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Non Ruminant Nutrition

Microbial phytase reduces basal endogenous loss of calcium in pigs fed diets containing phytate phosphorus at commercial levels

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Abstract

The objective of this experiment was to test the hypothesis that increasing dietary phytase reduces basal endogenous loss of Ca and increases P balance in pigs. Seventy barrows (initial body weight: 17.66 ± 1.69 kg) were allotted to seven Ca-free diets using a randomized complete block design with two blocks and five pigs per diet in each block. All diets were based on corn, potato protein concentrate, and full-fat rice bran. A positive control (**PC**) diet was formulated to contain P at the requirement for standardized total tract digestible (**STTD**) P by 11 to 25 kg pigs. Six negative control (**NC**) diets were formulated by reducing the provision of digestible P by 0.15% and adding 0, 250, 500, 1,000, 2,000, or 4,000 phytase units/kg diet. Pigs were housed individually in metabolism crates that allowed for total, but separate, collection of urine and feces. Daily feed allowance was 3.0 times the maintenance requirement for metabolizable energy and was divided into two equal meals. Diets were fed for 12 d with the first 5 d considered the adaptation period. Urine collections started on day 6 in the morning and ceased on day 10 in the morning. Fecal markers were also included in the morning meals on day 6 and day 10 and feces were collected according to the marker-to-marker procedure. Results indicated that the apparent total tract digestibility of dry matter was not affected by dietary P or phytase levels. The basal endogenous loss of Ca was not affected by dietary P, but exponentially decreased ($P = 0.030$) as phytase level increased in the diets. Phosphorus retention (g/d) and standardized total tract digestibility of phosphorus were greater ($P < 0.05$) in pigs fed the PC diet compared with pigs fed the NC diet with no phytase. The STTD of P exponentially ($P < 0.001$) increased as phytase level increased in the diets, but because of the lack of Ca, retention of P (% of absorbed) linearly decreased ($P = 0.006$) as phytase increased. In conclusion, basal endogenous loss of Ca decreased as dietary phytase increased demonstrating that endogenous Ca can be bound to phytate in the intestinal tract of pigs. However, STTD of P increased as phytase level in the diets increased.

Lay Summary

Phytate in plant-based ingredients limits the amount of phosphorus available for absorption and can form indigestible complexes with endogenous calcium. However, breakdown of the phytate molecule by phytase increases digestibility of phosphorus and may also reduce endogenous loss of calcium. Therefore, the objective of this experiment was to test the hypothesis that level of phytase influences the utilization of phosphorus and basal endogenous loss of calcium in growing pigs fed calcium-free diets. Results demonstrated that total tract digestibility of phosphorus increased as phytase increased in the diets, but not all of the absorbed P was retained in the pigs due to a lack of calcium. Dietary concentration of phosphorus did not affect basal endogenous loss of calcium, but increasing concentrations of microbial phytase reduced basal endogenous loss of calcium. These results demonstrate that some endogenous calcium is bound to phytate, but including microbial phytase in diets helps release this calcium and therefore reduce endogenous loss of calcium.

Key words: calcium, digestibility, endogenous loss, phosphorus, phytase, phytate

Abbreviations: ATTD, apparent total tract digestibility; DM, dry matter; DMI, dry matter intake; FTU, phytase unit; NC, negative control; PC, positive control; STTD, standardized total tract digestibility

Introduction

Most P in plant-based feed ingredients is bound to phytate, which limits the amount of P that is available for absorption (Selle and Ravindran, 2008), but microbial phytase in pig diets increases the digestibility of P (Poulsen et al., 2010; Rojas and Stein, 2012). Digestibility of P may be negatively affected by excess dietary Ca (Stein et al., 2011; Lee et al., 2020), but to a lesser extent if phytase is included in the diet than if no phytase is used (González-Vega et al., 2013). A molecule of phytate can chelate Ca cations resulting in the formation of insoluble Ca-phytate complexes that reduce digestibility of both Ca and P (Selle et al., 2009). Although inclusion of 1,500 units/kg of an *Escherichia coli* microbial phytase to diets based on canola meal did not influence the total endogenous loss of Ca in growing pigs (González-Vega et al., 2013), addition of 500 units of an *E. coli* microbial phytase

