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## NON RUMINANT NUTRITION

Formulation of diets for pigs based on a ratio between digestible calcium and digestible phosphorus results in reduced excretion of calcium in urine without affecting retention of calcium and phosphorus compared with formulation based on values for total calcium

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## Abstract

An experiment was conducted to test the hypothesis that formulating diets for pigs based on a ratio between standardized total tract digestible (STTD) Ca and STTD P instead of total Ca and STTD P does not decrease Ca retention, but increases P utilization. Forty barrows (59.4 ± 3.8 kg) were individually housed in metabolism crates and allotted to four corn-soybean meal-based diets in a randomized complete block design with two blocks and five pigs per diet in each block. Diets were formulated using a 2 × 2 factorial design with two diet formulation principles (total Ca or STTD Ca) and two inclusion levels of microbial phytase (0 or 500 units per kg of feed). Phytase was assumed to release 0.11% STTD P and 0.16% total Ca. Diets were formulated based on requirements for total Ca and STTD P or a ratio between STTD Ca and STTD P of 1.25:1. Diets were fed for 11 d and fecal and urine samples were collected from feed provided from day 6 to day 10. Interactions (P < 0.05) between diet formulation principle and phytase level were observed for Ca intake, Ca in feces, Ca absorbed, Ca retained, P digestibility, P absorbed, and P in urine. Phytase increased (P < 0.05) the digestibility of Ca in both total Ca and STTD Ca diets. Without phytase, Ca intake, Ca in feces, and Ca absorbed was greater (P < 0.05) from pigs fed total Ca diets than from pigs fed STTD Ca diets, but P absorbed, P digestibility, and P in urine was greater (P < 0.05) from pigs fed STTD Ca diets than from pigs fed total Ca diets. However, in the presence of phytase, no differences between diet formulation principles were observed in these variables. Regardless of phytase, Ca in urine was lower (P < 0.05) from pigs fed STTD Ca diets than from pigs fed total Ca diets. There were no differences in Ca retention between pigs fed STTD Ca diets and total Ca diets, but pigs fed total Ca diets retained less (P < 0.05) Ca if diets contained phytase. No differences in P retention were observed between diet formulation principles, but pigs fed non-phytase diets retained more (P < 0.05) P than pigs fed diets with phytase. In conclusion, because diets formulated based on STTD Ca contain less Ca than total Ca diets, pigs fed STTD Ca diets excreted less Ca in urine, but retention of Ca was not affected. Formulating non-phytase diets based on STTD Ca instead of total Ca increased P absorption, which confirms the detrimental effect of excess Ca on P digestibility. However, P retention was not improved if pigs were fed STTD Ca diets.

Key words: calcium, digestible calcium, phosphorus, phytase, pigs, retention

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Abbreviations	
ATTD	apparent total tract digestibility
BW	body weight
EPL	endogenous loss of P
FTU	phytase units per kilogram of feed
STTD	standardized total tract digestibility

#### Introduction

Requirements for P by pigs are expressed on the basis of standardized total tract digestibility (STTD) of P, but because of a lack of data for the digestibility of Ca in feed ingredients, Ca requirements are expressed on the basis of total Ca (NRC, 2012). However, it was recognized that a more accurate way to express Ca and P requirements may be to use a ratio between STTD Ca and STTD P (NRC, 2012). Therefore, research has been conducted to determine digestibility values in feed ingredients that contain Ca and to estimate the effect of phytase on the digestibility of Ca in ingredients fed to pigs (Stein et al., 2016). Data for the concentration of STTD Ca in feed ingredients have been used to formulate diets based on STTD Ca and to estimate Ca requirements expressed as a ratio between STTD Ca and STTD P by pigs from 11 to 130 kg (González-Vega et al., 2016c; Merriman et al., 2017; Lagos et al., 2019a, 2019b). Results from these studies indicated that ratios for STTD Ca to STTD P that maximize bone mineralization are greater than those that maximize growth performance, and it was suggested that to maximize Ca retention, a ratio greater than the ratio that maximizes bone mineralization is needed (González-Vega et al., 2016a, 2016c). Therefore, it is important to not only evaluate the effect of using STTD Ca:STTD P ratios that maximize growth performance on bone mineralization, but also on Ca retention. Another observation from requirement studies was that excess dietary Ca has detrimental effects on growth performance of pigs, which is likely because excess Ca reduces P digestibility (Stein et al., 2011). Excess Ca may, therefore, induce a P deficiency and result in decreased feed intake (Sørensen et al., 2018). Thus, the use of STTD Ca to STTD P ratios in diet formulation may prevent oversupplying Ca in diets. Therefore, the objective of this experiment was to test the hypothesis that formulating diets for growing pigs based on a ratio between STTD Ca and STTD P instead of values for total Ca and STTD P does not decrease Ca retention, but increases P utilization.

