca and P in the PC diet were 7.8 and 4.8 g/kg, respectively. The NC diet contained Ca and P that were close to expected values (i.e., 5.4 g/kg Ca and 3.8 g/kg P).

Basal endogenous loss of P by gestating sows was 0.82 g/kg DM intake (Table 6). Feed intake of sows was not affected by dietary treatment. Sows fed the diet containing the commercial phytase had less ($P < 0.05$) excretion of feces compared with sows fed the diet containing the novel phytase at 2000 FYT/kg. Due to increased concentration of dietary P, sows fed the PC diet had greater ($P < 0.01$) P intake compared with sows fed the NC diet or sows fed diets containing phytase. The analyzed concentration of fecal P was greatest ($P < 0.01$) in feces from sows fed the PC diet, followed by sows fed the NC diet, and sows fed diets containing phytase had the least ($P < 0.01$) concentration of P in feces. As a result, sows fed diets containing phytase had less ($P < 0.01$) fecal P output compared with sows fed the PC or the NC diet. Sows fed the NC diet tended to have less ($P < 0.10$) P absorbed compared with sows fed the other diets. Consequently, the NC diet had reduced ($P < 0.05$) ATTD of P compared with diets containing phytase. Diets containing phytase also had greater ($P < 0.01$) STTD of P compared with the PC or the NC diet. Sows fed the diet with the commercial phytase had greater ($P < 0.05$) excretion of urine compared with sows fed the PC diet or sows fed diets with the novel phytase. Sows fed diets containing phytase had greater ($P < 0.01$) urine P output compared with sows fed the NC diet; however, P retention was not different among experimental diets.

Sows fed the PC diet had greater ($P < 0.01$) Ca intake and absorbed more ($P < 0.01$) Ca compared with sows fed the NC diet or sows fed diets containing phytase (Table 7). Feces from sows fed diets containing the novel phytase had the least ($P < 0.01$) concentration of analyzed Ca, followed by feces from sows fed the NC or the commercial phytase diets, and feces from sows fed the PC diet had the greatest ($P < 0.01$) concentration of analyzed Ca. Therefore, sows fed diets containing phytase had less ($P < 0.05$) fecal Ca output compared with sows fed the PC diet. However, the ATTD of Ca did not differ among experimental diets. Sows fed the NC diet had greater ($P = 0.01$) urine Ca output compared with sows fed the other diets. As a result, sows fed the NC diet tended to have less ($P < 0.10$) Ca retention compared with sows fed the PC diet or sows fed diets containing phytase.

Dietary treatments did not influence concentrations of cytokines in serum from sows (Table 8). However, the concentration of CTX-I was greater ($P < 0.05$) in serum from sows fed the NC diet compared with sows fed the PC diet or sows fed diets containing phytase. Neither phytase supplementation nor dietary levels of Ca and P influenced concentrations of BAP, OC, or FGF23 in serum.

3.2. Lactating sows

Diet analyses indicated that the intended concentrations of Ca and P were present in all diets, and concentrations of other nutrients were not affected by dietary treatments indicating that the expected nutrient compositions of ingredients (NRC, 2012) were accurate. Diets also contained the intended quantities of phytase. Analyzed concentrations of Ca and P in the PC diet were 7.5 and 5.9 g/kg, respectively. The NC diets without or with phytase contained Ca and P that were close to expected values (i.e., 6.0 to 6.4 g/kg Ca and 4.4 to 4.9 g/kg P).

Basal endogenous loss of P from lactating sows was 0.65 g/kg DM intake (Table 9). Diets containing phytase had greater ($P < 0.01$) digestibility of DM compared with the NC or the PC diets; however, digestibility of gross energy did not differ among experimental

Table 6
Apparent total tract digestibility (ATTD), standardized total tract digestibility (STTD), and retention of P in experimental diets fed to sows in late gestation, experiment 1[a].

Item	PC[b]	NC[b]	NC + 1000 FYT[b]/kg novel phytase	NC + 2000 FYT/kg novel phytase	NC + 1000 FYT/kg commercial phytase	SEM	P-value
Feed intake, kg/day	2.83	2.99	2.90	2.91	2.77	0.163	0.399
P intake, g/day	13.62^{a}	11.19^{b}	10.87^{b}	10.91^{b}	10.36^{b}	0.640	< 0.001
Dry fecal excretion, kg/day	0.33^{ab}	0.35^{ab}	0.35^{ab}	0.37^{a}	0.31^{b}	0.019	0.039
P in feces, g/kg	32.01^{a}	25.52^{b}	21.63^{c}	19.99^{c}	22.41^{c}	0.104	< 0.001
Fecal P output, g/day	9.93^{a}	8.59^{b}	7.01^{c}	7.19^{c}	6.54^{c}	0.433	< 0.001
Absorbed P, g/day	3.72^{x}	2.62 ^{y}	3.86^{x}	3.72^{x}	3.83^{x}	0.594	0.077
ATTD of P	0.27^{bc}	0.24^{c}	0.34^{ab}	0.34^{ab}	0.36^{a}	0.037	0.028
Basal endogenous P loss, g/day	2.06	2.18	2.11	2.12	2.02	0.119	0.399
STTD of P[c]	0.42^{b}	0.43^{b}	0.59^{a}	0.53^{a}	0.56^{a}	0.037	0.006
Urine excretion, kg/day	4.32^{b}	5.92^{ab}	3.76^{b}	3.51^{b}	8.13^{a}	1.071	0.015
Urine P output, g/day	0.55^{bc}	0.31^{c}	0.60^{ab}	0.61^{ab}	0.80^{a}	0.089	0.004
P retained, g/day	3.18	2.31	3.26	3.11	3.03	0.559	0.267
P retention, g/100 g of P intake	23.06	21.01	29.09	28.23	28.66	3.746	0.197