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reduced the basal endogenous loss of Ca and increased digestibility of Ca and P (Lee et al., 2019a, 2019b). The basal endogenous loss of Ca is determined using a Ca-free diet, whereas the total endogenous loss often is determined using the regression procedure (NRC, 2012). The decrease in basal endogenous loss of Ca may be due to a reduction in the amount of phytate (i.e., inositol hexakisphosphate) that can form Ca-phytate complexes if phytase is added to the diet, resulting in an increase in absorption of endogenous Ca and a reduced amount of endogenous Ca being excreted in feces (Lee et al., 2019a). If indeed the reduced endogenous loss of Ca is a result of degradation of phytate, it is expected that increased doses of dietary phytase will linearly reduce endogenous losses of Ca, but this hypothesis has not been experimentally verified. Therefore, an experiment was conducted to test the hypothesis that increasing dietary phytase reduces basal endogenous loss of Ca and increases P digestibility in growing pigs.

Table 1. Ingredient composition of experimental diets[1]

Item,%	Positive control	Negative control
Ground yellow corn	71.88	72.04
Full-fat rice bran	6.00	6.00
Potato protein concentrate	18.20	18.20
Soybean oil	2.00	2.00
Monosodium phosphate	1.02	0.36
Sodium chloride	0.40	0.40
Vitamin mineral premix[2]	0.50	0.50
Corn-phytase premix	-	0.50

[1]There were six negative control diets containing 0, 250, 500, 1,000, 2,000, or 4,000 units of phytase/kg diet, but the positive control diet did not contain phytase.
[2]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 10,662 IU; Vitamin D_3 as cholecalciferol, 1,660 IU, vitamin E as selenium yeast, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.40 mg; thiamin as thiamine mononitrate, 1.08 mg; riboflavin, 6.49 mg; pyridoxine as pyridoxine hydrochloride, 0.98 mg; vitamin B_{12}, 0.03 mg; D-panthothenic acid as D-calcium pantothenate, 23.2 mg; niacin, 43.4 mg; folic acid, 1.56 mg; biotin, 0.44 mg; cu, 20 mg as copper chloride; Fe, 123 mg as iron sulfate; I, 1.24 mg as ethylenediamine dihydriodide; Mn, 59.4 mg as manganese hydroxychloride; Se, 0.27 mg as sodium selenite and selenium yeast; and Zn, 124.7 mg as zinc hydroxychloride.

Materials and Methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for this experiment. The pigs used in the experiment were the offspring of Line 800 boars mated to Camborough females (Pig Improvement Company, Hendersonville, TN).

Diets, animals, and experimental design

Seven Ca-free diets were formulated (Tables 1 and 2). The positive control (**PC**) diet contained P at the requirement for standardized total tract digestible (**STTD**) P for 11 to 25 kg pigs (i.e., 0.33% STTD P; NRC, 2012). A negative control (**NC**) diet was formulated by reducing the provision of STTD P by 0.15 percentage units, which was the assumed release of STTD P obtained by inclusion of 1,000 phytase units (**FTU**)/kg. The NC diet, therefore, contained 0.18% STTD P. Five additional diets were formulated by adding 250, 500, 1,000, 2,000, or 4,000 FTU/kg of a novel consensus bacterial 6-phytase variant (Danisco Animal Nutrition & Health – IFF, Oegstgeest, The Netherlands) to the NC diet. All diets were based on corn, potato protein concentrate, and full-fat rice bran (Table 3). Vitamins and minerals, with the exception of Ca and P, were included in all diets to meet or exceed current requirement estimates (NRC, 2012).

Seventy barrows (initial body weight: 17.66 ± 1.69 kg) were allotted to the seven diets using a randomized complete block design with two blocks of 35 pigs. Weaning group was the block and there were five replicate pigs per diet in each block for a total of 10 replicate pigs per diet. Pigs were housed individually in metabolism crates that were equipped with a feeder, a nipple drinker, and a fully slatted floor. A screen floor and a urine tray were placed under the slatted floor to allow for total, but separate, collection of urine and feces.