### **Materials and Methods**

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

#### Animals, housing, and diets

Forty barrows [body weight (BW):  $59.4 \pm 3.8$  kg] were randomly allotted to four diets and two blocks in a randomized complete block design with 20 pigs per block. Each block had five replicate pigs per diet for a total of 10 replicate pigs per diet in the two blocks. Pigs were individually housed in metabolism crates that were equipped with a feeder, a nipple drinker, and a slatted floor to allow for the total, but separate, collection of urine and fecal materials. Pigs had free access to water throughout the experiment.

Diet formulation followed a 2 × 2 factorial design with two diet formulation principles (based on requirements for total Ca or for STTD Ca) and two inclusion levels of microbial phytase [0 or 500 phytase units per kg of feed (FTU)]. Therefore, four corn-soybean meal based diets were formulated (Table 1). Requirements for total Ca and STTD P were based on requirements for 50- to 75-kg pigs (NRC, 2012) and requirements for STTD Ca were calculated by multiplying the requirement for STTD P by 1.25 according to Lagos et al. (2019b). As a consequence, diets 1 and 2 contained no phytase and were formulated based on either the requirement for total Ca (0.59%) or the calculated requirement for STTD Ca (0.34%). Both diets were formulated to contain 0.27% STTD P. Diets 3 and 4 both contained 500 FTU of microbial phytase (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK). Diet 3 was formulated based on the same requirements for total Ca and STTD P as diet 1, with the exception that it was assumed that phytase would release 0.16% total Ca and 0.11% STTD P. This diet was, therefore, calculated to contain 0.43% total Ca and 0.16% STTD P. Diet 4 was formulated as diet 2 with the exception that the release of STTD P by phytase was taken into account so 0.16% STTD P was included in this diet as was the case for diet 3. The provision of STTD Ca in diet 4 was calculated as in diet 2, i.e., by multiplying STTD P by 1.25, after taking the assumed release of STTD P by phytase into account, so the provision of STTD Ca was calculated by multiplying 0.27 by 1.25. Values for STTD Ca in the ingredients were from Stein et al. (2016). Values obtained in the absence of phytase were used to formulate diet 2 and values for STTD Ca in the presence of phytase were used to formulate diets for diet 4 (Table 2). Because phytase does not increase the STTD of Ca in monocalcium phosphate, values for STTD of Ca in monocalcium phosphate was the same without and with phytase, whereas the STTD values for corn, soybean meal, and calcium carbonate were greater in the presence of phytase than without phytase. Using these formulation principles, it turned out that concentrations of total and STTD Ca in diets 3 and 4 were practically identical, although calculated using different formulation principles. All diets were formulated to have identical concentrations of net energy, Na, Cl, K, and vitamin D.