[a-c]Means within a row lacking a common letter are different ($P \leq 0.05$).
[x-y]Means within a row lacking a common letter tend to be different ($P \leq 0.10$).
[a]Data are means of 9 to 12 observations per measurement.
[b]PC, positive control; NC, negative control; FYT, phytase units.
[c]The basal endogenous loss of P (i.e., 0.82 g/kg dry matter intake) was determined from sows fed the P-free diet. This value was used to correct the ATTD of P to calculate STTD of P (Almeida and Stein, 2010).

Table 7
Apparent total tract digestibility (ATTD) and retention of Ca in experimental diets fed to sows in late gestation, experiment 1[a].

Item	PC[b]	NC[b]	NC + 1000 FYT[b]/kg novel phytase	NC + 2000 FYT/kg novel phytase	NC + 1000 FYT/kg HiPhos	SEM	P-value
Ca intake, g/day	22.14[a]	16.70[b]	16.23[b]	16.28[b]	15.46[b]	0.974	< 0.001
Ca in feces	46.51[a]	39.20[b]	35.34[bc]	32.76[c]	38.55[b]	0.187	< 0.001
Fecal Ca output, g/day	15.29[a]	13.83[ab]	12.10[b]	12.33[b]	11.67[b]	0.773	0.016
Absorbed Ca, g/day	6.96[a]	2.59[b]	4.13[b]	3.96[b]	3.80[b]	0.849	< 0.001
ATTD of Ca,	0.31	0.18	0.25	0.24	0.24	0.040	0.158
Urine Ca output, g/day	0.39[b]	0.72[a]	0.36[b]	0.40[b]	0.51[ab]	0.077	0.010
Ca retained, g/day	6.58[a]	2.17[b]	3.77[b]	3.56[b]	3.29[b]	0.842	< 0.001
Ca retention, g/100 g of Ca intake	29.68[x]	13.42 [y]	22.45[xy]	21.95[xy]	20.73[xy]	4.065	0.073

[a-c]Means within a row lacking a common letter are different ($P \leq 0.05$).
[x-y]Means within a row lacking a common letter tend to be different ($P \leq 0.10$).
[a]Data are means of 9 to 12 observations per measurement.
[b]PC, positive control; NC, negative control; FYT, phytase units.

Table 8
Concentrations of cytokines and biomarkers for bone resorption and formation in serum samples of late gestation sows, experiment 1[a,b].

Item	PC[c]	NC[c]	NC + 1000 FYT[c]/kg novel phytase	NC + 2000 FYT/kg novel phytase	NC + 1000 FYT/kg HiPhos	SEM	P-value
Cytokines							
Interferon γ, ng/mL							
day 90 of gestation	64.61	51.19	48.35	53.82	51.27	-	-
day 105 of gestation	65.71	48.74	68.31	43.68	50.03	9.578	0.291
Interleukin 1β, ng/mL							
day 90 of gestation	6.05	5.10	5.96	7.19	5.79	-	-
day 105 of gestation	5.81	6.19	6.63	5.91	5.50	0.677	0.753
Interleukin 4, ng/mL							
day 90 of gestation	41.18	26.78	26.22	42.03	31.46	-	-
day 105 of gestation	31.17	36.10	37.89	33.25	31.80	3.974	0.662
Interleukin 6, ng/mL							
day 90 of gestation	3.10	2.13	1.93	3.15	2.32	-	-
day 105 of gestation	2.41	2.69	2.79	2.50	2.46	0.240	0.761
Interleukin 10, ng/mL							
day 90 of gestation	10.41	7.21	6.45	10.41	8.00	-	-
day 105 of gestation	7.72	9.06	9.8	8.37	8.41	0.766	0.638
Tumor necrosis factor-α, ng/mL							
day 90 of gestation	0.41	0.26	0.34	0.38	0.25	-	-
day 105 of gestation	0.25	0.31	0.34	0.28	0.24	0.047	0.549
Biomarkers							
CTX-I[4], μg/L							
day 90 of gestation	1.19	1.40	0.98	0.95	1.32	-	-
day 105 of gestation	1.06[b]	1.44[a]	1.12[b]	1.08[b]	1.12[b]	0.086	0.033
Bone alkaline phosphatase, μg/L							
day 90 of gestation	4.56	5.66	6.57	4.98	5.45	-	-
day 105 of gestation	5.97	6.23	8.13	8.25	7.79	1.203	0.430
Osteocalcin, μg/L							
day 90 of gestation	15.75	18.48	18.09	16.90	17.80	-	-
day 105 of gestation	17.26	17.94	17.14	16.89	18.62	0.754	0.419
FGF23[d], μg/L							
day 90 of gestation	0.15	0.15	0.15	0.16	0.16	-	-
day 105 of gestation	0.15	0.16	0.16	0.18	0.16	0.012	0.224