Table 2. Nutrient composition of experimental diets (as-fed basis)[1]

Item	Positive control	Negative control (phytase, FTU/kg)[2]					
		0	250	500	1,000	2,000	4,000
Dry matter, %	90.84	90.62	90.56	90.58	90.83	90.85	90.72
Crude protein, %	19.28	19.83	19.67	19.76	19.71	20.96	20.40
Ash, %	8.83	8.97	7.67	7.51	8.82	7.69	7.21
Ca, %	0.03	0.03	0.03	0.03	0.03	0.05	0.04
P, %	0.66	0.46	0.45	0.46	0.48	0.49	0.45
Phytate[3], %	0.96	0.96	0.96	0.96	0.96	0.96	0.96
Phytate P[4], %	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Non-phytate P[5], %	0.39	0.19	0.18	0.19	0.21	0.22	0.18
Phytase activity, FTU/kg	< 70	< 70	260	560	1,200	1,800	3,900

[1]All analyzed data are the average of two analyses.
[2]FTU, phytase unit.
[3]Phytate was calculated by multiplying the analyzed phytate in the ingredients by the inclusion rate of the ingredients in the diet.
[4]Phytate-P was calculated by multiplying phytate by 0.282 (Tran and Sauvant, 2004).
[5]Non-phytate P was calculated as the difference between total P and phytate.

Table 3. Nutrient composition of ingredients (as-fed basis)[1]

Item	Ground yellow corn	Potato protein concentrate	Full-fat rice bran
Dry matter, %	89.13	93.48	96.96
Crude protein, %	6.46	81.18	15.27
Ash, %	2.08	1.48	10.92
Ca, %	0.02	0.02	0.06
P, %	0.30	0.10	2.10
Phytate, %	0.78	0.23	5.98
Phytate P[2], %	0.22	0.06	1.68
Non-phytate P[3], %	0.08	0.04	0.42

[1]All analyzed data are the average of two analyses.
[2]Phytate-P was calculated by multiplying analyzed phytate by 0.282 (Tran and Sauvant, 2004).
[3]Non-phytate P was calculated as the difference between total P and phytate P.

Feeding and sample collection

Pigs were provided feed at 3.0 times the daily maintenance requirement for metabolizable energy (i.e., 197 kcal metabolizable energy per kg body weight$^{0.60}$; NRC, 2012). Daily feed allotments were divided into two equal meals provided at 0800 and 1600 h. Pigs were fed experimental diets for 12 d, with the initial 5 d considered the adaptation period. Feces were collected for 4 d following the adaptation period using the marker-to-marker procedure (Adeola, 2001). Indigo carmine was fed in the morning of day 6 and fecal collection began when the marker appeared in the feces. Fecal collection ceased when the second marker, ferric oxide, which was fed in the morning of day 10, appeared in the feces. Urine was collected from day 6 to day 10. Feed consumption was recorded daily and orts were collected to determine feed intake from day 6 to day 10. Pigs had free access to water throughout the experiment.

Fecal collection occurred twice daily and samples were stored at –20 °C immediately after collection. Urine was collected in buckets containing a preservative of 50 mL of 6*N* HCl that were placed under each metabolism crate. The buckets were weighed and emptied once per day and 10% of the collected urine was stored at –20 °C.

Chemical analysis

Samples of the main ingredients and diets were collected at the time of diet mixing for chemical analysis. Ingredients were analyzed for phytic acid before diets were formulated (Ellis et al., 1977), and diets were analyzed for phytase activity (method 2000.12; AOAC Int., 2019) before feeding was initiated. Fecal samples were thawed and then dried in a 65 °C forced air drying oven and ground using a 500G stainless steel mill grinder (RRH, Zhejiang, China). Urine samples were thawed at room temperature and subsamples were collected and filtered for analysis. Diet, ingredient, and dried fecal samples were analyzed in duplicate for dry matter (**DM**) by oven drying at 135 °C for 2 h (method 930.15; AOAC Int., 2019). These samples were also analyzed for ash at 600 °C (Method 942.05; AOAC Int., 2019), and for N using the combustion procedure (Method 990.03; AOAC Int., 2019). Crude protein was calculated as N × 6.25. Fecal, urine, diet, and ingredient samples were analyzed for Ca and P (Method 985.01 A, B, and C; AOAC Int., 2019) using inductively coupled plasma-optical emission spectrometry (ICP-OES; Avio 200, PerkinElmer, Waltham, MA, USA) after wet digestion with nitric acid (method 3050 B; U.S. Environmental Protection Agency, 2000).