#### Feeding and sample collection

Pigs were fed three times the daily maintenance energy requirement (i.e., 197 kcal of metabolizable energy per kg of BW<sup>0.60</sup>; NRC, 2012). The daily allotments of feed were divided into two equal meals that were provided at 0800 and 1600 h. Pigs were fed each diet for 11 d. The initial 5 d were considered the adaptation period to the diets and fecal samples were collected quantitatively from the feed provided on d 6, 7, 8, and 9 using the marker-tomarker approach (Kong and Adeola, 2014). The beginning of fecal collections was marked by adding a color marker (indigo carmine) to the morning meal on d 6, and the conclusion of fecal collection was marked by adding ferric oxide to the morning meal on d 10. Urine was collected in urine buckets that contained 50 mL of 6N HCl and urine collections started after feeding the morning meal on d 6 and ceased after feeding the morning meal on d 10. Fecal samples and 20% of the collected urine was stored at -20 °C. At the conclusion of the experiment, fecal samples and orts were dried at 65 °C in a forced air oven. Fecal samples were then finely ground through a 1-mm screen before analysis using a Wiley mill (Model 4; Thomas Scientific, Swedesboro, NJ).

## Sample analysis

Ingredient, diet, urine, and fecal samples were analyzed for Ca and P by inductively coupled plasma-optical emission **Table 1.** Ingredient composition and calculated and analyzed values of experimental diets formulated based on total Ca or standardized total tract digestible (STTD) Ca, without microbial phytase or with 500 units of microbial phytase per kilogram of feed (FTU)

Item Phytase inclusion:	0 1	FTU	500 FTU		
Ca requirement:	Total Ca	STTD Ca	Total Ca	STTD Ca	
Ingredients, %					
Ground corn	76.22	76.89	77.68	77.74	
Soybean meal, 48% CP1	18.50	18.50	18.50		
Choice white grease	2.50	2.18	1.78	1.75	
Calcium carbonate	1.00	0.65	0.86	0.83	
Monocalcium	0.76	0.76	0.15 0.		
phosphate					
Sodium bicarbonate	0.10	0.10	0.10	0.10	
L-Lys HCl, 78% Lys	0.28	0.28	0.28	0.28	
DL-Met	0.03	0.03	0.03	0.03	
L-Thr	0.06	0.06	0.06	0.06	
Sodium chloride	0.40	0.40	0.40	0.40	
Vitamin mineral	0.15	0.15	0.15	0.15	
premix <sup>2</sup>					
Phytase concentrate <sup>3</sup>	-	-	0.01	0.01	
Calculated values					
Ca, %	0.59	0.45	0.43	0.42	
P, %	0.49	0.49	0.36	0.37	
Phytate, %	1.00	1.00	1.00	1.00	
STTD Ca⁴, %	0.43	0.34	0.35	0.34	
STTD P4, %	0.27	0.27 0.27		0.27	
STTD Ca:STTD P	1.59	1.59 1.25 1.		1.25	
Analyzed values					
Gross energy, kcal/kg	3,988	3,977	3,969	3,970	
Dry matter, %	89.42	88.10	87.83	87.82	
Ash,%	3.77	3.76	3.43	3.64	
CP, %	13.93	.93 14.22 14.19		13.62	
AEE, <sup>1</sup> %	5.24 4.24		4.28	4.36	
Ca, %	0.61	0.48	0.46	0.47	
Total P, %	0.52	0.53	0.38	0.39	
Phytate-bound P, %	0.25	.25 0.24		0.25	
Phytate,⁵ %	0.89	0.85	0.89	0.89	
Non-phytate P,6 %	0.27	0.30 0.13		0.14	
Phytase activity, FTU	< 50	< 50	567	464	

<sup>1</sup>AEE = acid hydrolyzed ether extract; CP = crude protein. <sup>2</sup>The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D<sub>3</sub> as cholecalciferol, 2,210 IU; vitamin E as <sub>DL</sub> alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin,6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B<sub>12</sub>, 0.03 mg; <sub>D</sub> pantothenic acid as <sub>D</sub> calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper sulfate; Fe, 125 mg as ironsulfate; I, 1.26mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganous sulfate; Se, 0.30mg as sodium selenite and selenium yeast; and Zn, 125.1mg as zinc sulfate.

<sup>3</sup>The phytase concentrate contained 5,000 units of phytase/g (Quantum blue, AB Vista, Marlborough, UK).