[a-b]Means within a row lacking a common letter are different ($P \leq 0.05$).
[a]Data are means of 10 to 12 observations per measurement.
[b]Data from d 90 of gestation were used as a covariate for data obtained on d 105 of gestation.
[c]PC, positive control; NC, negative control; FYT, phytase units.
[d]CTX-I, cross-linked C-telopeptide of type I collagen; FGF23, fibroblast growth factor 23.

diets. Sows fed the PC diet had the greatest ($P < 0.01$) concentration of analyzed Ca in feces, followed by sows fed the NC diet, and sows fed diets containing phytase had the least ($P < 0.01$) concentration of analyzed Ca in feces. Diets containing phytase had greater ($P < 0.01$) ATTD of Ca compared with the NC or the PC diets. Diets containing phytase also had reduced ($P < 0.01$) concentration of analyzed P in feces compared with diets without phytase. As a result, diets containing the novel phytase or the commercial phytase had greater ($P < 0.01$) ATTD and STTD of P compared with the PC or the NC diet. The ATTD of DM, Ca, and P, as well as STTD P were not

Table 9
Apparent total tract digestibility (ATTD) of dry matter (DM), gross energy, Ca, and P and standardized total tract digestibility (STTD) of P in experimental diets fed to lactating sows, experiment 2[a].

Item	PC[b]	NC[b]	NC + 1000 FYT[b]/kg novel phytase	NC + 2000 FYT/kg novel phytase	NC + 1000 FYT/kg commercial phytase	SEM	P-value
ATTD of DM	0.87[c]	0.88[b]	0.89[a]	0.89[a]	0.89[a]	0.034	< 0.001
ATTD of gross energy	0.89	0.90	0.90	0.90	0.90	0.030	0.338
Ca in feces, g/kg	50.33[a]	44.73[b]	39.22[c]	38.24[c]	35.84[c]	0.210	< 0.001
ATTD of Ca	0.27[b]	0.24[b]	0.41[a]	0.42[a]	0.45[a]	0.032	< 0.001
P in feces, g/kg	42.08[a]	38.84[a]	27.70[b]	26.54[b]	26.80[b]	0.150	< 0.001
ATTD of P	0.23[b]	0.15[c]	0.44[a]	0.47[a]	0.44[a]	0.024	< 0.001
STTD of P[c]	0.33[b]	0.27[b]	0.56[a]	0.60[a]	0.57[a]	0.024	< 0.001

[a-c]Means within a row lacking a common letter are different ($P \leq 0.01$).
[a]Data are means of 9 to 11 observations per measurement.
[b]PC, positive control; NC, negative control; FYT, phytase units.
[c]The basal endogenous loss of P (i.e., 0.65 g/kg DM intake) was determined from sows fed the P-free diet. This value was used to correct the ATTD of P to calculate STTD of P (Almeida and Stein, 2010).

different between the diet containing 1000 FYT/kg of the novel phytase and the diet containing 2000 FYT/kg of the novel phytase. Likewise, no difference was observed for nutrient digestibility between the diets containing the novel phytase and the diet containing the commercial phytase.

Dietary treatments did not influence concentrations of IL-4, IL-10, or tumor necrosis factor-α in serum (Table 10). Sows fed the NC diet tended to have greater ($P < 0.10$) concentration of interferon-gamma compared with that of sows fed the PC diet; however,

Table 10
Concentrations of cytokines and biomarkers for bone resorption and formation in serum samples of lactating sows fed experimental diets, experiment 2[a,b].

Item	PC[c]	NC[c]	NC + 1000 FYT[c]/kg novel phytase	NC + 2000 FYT/kg novel phytase	NC + 1000 FYT/kg commercial phytase	SEM	P-value
Cytokines							
Interferon γ, ng/mL							
day 5 post-farrowing	31.51	39.07	33.72	33.21	36.40	-	-
day 14 post-farrowing	28.58[y]	42.43[x]	47.24[x]	32.88[xy]	37.78[xy]	5.373	0.097
Interleukin 1β, ng/mL							
day 5 post-farrowing	2.52	3.28	3.03	4.23	4.20	-	-
day 14 post-farrowing	2.47[y]	3.71[x]	3.29[xy]	2.54[y]	3.31[xy]	0.402	0.092
Interleukin 4, ng/mL							
day 5 post-farrowing	14.34	18.77	16.13	23.53	25.14		
day 14 post-farrowing	14.67	22.87	21.00	16.36	22.18	2.761	0.143
Interleukin 6, ng/mL							
day 5 post-farrowing	0.93	1.38	1.24	1.81	1.38	-	-
day 14 post-farrowing	1.15[ab]	1.44[a]	1.37[a]	0.87[b]	0.96[b]	0.153	0.014
Interleukin 10, ng/mL							
day 5 post-farrowing	4.95	6.94	5.46	7.86	8.80	-	-
day 14 post-farrowing	5.18	7.44	7.24	6.01	7.25	0.838	0.206
Tumor necrosis factor-α, ng/mL							
day 5 post-farrowing	0.32	0.37	0.44	0.43	0.40	-	-
day 14 post-farrowing	0.23	0.47	0.41	0.42	0.40	0.097	0.467
Biomarkers							
CTX-I[d], μg/L							
day 5 post-farrowing	4.85	2.89	4.09	3.00	2.61	-	-
day 14 post-farrowing	2.41[y]	4.40[x]	2.98[xy]	3.14[xy]	2.39[y]	0.541	0.068
Bone alkaline phosphatase, μg/L							
day 5 post-farrowing	8.58	10.15	8.04	8.63	8.02	-	-
day 14 post-farrowing	6.80	6.35	6.10	6.15	6.22	0.699	0.953
Osteocalcin, μg/L							
day 5 post-farrowing	22.92	22.47	22.51	21.90	20.69	-	-
Day 14 post-farrowing	20.94	22.61	20.50	20.37	19.25	1.354	0.524