Calculations

Phytate in the diets was calculated by multiplying the analyzed phytate in corn, potato protein concentrate, and rice bran by the inclusion rate of each ingredient in the diet and by adding the values. Phytate-P was calculated by multiplying phytate by 0.282 (Tran and Sauvant, 2004) and non-phytate P was calculated as the difference between phytate-P and total P.

The basal endogenous loss of Ca was calculated using the fecal flow of Ca and feed intake of pigs and was expressed as mg/kg DM intake (**DMI**) using the following equation (adapted from Almeida and Stein, 2010):

$$\text{Basal endogenous loss} = \frac{\text{Fecal output of Ca}}{\text{DMI}} \times 1,000,$$

where basal endogenous loss is in mg/kg DMI, DMI is in kg DM per day, and fecal output of Ca is in gram per day.

The apparent total tract digestibility (**ATTD**) of P in each experimental diet was calculated according to the following equation (Almeida and Stein, 2010):

$$ATTD\ (\%) = [(P\ intake - fecal\ P\ output)/P\ intake] \times 100,$$

where both P intake and fecal P output are expressed in grams per day.

The STTD (%) of P in each experimental diet was calculated by correcting the ATTD of P for the average basal endogenous loss of P (i.e., 190 mg/kg DMI; NRC, 2012).

Retention of P (%) was calculated using the following equation (Fernández, 1995):

$$\text{Retention} = \frac{\text{P intake- (fecal P output + urine P output)}}{\text{P intake}} \times 100,$$

where P intake, fecal P output, and urine P output are expressed in grams per day.

Statistical analysis

Normality and homogeneity of data were verified using the UNIVARIATE and MIXED procedures (SAS Inst. Inc., Cary, NC, USA) and outliers were identified using Internally Studentized Residuals (Tukey, 1977). Identified outliers were excluded from the final statistical analysis; if the number of observations was not identical among the dietary treatments, an average SEM was used. Data were analyzed using the PROC MIXED procedure (SAS Inst. Inc.). The model included diet as fixed effect and block as random effect. Mean values were calculated using the LSMeans statement. Contrast statements were used to analyze PC vs. NC diets and linear effects of increasing phytase in NC diets. Using JMP software in SAS, exponential curve fitting was also analyzed with level of phytase in NC diets as the X variable and response criteria as the Y variable. Pig was the experimental unit for all analyses and results were considered significant at $P \leq 0.05$ and considered a trend at $P \leq 0.10$.

Results

The basal endogenous loss of Ca by one pig fed the PC diet and one pig fed the diet containing 2,000 FTU/kg were identified as outliers and removed. Therefore, these diets had nine observations for the endogenous loss of Ca. No other outliers were identified and there were, therefore, 10 observations for all other criteria. Feed intake, weights of fecal and urine excretion, and the ATTD of DM were not affected by experimental diets (Table 4). Calcium excretion in feces expressed as percent of feces and as g/d was not different between the PC and NC diets, but linearly ($P < 0.05$) decreased as phytase increased in the diets. Basal endogenous loss of Ca was not different between the PC and NC diet. However, as dietary phytase increased, basal endogenous loss of Ca exponentially ($P = 0.030$) decreased with the basal endogenous loss of Ca in the diet containing 4,000 FTU/kg being the least among all diets. Calcium excretion in urine expressed in mg/kg and g/d was not affected by increasing phytase in the NC diets, but Ca in urine tended ($P = 0.06$) to be less from pigs fed the NC diet compared with pigs fed the PC diet.

Phosphorus intake, concentration of P in feces, absorbed P, the ATTD of P, and the STTD of P were greater ($P < 0.05$) for pigs fed the PC diet compared with pigs fed the NC diet (Table 5). Concentration of P in the urine and P retention (g/d) were greater ($P < 0.001$) for pigs fed the PC diet compared with those fed the NC diet. However, P retention calculated as percent of intake and absorbed P was not different between the two diets.