<sup>4</sup>Values for STTD Ca and STTD P in the phytase diets include expected release of Ca and P by phytase.

<sup>5</sup>Phytate was calculated by dividing the phytate-bound P by 0.282 (Tran and Sauvant, 2004).

 $^6\mathrm{Non-phytate}$  P was calculated as the difference between total P and phytate-bound P.

spectrometry (Method 985.01 A, B, and C; AOAC Int., 2007) after wet ash sample preparation [Method 975.03 B(b); AOAC Int., 2007]. Diets and ingredients were analyzed for dry matter

(Method 930.15; AOAC Int., 2007) and ash (Method 942.05; AOAC Int., 2007). Diets were also analyzed for acid hydrolyzed ether extract (Method 2003.06; AOAC Int., 2007) using 3 N HCl in an Ankom<sup>HCl</sup> hydrolyzer and petroleum ether in an Ankom<sup>XTIS</sup> extractor (Ankom Technology, Macedon, NY), gross energy using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL), and N (method 990.03; AOAC Int., 2007) using a LECO FP628 (LECO Corp., Saint Joseph, MI); crude protein was calculated as N × 6.25. Phytate-bound P in diets and ingredients was predicted by near infra-red reflectance spectroscopy using AUNIR calibration standards (AB Vista, Plantation, FL). Phytase activity was analyzed in diets by the ELISA method using Quantiplate Kits for Quantum Blue (Enzyme Services and Consultancy Ltd, Ystrad Mynach, UK).

#### Calculations and statistical analyses

Dietary concentration of phytate was calculated by dividing the analyzed phytate-bound P by 0.282 (Tran and Sauvant, 2004) and non-phytate P was then calculated by subtracting the amount of phytate-bound P from the concentration of total P. Feed intake was calculated by subtracting the weight of dried orts from feed provisions. The apparent total tract digestibility (ATTD) of Ca and P in diets was calculated (Almeida and Stein, 2010) and values for STTD of P were calculated by correcting the ATTD values for the basal endogenous loss of P (EPL; i.e.,190 mg/kg dry matter intake; NRC, 2012). Values for retention of Ca and P were calculated as explained by González-Vega et al. (2013).

Normality of residuals and model assumptions were tested using INFLUENCE, PROC GPLOT, and PROC UNIVARIATE options of SAS (SAS Inst. Inc., Cary, NC). Data for Ca and P balance were analyzed using the PROC MIXED of SAS with the experimental unit being the pig. The model included diet formulation principle (total Ca or STTD Ca) and phytase inclusion (0 or 500 FTU), and the interaction between diet formulation principle and phytase inclusion. The model also included the random effects of block and replicate within block. Treatment means were calculated using the LSMEANS statement and means were separated using the PDIFF option in SAS. Statistical significance and tendency were considered at P < 0.05 and  $0.05 \le P < 0.10$ , respectively.