[a-b]Means within a row lacking a common letter are different ($P \leq 0.05$).
[x-y]Means within a row lacking a common letter tend to be different ($P \leq 0.10$).
[a]Data are means of 10 to 11 observations per measurement.
[b]Data from d 5 post-farrowing were used as a covariate for data obtained on d 14 post-farrowing.
[c]PC, positive control; NC, negative control; FYT, phytase units.
[d]CTX-I, cross-linked C-telopeptide of type I collagen.

interferon-gamma concentration from sows fed the 2000 FYT/kg of novel phytase diet or the commercial phytase diet did not differ from that of sows fed the PC diet. Concentration of IL-1β in serum from sows fed the 2000 FYT/kg of novel phytase or the PC diet tended to be less ($P < 0.10$) compared with that of sows fed the NC diet. Sows fed the diet containing 2000 FYT/kg of novel phytase or commercial phytase had reduced ($P < 0.05$) concentration of IL-6 compared with sows fed the NC diet or the diet containing 1000 FYT/kg of novel phytase. Neither phytase supplementation nor dietary levels of Ca and P influenced concentrations of BAP or OC in serum. However, the concentration of CTX-I tended to be greater ($P < 0.10$) in sows fed the NC diet compared with sows fed the PC diet or sows fed the diet containing the commercial phytase.

4. Discussion

4.1. Gestating sows

The basal endogenous loss of P obtained for gestating sows in this experiment concurs with values reported by Bikker et al. (2017); i.e., 0.65 g/kg DM intake) and Lee et al. (2021); i.e., 0.78 g/kg DM intake) for sows in mid-gestation. The obtained basal endogenous loss of P in gestating sows was greater compared with growing pigs, who on average have a basal endogenous loss of 0.19 g/kg DM intake (NRC, 2012). The difference between growing pigs and sows may be a result of differences in body size and physiological state of the animals. Therefore, if the average basal endogenous loss of P for growing pigs is applied to gestating sows, values for STTD of P in diets and feed ingredients obtained for gestating sows will be underestimated.

Values obtained for the ATTD of P in experimental diets concur with data reported for gestating sows (Nyachoti et al., 2006; Jang et al., 2014; Lee et al., 2020; Lee and Stein, 2022). The observed reduction in fecal P output and subsequent increase in the STTD of P in diets upon phytase supplementation indicates that the exogenous phytase hydrolyzed some of the ester bonds between P and the inositol ring of phytate that resulted in increased absorption of P (Adeola et al., 1995). The observed increase in P digestibility upon inclusion of phytase in diets for gestating sows is also in agreement with results from previous experiments (Jongbloed et al., 2004; Nyachoti et al., 2006). However, this is in contrast with results by Lee et al. (2019) who reported that supplementation of phytase at 500 phytase units/kg had limited effects on P digestibility in diets for gestating sows in early- mid-, or late-gestation periods. Gestation period, dietary Ca and P, and phytase dose and source may influence responses to phytase fed to gestating sows; therefore, further research is warranted to elucidate factors that may influence phytase efficacy in diets for gestating sows. Urinary P excretion is highly associated with dietary P intake because sows and growing pigs fed P-deficient diets excrete low amounts of P in urine (Grez-Capdeville and Crenshaw, 2022). Therefore, the observed reduction of P excretion in urine by sows fed the NC diet compared with sows fed the PC diet indicates that sows excrete a minimal quantity of urinary P until P intake is sufficient to restore the level of P in the body (Rodehutscord et al., 1999). It is not clear why inclusion of 1000 FYT/kg of commercial phytase in the NC diet resulted in increased excretion of urine from sows, but the observation that P retention was not influenced by source of phytase indicates that the two sources were equally efficient in making Ca and P available when at least 1000 FYT were used. Because sows fed the diet with 2000 FYT/kg did not have STTD or retention of P that was different from that of sows fed diets with 1000 FYT/kg, it is likely that the maximum response to the new phytase in diets containing approximately 2.3 g/kg of phytate-bound P was obtained at around 1000 FYT/kg, which is in agreement with what has been observed with commerical phytases fed to growing pigs (Almeida et al., 2013).