As phytase level increased in the NC diets, P excretion in feces, expressed as percent of feces or as g/d, exponentially ($P < 0.001$) decreased, which resulted in an exponential increase ($P < 0.001$) in the ATTD and STTD of P. Phosphorus excretion in urine (g/d) exponentially ($P = 0.009$) increased as phytase in the NC diet increased. Retention of P (percent of intake) was not affected by dietary phytase, but retention of P calculated as percent of absorbed P linearly ($P = 0.006$) decreased as dietary phytase increased.

Discussion

The basal endogenous loss of Ca is a loss of Ca that is diet-independent whereas the specific endogenous loss is diet-dependent as has been described for amino acids and P (Stein et al., 2007; NRC, 2012). Total endogenous loss of Ca, thus, includes both the basal and specific endogenous losses. The basal endogenous loss of Ca by pigs fed cornstarch-based Ca-free diets is less compared with pigs fed a corn-based diet (González-Vega et al., 2015), but because corn-based diets have concentrations of fiber and phytate resembling commercial diets, corn based diets usually are used to determine the basal endogenous loss of Ca (Lee et al., 2019a, 2019b). The very low levels of Ca (0.02% to 0.04%) in the diets used in the experiment was assumed not to impact calculated endogenous losses because only approximately 25% of diet Ca will be excreted in the feces.

Analyzed concentrations of P and Ca in corn, potato protein concentrate, and full-fat rice bran were consistent with previous data (Casas and Stein, 2015; Lee et al., 2019a). The basal endogenous loss of Ca in pigs fed the PC and NC diets was within the range of values observed in growing pigs fed corn-based, Ca-free diets (i.e., 329 to 659 mg per kg DMI; Merriman and Stein, 2016; Lee et al., 2019b; Sung et al., 2020). The observation that the basal endogenous loss of Ca was reduced by inclusion of microbial phytase in the diets was consistent with results from both gestating sows and growing pigs (Lee et al., 2019a, 2019b). The negatively charged phytate molecule has the ability to chelate endogenous Ca cations resulting in formations of insoluble Ca-phytate complexes (Selle et al., 2009). Therefore, it is likely that the observed reduction in basal endogenous loss of Ca that was caused by dietary phytase is due to the decreased concentration of phytate molecules that may form non-digestible complexes with Ca when phytase was added to experimental diets, resulting in less endogenous Ca being excreted (Lee et al., 2019a).

The STTD of P in the PC diet was greater than the calculated STTD of P (NRC, 2012), which may be due to the lack

Table 4. Basal endogenous loss of Ca and excretion of Ca in urine by pigs fed Ca-free diets[1,2]

Item, %	PC	NC (FTU/kg)						SEM	Contrast *P*-value		SE[3]	Exponential *P*-value
		0	250	500	1,000	2,000	4,000		PC vs. NC	Linear		
Feed intake, kg/d	0.90	0.91	0.89	0.89	0.92	0.87	0.90	0.02	0.763	0.712	0.02	0.987
Fecal excretion, g DM/d	58.15	60.41	61.80	65.72	70.78	60.47	64.09	5.86	0.692	0.922	0.01	0.659
ATTD of DM, %	92.92	92.71	92.27	91.88	91.52	92.35	92.14	0.76	0.758	0.832	0.01	0.629
Urine excretion, kg/d	2.89	4.05	3.98	4.24	4.11	4.04	4.95	0.83	0.287	0.363	0.01	0.685
Ca excretion												
Ca in feces, %	0.75	0.67	0.35	0.29	0.24	0.40	0.22	0.11	0.382	0.010	0.004	0.133
Fecal Ca output, g/d	0.48	0.42	0.23	0.20	0.17	0.25	0.15	0.06	0.427	0.023	0.01	0.187
Basal endogenous loss of Ca, mg/kg DMI	512	501	282	246	211	239	186	63	0.885	0.002	0.002	0.030
Ca in urine, mg/kg	65	23	45	25	34	45	18	18	0.060	0.601	0.002	0.761
Urine Ca output, g/d	0.22	0.09	0.23	0.09	0.15	0.17	0.14	0.09	0.232	0.901	0.002	0.801