Values obtained for ATTD of Ca are in agreement with published values obtained from gestating sows (Jongbloed et al., 2004; Jang et al., 2014; Zhai et al., 2021; Lee and Stein, 2022; Lee et al., 2022), but these values were less than the ATTD of Ca in growing pigs (Rojas and Stein, 2012; Gonzalez-Vega et al., 2015; Lee et al., 2021). The low values for ATTD of Ca may be a result of increased quantities of Ca coming into circulation from bone demineralization (Jongbloed et al., 2004), but the mechanism regulating the impact of serum Ca on the ATTD of Ca has not been elucidated. The observed reduction of Ca in feces and subsequent increase in Ca retention by sows fed diets containing phytase compared with sows fed the NC diet indicates that inclusion of phytase at 1000 or 2000 FYT/kg was effective in hydrolyzing the Ca-phytate complexes (Selle et al., 2009). However, because Ca is mostly stored in bone tissue, which requires both Ca and P for synthesis, the increased Ca retention indicates that phytase also released sufficient P to allow for increased bone tissue synthesis.

To the best of our knowledge, no data demonstrating effects of phytase on concentrations of cytokines in serum of gestating sows have been reported. However, phytate is an antinutrient that has negative impacts on intestinal functions (Bedford and Walk, 2016) and it was, therefore, speculated that destruction of phytate may improve the immune function of sows. However, the lack of effects of phytase on concentrations of serum cytokines indicates that under the conditions of this experiment, phytase did not influence the immune status of sows. It is possible that this is a result of the fact that sows used in this experiment had high health status, but it is also possible that more than 2000 FYT is needed to impact plasma cytokine concentrations.

The CTX-I is a peptide that is a biomarker for bone resorption (Sørensen et al., 2018). Therefore, the observed increase in CTX-I in sows fed the NC diet indicates that bone tissue breakdown was increased due to a deficiency in dietary Ca and P (Lee at al, 2020). However, the observation that CTX-I in sows fed the phytase-containing diets was not different from the concentration in sows fed the PC diet demonstrates that addition of phytase to the NC diet resulted in sufficient P availability to preserve bone integrity at the same level as sows fed the PC diet.

Osteocalcin and BAP are biomarkers for bone formation (Robey et al., 1993), whereas FGF23 is a hormone-like regulator of P homeostasis that is produced primarily in bone osteoblast and osteocytes (Crenshaw et al., 2011). The observation that these biomarkers were not affected by dietary treatments is in contrast with data by Sørensen et al. (2018) that indicating that OC was reduced in growing pigs fed a low P diet. The different responses between sows and growing pigs may be attributed to breed, physiological status, and feeding duration of experimental diets (Sørensen et al., 2018; Lee et al., 2020).

The observation that there were no differences in STTD of P, ATTD of Ca, absorption of P and Ca, or in serum concentrations of cytokines and bone biomarkers, between sows fed the novel phytase and the commercial phytase indicates that under the conditions of this experiment, the two phytases were equally effective. We are not aware of other experiments with gestating sows in which these two phytases were compared.

4.2. Lactating sows

To the best of our knowledge, the basal endogenous loss of P for lactating sows has not been reported previously. However, the obtained basal endogenous loss of P (i.e., 0.65 g/kg DM intake) concurs with values published for gestating sows (Lee et al., 2021). Therefore, the value obtained is greater compared with that of growing pigs (NRC, 2012), which indicates that values for STTD of P in feed ingredients obtained in growing pigs cannot be applied to lactating sows.

The observation that phytase increased the ATTD of DM is in agreement with data indicating that phytase increased the ATTD of DM in corn-soybean meal based diets for growing pigs (Velayudhan et al., 2015). The lack of an increase in digestibility of gross energy when phytase was used is in agreement with data reported by Yang et al. (2017) and Lu et al. (2020), but in contrast with data indicating that phytase increased the ATTD of gross energy and concentration of digestible energy in diets for growing pigs (Velayudhan et al., 2015; Arredondo et al., 2019). Further research is, therefore, needed to determine why phytase sometimes appears to influence gross energy digestibility, whereas this effect is not observed in other experiments.

Values obtained for the ATTD of Ca and P in experimental diets (i.e., without or with phytase) are within the reported range for lactating sows (Kemme et al., 1997a, 1997b; Jongbloed et al., 2004; Zhai et al., 2021). The observed reduction in the analyzed Ca and P in feces and the subsequent increase in Ca and P digestibility in diets upon phytase supplementation indicates that the exogenous phytase hydrolyzed some of the ester bonds between P and the inositol ring of phytate that resulted in increased absorption of these minerals (Adeola et al., 1995). At pH 5.5 and above, phytic acid occurs mainly as a complex with metal cations such as Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, K^{+}, and Mn^{2+} (Humer et al., 2015). Therefore, formation of these complexes reduces the solubility and digestibility of minerals in the gastrointestinal tract of pigs. With the reduced ability of phytate to chelate Ca upon phytase supplementation, increased digestibility of Ca from calcium carbonate may be the reason for the observed increase in Ca digestibility in diets with phytase. The observed increase in P digestibility upon inclusion of phytase in sow diets is also in agreement with results from previous experiments (Kemme et al., 1997a, 1997b; Jongbloed et al., 2004; Zhai et al., 2021). However, the observed increase in Ca digestibility is in contrast with results by Kemme et al. (1997a) who reported that supplementation of phytase at 500 phytase units/kg did not influence Ca digestibility by lactating sows. Because sows fed the diet with 2000 FYT/kg did not have digestibility of DM, Ca, or P that was different from that of sows fed diets with 1000 FYT/kg, it is likely that the maximum response to the novel phytase was obtained at 1000 FYT/kg. The present data from gestating and lactating sows are in agreement with data from weanling and growing pigs indicating that the ATTD of Ca and P was maximized at 800 to 1100 FYT/kg (Almeida et al., 2013). The fact that the lowest level of phytase used in the present experiments maximised the response to phytase may have been the reason no differences between the two sources of phytase were observed, but it is possible that at lower inclusion levels, differences may be observed.

Pro-inflammatory cytokines include IL-1β and IL-6 (Chen et al., 2019), and the observed reduction of these cytokines in lactating sows fed the diet containing the commercial phytase or the diet with 2000 FYT/kg of the novel phytase may be a result of phytase improving the immune response of lactating sows. This is likely due to the ability of phytase to reduce the anti-nutritional effect of phytate and consequently increase nutrient digestibility and overall health of sows. The observed increase in CTX-I in lactating sows fed the NC diet indicates an increase in bone tissue breakdown due to a deficiency in dietary Ca and P (Lee at al, 2020), and is also in agreement with data observed for gestating sows. The observation that OC and BAP were not affected by dietary treatments is in contrast with data by Sørensen et al. (2018) who demonstrated that concentration of OC in the serum of pigs was reduced when long-term feeding of low P diets was used. Therefore, it is likely that experimental diets need to be fed to sows for a longer period of time than what was done in this experiment to influence concentrations of OC and BAP.

5. Conclusion

Results from the two experiments indicated that inclusion of phytase in diets for gestating and lactating sows increased the apparent total tract digestibility and the standardized total tract digestibility of P. Phytase also increased retention of Ca and P in gestating sows and increased apparent total tract digestibility of dry matter and Ca in diets for lactating sows. Sows fed the negative control diet had increased concentration of carboxyterminal cross-linked telopeptide of type I collagen, which indicates that sows fed the negative control diet had elevated resorption of bone tissue compared with sows fed the positive control diet or phytase diets. Phytase did not affect serum concentrations of cytokines, bone formation markers, or fibroblast growth factor 23 in gestating sows, but concentrations of some pro-inflammatory cytokines (i.e., interleukin-1β and interleukin-6) in lactating sows were reduced by phytase supplementation, which may be a result of decreased anti-nutritional effects associated with phytate. There were, however, no differences between the novel phytase and the commercial phytase.

CRediT authorship contribution statement

Stein Hans H: Conceptualization, Writing – review & editing, Funding acquisition, Investigation, Resources, Supervision. **Torres-Mendoza Leidy J.:** Formal analysis, Resources. **Espinosa Charmaine D.:** Conceptualization, Formal analysis, Project administration, Writing – original draft. **Bergstrom Jonathan R.:** Conceptualization, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

JRB is an employee at DSM Nutritional Products, Parsipanny, NJ, USA, a global supplier of microbial phytase. CDE, LJTM, and HHS have no conflicts of interest.

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References

Adeola, O., Lawrence, B.V., Sutton, A.L., Cline, T.R., 1995. Phytase-induced changes in mineral utilization in zinc-supplemented diets for pigs. J. Anim. Sci. 73, 3384–3391. https://doi.org/10.2527/1995.73113384x.

Almeida, F.N., Stein, H.H., 2010. Performance and phosphorus balance of pigs fed diets formulated on the basis of values for standardized total tract digestibility of phosphorus. J. Anim. Sci. 88, 2968–2977. https://doi.org/10.2527/jas.2009-2285.

Almeida, F.N., Sulabo, R.C., Stein, H.H., 2013. Effects of a novel bacterial phytase expressed in Aspergillus Oryzae on digestibility of calcium and phosphorus in diets fed to weanling or growing pigs. J. Anim. Sci. Biotechnol. 4, 8 https://doi.org/10.1186/2049-1891-4-8.

AOAC Int, 2019. Official methods of analysis of AOAC Int, 21th ed. AOAC Int., Rockville, MD, USA.

Arredondo, M.A., Casas, G.A., Stein, H.H., 2019. Increasing levels of microbial phytase increases the digestibility of energy and minerals in diets fed to pigs. Anim. Feed Sci. Technol. 248, 27–36. https://doi.org/10.1016/j.anifeedsci.2019.01.001.

Bedford, M.R., Walk, C.L., 2016. Reduction of phytate to tetrakisphosphate (IP_4) to triphosphate (IP_3), or perhaps even lower, does not remove its antinutritive properties. In: Walk, C.L., Kuhn, I., Stein, H.H., Kidd, M.T., Rodehutscord, M. (Eds.), Phytate Destruction - Consequences for Precision Animal Nutrition. Wageningen Academic Publishers, Wageningen, the Netherlands, pp. 45–51. https://doi.org/10.3920/978-90-8686-836-0_3.

Bikker, P., van der Peet-Schwering, C.M.C., Gerrits, W.J.J., Sips, V., Walvoort, C., van Laar, H., 2017. Endogenous phosphorus losses in growing-finishing pigs and gestating sows. J. Anim. Sci. 95, 1637–1643. https://doi.org/10.2527/jas.2016.1041.