[1]NC, negative control; PC, positive control; DM, dry matter; ATTD, apparent total tract digestibility; FTU, phytase unit; DMI, dry matter intake.
[2]Least squares means represent 10 observations with the exception that there were 9 observations for basal endogenous loss of Ca by pigs fed the PC diet and pigs fed the NC diet containing 2,000 FTU/kg.
[3]Standard error for the exponential model.

of dietary Ca in the PC diet. Increasing dietary Ca reduces the digestibility of P in gestating sows and growing pigs (Stein et al., 2011; Lee et al., 2020) and the ATTD of P in a Ca-free diet is greater than in diets containing calcium carbonate as a Ca source (Lee et al., 2019a). An increase in dietary Ca may result in increased formations of Ca-P complexes that prevent P from being absorbed, resulting in decreased P digestibility (Stein et al., 2011). It is, therefore, likely that the greater than expected STTD of P is a result of the lack of Ca in the PC diet.

The observation that STTD of P increased and P excretion in feces decreased as dietary phytase increased was expected due to the liberation of phytate-bound P by microbial phytase and is consistent with previous data (Casas and Stein, 2015; Blavi et al., 2017; She et al., 2017). Likewise, the exponential increase in the ATTD of P that was observed as microbial phytase was included in the diet is consistent with data demonstrated that using from 500 to 4,000 FTU of the same consensus bacterial 6-phytase variant as used in the present experiment resulted in an increased ATTD of P regardless of the phytate concentration in the diet (Espinosa et al., 2021). The majority of P stored in plant-based ingredients is bound to phytate, limiting the amount of P available for utilization by pigs (Selle and Ravindran, 2008). Phytase hydrolyzes the bonds between P and phytate, releasing some of the phytate-bound P, which increases digestibility of P (Poulsen et al., 2010). Although pigs have some mucosal phytase activity, it is not enough to effectively release phytate-bound P in plant based feed ingredients (Selle and Ravindran, 2008). As expected, phytase released some of the P from phytate and the quantities of released P (between 0.04% and 0.10%, depending on the level of phytase in the diet) was within the range of values previously reported (Rojas and Stein, 2012; She et al., 2017). However, the released P in this experiment was less than sometimes observed because the ATTD and STTD of P in the control diets were greater than usual as discussed above, and there was, therefore, not as much opportunity to increase digestibility as in more practical diets.

Calcium and P must both be available in sufficient quantities for bone tissue synthesis to occur (Crenshaw, 2001). Because no dietary Ca was included in the experimental diets, absorbed P was not used to synthesize bone tissue, which resulted in an increase in P excretion in the urine as well as a decrease in P retention, calculated as a percentage of absorption, as dietary phytase levels increased. This observation demonstrates that the extra P that was absorbed as phytase was included in the diet had to be excreted in the urine due to the lack of Ca for bone tissue synthesis. Stein et al. (2006) reported a decrease in Ca retention in pigs fed a P-free diet compared with pigs fed diets containing field peas without or with phytase. Thus, an insufficient amount of Ca or P in diets will result in decreased retention of P or Ca, respectively.

The observation that phytase reduced endogenous losses of Ca and at the same time increased digestibility of P, indicates that less Ca is needed in diets containing microbial phytase because a greater proportion of both dietary Ca and reabsorbed endogenous Ca can be used for bone tissue synthesis. Because the response to increasing doses of microbial phytase on the endogenous loss of Ca was linear and exponential, it will be necessary to use different values for the reduction in the endogenous loss of Ca as the phytase dose is increased to capture the true value of phytase. Although the reductions in endogenous loss of Ca caused by phytase may seem small, it is important that these values are accounted for in diet formulations and that Ca inclusion is reduced accordingly because even a small excess of Ca will reduce growth performance of growing pigs (González-Vega et al., 2016a, 2016b; Merriman et al., 2017; Lagos et al., 2019a, 2019b). To quantify the effect of phytase on Ca digestibility it will, therefore, be necessary to use values for STTD Ca rather than ATTD values

Table 5. Phosphorus balance in growing pigs fed Ca-free diets[1,2]