Chen, J., Xu, Q., Li, Y., Tang, Z., Sun, W., Zhang, X., Sun, J., Sun, Z., 2019. Comparative effects of dietary supplementations with sodium butyrate, medium-chain fatty acids, and n-3 polyunsaturated fatty acids in late pregnancy and lactation on the reproductive performance of sows and growth performance of suckling piglets. J. Anim. Sci. 97, 4256–4267. https://doi.org/10.1093/jas/skz284.

Crenshaw, T.D., Rortvedt, L.A., Hassen, Z., 2011. Triennial growth symposium: a novel pathway for vitamin D-mediated phosphate homeostasis: implications for skeleton growth and mineralization. J. Anim. Sci. 89, 1957–1964. https://doi.org/10.2527/jas.2010-3411.

Dias, R.S., Lopez, S., Moreira, J.A., Schulin-Zeuthen, M., Vitti, D.M.S.S., Kebreab, E., France, J., 2010. Application of a kinetic model to describe phosphorus metabolism in pigs fed a diet with a microbial phytase. J. Agric. Sci. 148, 277–286. https://doi.org/10.1017/S0021859610000195.

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

Espinosa, C.D., Oliveira, M.S.F., Velayudhan, D.E., Dersjant-Li, Y., Stein, H.H., 2021. Influence of a novel consensus bacterial 6-phytase variant on mineral digestibility and bone ash in young growing pigs fed diets with different concentrations of phytate-bound phosphorus. J. Anim. Sci. 99, 1–12. https://doi.org/10.1093/jas/skab211.

Gonzalez-Vega, J.C., Walk, C.L., Stein, H.H., 2015. Effect of phytate, microbial phytase, fiber, and soybean oil on calculated values for apparent and standardized total tract digestibility of calcium and apparent total tract digestibility of phosphorus in fish meal fed to growing pigs. J. Anim. Sci. 93, 4808–4818. https://doi.org/10.2527/jas.2015-8992.

Grez-Capdeville, M., Crenshaw, T.D., 2022. Estimation of phosphorus requirements of sows based on 24-h urinary phosphorus excretion during gestation and lactation. Br. J. Nutr. 128, 377–388. https://doi.org/10.1017/S0007114521003421.

Humer, E., Schwarz, C., Schedle, K., 2015. Phytate in pig and poultry nutrition. J. Anim. Physiol. Anim. Nutr. 99, 605–625. https://doi.org/10.1111/jpn.12258.

Jang, Y.D., Lindemann, M.D., Heugten, Ev, Jones, R.D., Kim, B.G., Maxwell, C.V., Radcliffe, J.S., 2014. Effects of phytase supplementation on reproductive performance, apparent total tract digestibility of Ca and P and bone characteristics in gestating and lactating sows. Rev. Colomb. Cienc. Pecu. 27, 178–193.

Jongbloed, A.W., van Diepen, J.T.M., Kemme, P.A., Broz, J., 2004. Efficacy of microbial phytase on mineral digestibility in diets for gestating and lactating sows. Livest. Prod. Sci. 91, 143–155. https://doi.org/10.1016/j.livprodsci.2004.07.017.

Kemme, P.A., Jongbloed, A.W., Mroz, Z., Beynen, A.C., 1997a. The efficacy of Aspergillus niger phytase in rendering phytate phosphorus available for absorption in pigs is influenced by pig physiological status. J. Anim. Sci. 75, 2129–2138. https://doi.org/10.2527/1997.7582129x.

Kemme, P.A., Radcliffe, J.S., Jongbloed, A.W., Mroz, Z., 1997b. The effects of sow parity on digestibility of proximate components and minerals during lactation as influenced by diet and microbial phytase supplementation. J. Anim. Sci. 75, 2147–2153. https://doi.org/10.2527/1997.7582147x.

Lee, S.A., Stein, H.H., 2022. Effects of dietary levels of calcium, phosphorus, and 1-alpha-hydroxycholecalciferol on digestibility, retention of calcium and phosphorus, and concentration of metabolizable energy in diets fed to sows in late-gestation. Can. J. Anim. Sci. 102, 184–188. https://doi.org/10.1139/cjas-2021-0018.

Lee, S.A., Lagos, L.V., Walk, C.L., Stein, H.H., 2019. Basal endogenous loss, standardized total tract digestibility of calcium in calcium carbonate, and retention of calcium in gestating sows change during gestation, but microbial phytase reduces basal endogenous loss of calcium. J. Anim. Sci. 97, 1712–1721. https://doi.org/10.1093/jas/skz048.

Lee, S.A., Lagos, L.V., Bedford, M.R., Stein, H.H., 2020. Increasing calcium from deficient to adequate concentration in diets for gestating sows decreases digestibility of phosphorus and reduces serum concentration of a bone resorption biomarker. J. Anim. Sci. 98, 1–8. https://doi.org/10.1093/jas/skaa076.

Lee, S.A., Bedford, M.R., Stein, H.H., 2021. Comparative digestibility and retention of calcium and phosphorus in normal- and high-phytate diets fed to gestating sows and growing pigs. Anim. Feed Sci. Technol. 280, 1–9. https://doi.org/10.1016/j.anifeedsci.2021.115084.

Lee, S.A., Torres-Mendoza, L.J., Stein, H.H., 2022. Effects of 25-hydroxycholecalciferol (25-OH-D_3) and 1-hydroxycholecalciferol (1-OH-D_3) on serum bone biomarkers and calcium and phosphorus balance and concentrations of energy in diets without or with microbial phytase fed to sows in late gestation. J. Anim. Sci. 100, 1–8. https://doi.org/10.1093/jas/skac299.

Lu, H., Shin, S., Kuehn, I., Bedford, M., Rodehutscord, M., Adeola, O., Ajuwon, K.M., 2020. Effect of phytase on nutrient digestibility and expression of intestinal tight junction and nutrient transporter genes in pigs. J. Anim. Sci. 98, 1–12. https://doi.org/10.1093/jas/skaa206.

NRC, 2012. Nutrient Requirements of Swine, 11th rev. Natl. Acad. Press, Washington, D.C., USA.

Nyachoti, C.M., Sands, J.S., Connor, M.L., Adeola, O., 2006. Effect of supplementing phytase to corn- or wheat-based gestation and lactation diets on nutrient digestibility and sow and litter performance. Can. J. Anim. Sci. 86, 501–510. https://doi.org/10.4141/A04-500.

Pallauf, J., Rimbach, G., Pippig, S., Schindler, B., Höhler, D., Most, E., 1994. Dietary effect of phytogenic phytase and an addition of microbial phytase to a diet based on field beans, wheat, peas and barley on the utilization of phosphorus, calcium, magnesium, zinc and protein in piglets. Z. Ernahr. Suppl. 33, 128–135. https://doi.org/10.1007/BF01622225.

Robey, P.G., Fedarko, N.S., Hefferan, T.E., Bianco, P., Vetter, U.K., Grzesik, W., Friedenstein, A., van der Pluijm, G., Mintz, K.P., Young, M.F., Kerr, J.M., Ibaraki, K., Heegaard, A.-M., 1993. Structure and molecular regulation of bone matrix proteins. J. Bone Miner. Res. 8, S483–S487. https://doi.org/10.1002/jbmr.5650081310.

Rodehutscord, M., Faust, M., Pfeffer, E., 1999. The course of phosphorus excretion in growing pigs fed continuously increasing phosphorus concentrations after a phosphorus depletion. Arch. Anim. Nutr. Tierernahr. 52, 323–334. https://doi.org/10.1080/17450399909386171.

Rojas, O.J., Stein, H.H., 2012. Digestibility of phosphorus by weanling pigs of fermented and conventional soybean meal without and with microbial phytase. J. Anim. Sci. 90, 1506–1512. https://doi.org/10.2527/jas.2011-4103.

Rosenfelder-Kuon, P., Siegert, W., Rodehutscord, M., 2020. Effect of microbial phytase supplementation on P digestibility in pigs: a meta-analysis. Arch. Anim. Nutr. 74, 1–18. https://doi.org/10.1080/1745039X.2019.1687249.

SAS Institute Inc, 2016. SAS® 9.4 SQL Procedure User's Guide, 4th ed. SAS Institute Inc, Cary, NC, USA.

Selle, P.H., Cowieson, A.J., Ravindran, V., 2009. Consequences of calcium interactions with phytate and phytase for poultry and pigs. Livest. Sci. 124, 126–141. https://doi.org/10.1016/j.livsci.2009.01.006.

Sørensen, K.U., Kruger, M.C., Hansen-Møller, J., Poulsen, H.D., 2018. Bone biochemical markers for assessment of bone responses to differentiated phosphorus supply in growing-finishing pigs. J. Anim. Sci. 96, 4693–4703. https://doi.org/10.1093/jas/sky311.

Tran, G., Sauvant, D., 2004. Chemical data and nutritional value. In: Sauvant, D., Perez, J.M., Tran, G. (Eds.), Tables of composition and nutritional value of feed materials, 2nd ed. Wageningen Acad. Publ., Wageningen, The Netherlands, pp. 17–24.

U.S. Environmental Protection Agency, 2000. Acid digestion of sediments, sludges, and soils. U.S. EPA, Washington, DC, USA. ⟨https://www.epa.gov/sites/production/files/2015-12/documents/3050b.pdf⟩. accessed January 21, 2024.

Velayudhan, D.E., Heo, J.M., Dersjant-Li, Y., Owusu-Asiedu, A., Nyachoti, C.M., 2015. Efficacy of novel 6-phytase from *Buttiauxella sp*. on ileal and total tract nutrient digestibility in growing pigs fed a corn-soy based diet. Anim. Feed Sci. Technol. 210, 217–224. https://doi.org/10.1016/j.anifeedsci.2015.10.005.

Yang, Y.Y., Fan, Y.F., Cao, Y.H., Guo, P.P., Dong, B., Ma, Y.X., 2017. Effects of exogenous phytase and xylanase, individually or in combination, and pelleting on nutrient digestibility, available energy content of wheat and performance of growing pigs fed wheat-based diets. Asian-Australas. J. Anim. Sci. 30, 57–63. https://doi.org/10.5713/ajas.15.0876.

Zhai, H., Cowieson, A.J., Pappenberger, G., Zhang, J., Wu, J., 2021. The effect of short-term phytase supplementation on the apparent total tract digestibility of calcium and phosphorus and the reproductive performance of late gestation and lactating sows fed diets without mineral phosphorus. J. Anim. Sci. 99, 1–9. https://doi.org/10.1093/jas/skab194.