Item, %	PC	NC (FTU/kg diet)						SEM	Contrast *P*-value		SE[3]	Exponential *P*-value
		0	250	500	1,000	2,000	4,000		PC vs. NC	Linear		
P intake, g/d	5.91	4.23	4.13	4.15	4.28	4.04	4.17	0.12	< 0.001	0.735	0.02	1.000
P in feces, %	2.30	2.15	1.55	1.22	1.02	1.00	0.74	0.04	0.009	< 0.001	0.0002	< 0.001
Fecal P output, g/d	1.42	1.38	1.01	0.85	0.75	0.64	0.50	0.08	0.685	< 0.001	0.0004	< 0.001
Absorbed P, g/d	4.48	2.85	3.12	3.30	3.52	3.40	3.68	0.14	< 0.001	< 0.001	0.0007	0.024
ATTD of P, %	75.96	67.49	75.48	79.63	82.28	84.12	87.97	1.95	< 0.001	< 0.001	0.0004	< 0.001
Digestible P in diet[4], %	0.50	0.31	0.35	0.37	0.38	0.39	0.41	0.01	< 0.001	< 0.001	0.0004	< 0.001
STTD of P[5], %	78.60	71.20	79.19	83.33	86.00	87.83	91.73	1.95	0.001	< 0.001	0.0004	< 0.001
Digestible P in diet[4], %	0.52	0.33	0.37	0.39	0.40	0.41	0.43	0.01	< 0.001	< 0.001	0.0004	< 0.001
P in urine, %	0.06	0.03	0.04	0.05	0.05	0.06	0.04	0.01	0.014	0.379	0.004	0.363
Urine P output, g/d	1.40	0.81	1.36	1.33	1.63	1.68	1.66	0.19	< 0.001	< 0.001	0.001	0.009
P retention, g/d	3.08	2.04	1.76	1.96	1.89	1.72	2.01	0.29	< 0.001	0.845	-	Not good fit
P retention, % of intake	52.25	48.22	42.53	47.31	44.05	42.47	47.71	6.00	0.338	0.808	-	Not good fit
P retention, % of absorbed	68.45	71.20	56.26	59.10	53.28	50.26	53.95	6.45	0.552	0.006	0.003	0.108

[1]ATTD, apparent total tract digestibility; STTD, standardized total tract digestibility; FTU, phytase unit; PC, positive control; NC, negative control.
[2]Least squares means represent 10 observations.
[3]Standard error for the exponential model.
[4]Digestible P was calculated by multiplying concentration of P in each diet by the ATTD or STTD of P.
[5]Calculated by correcting the ATTD of P for the average basal endogenous loss of P (i.e., 190 mg/kg DMI; NRC, 2012).

in diet formulation because values for STTD of Ca are additive in mixed diets. In addition, it is important to reduce the provision of dietary Ca as the dose of phytase is increased to avoid the negative effects of oversupplying Ca in the diets. However, by reducing Ca in diets containing microbial phytase, fecal excretion of P due to formation of Ca-P complexes will be reduced (Stein et al., 2011), and the provision of P can, therefore, also be reduced. Future research should be directed at quantifying the effects of increasing dietary provisions of microbial phytase on the STTD of both Ca and P.

Conclusion

The ATTD and STTD of P increased by increasing phytase doses in low-P Ca-free diets, but dietary concentration of P did not affect basal endogenous loss of Ca in pigs fed Ca-free diets. However, increasing dietary phytase reduced the basal endogenous loss of Ca. This indicates that phytate and endogenous Ca form insoluble complexes in the gastro intestinal tract of pigs, but if phytase is included in the diet, phytate is hydrolyzed by phytase before it forms an insoluble complex with Ca, which results in the reduced endogenous loss of Ca. Consideration should be given to the effect of dietary phytase on basal endogenous loss of Ca when formulating diets for pigs because the increased absorption of Ca caused by phytase indicates that dietary Ca can be reduced if phytase is added to the diet, which may increase P digestibility.

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Conflict of Interest Statement

Y.D.L. and J.R. are employees at Danisco-IFF, a global supplier of exogenous phytase. M.E.N., S.A.L., and H.H.S. have no conflicts of interests.

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