### **Results**

Pigs remained healthy throughout the experiment and consumed the diets without apparent problems. No interaction between diet formulation principle and phytase inclusion was observed for daily feed intake, ATTD of Ca, daily urine output, percentage and quantity (grams per day) of Ca in urine, or Ca retention as a percentage of intake (Table 3). For daily feed intake and urine output, no effect of diet formulation principle or phytase inclusion was observed, but pigs fed diets formulated based on total Ca had an increased (P < 0.05) percentage and quantity (grams per day) of Ca excreted in the urine compared with pigs fed diets formulated based on a ratio between STTD Ca and STTD P. The ATTD of Ca and the retention of Ca as a percentage of intake were greater (P < 0.05) in diets with phytase than in diets without phytase, and the retention of Ca as a percentage of intake was greater (P < 0.05) in diets based on a ratio between STTD Ca and STTD P than in total Ca based diets. Pigs fed diets without phytase had greater Ca intake if diets were formulated based on total Ca compared with diets formulated based on a ratio between STTD Ca and STTD P, but there were no differences between the two diet formulation principles for Ca intake in pigs fed diets with phytase (interaction, P < 0.01). Pigs fed diets formulated based on total Ca had reduced fecal output and reduced Ca retention (grams per day) if phytase was included in the diet compared with pigs fed diets without phytase, but for pigs fed diets formulated based on a ratio between STTD Ca and STTD P, no differences in fecal output or Ca retention between the two inclusion levels of phytase were observed (interaction, P < 0.05). For pigs fed diets with phytase, there were no differences in the percentage and quantity (grams per day) of Ca in feces or the quantity of Ca absorbed between the two diet formulation principles, but if phytase was not used, pigs fed diets formulated based on a ratio between STTD Ca and STTD P had a reduced (P < 0.05) quantity of Ca absorbed, and reduced percentage and quantity (grams per day) of Ca in feces compared with pigs fed diets formulated based on total Ca (interaction, P < 0.05). Pigs fed diets formulated based on total Ca had lower Ca intake, Ca absorption (grams per day), and percentage and quantity (grams per day) of Ca in feces, if phytase was used, but there was no effect of inclusion of phytase on these variables if pigs were fed diets formulated based on a ratio between STTD Ca and STTD P (interaction, P < 0.05).

Pigs fed diets without phytase had greater (P < 0.05) P intake and P in feces (percent and grams per day) than pigs fed diets with phytase (Table 4). Pigs fed diets formulated based on total Ca tended (P < 0.10) to ingest less P (grams) daily than pigs fed diets formulated based on a ratio between STTD Ca and STTD P. Pigs fed diets without phytase had greater ATTD of P, STTD of P, absorbed P (grams per day), and percentage and quantity (grams per day) of P in urine if diets were formulated based on a ratio between STTD Ca and STTD P compared with pigs fed diets formulated based on total Ca. However, no differences between the two diet formulation principles were observed for pigs fed diets containing phytase (interaction, P < 0.05). The ATTD of P was greater, but the quantity (grams per day) of P absorbed was

Table 2. Analyzed composition and calculated values for total and standardized total tract digestible (STTD) Ca and P in feed ingredients, as fed basis

Item	Corn	Soybean meal	Calcium carbonate	Monocalcium phospha	
Analyzed values					
Dry matter, %	84.50	88.36	99.96	92.15	
Ash, %	1.34	6.94	92.65	81.23	
Ca, %	0.03	0.35	38.96	17.70	
P, %	0.30	0.78	0.04	20.91	
Phytate-bound P, %	0.13	0.45	-	-	
Calculated values					
Phytate,1 %	0.46	1.60	-	-	
Non-phytate P,² %	0.16	0.24	-	-	
Total Ca, %	0.020	0.33	38.20	17.10	
Total P, %	0.26	0.71	0.02	21.00	
STTD without phytase					
Ca, %	Ca, % 0.013		26.74	14.71	
P, %	0.09	0.34	0.012	18.48	
STTD with phytase					
Ca, %	0.015	0.26	30.56	14.71	

<sup>1</sup>Phytate was calculated by dividing phytate-bound P by 0.282 (Tran and Sauvant, 2004). <sup>2</sup>Non-phytate P was calculated as the difference between total P and phytate-bound P.

without or with 500 units of microbial phytase per kilogram of feed (FTU)<sup>1,2</sup>

Table 3. Calcium balance for pigs fed diets formulated based on requirements for total Ca or standardized total tract digestible (STTD) Ca,

Item Phytase:	0 FTU		500 FTU			P-value		
Requirement:	ement: Total Ca STTD Ca Total Ca SEM	SEM	Req.	Phytase	Req. × phytase			
Feed intake, g/d	2,134	2,162	2,168	2,169	31.2	0.632	0.523	0.672
Ca intake, g/d	12.91ª	10.43 <sup>b</sup>	9.86°	10.29 <sup>bc</sup>	0.165	< 0.001	< 0.001	< 0.001
Dry fecal output, g/d	216.3ª	196.2 <sup>ab</sup>	195.4 <sup>b</sup>	211.1 <sup>ab</sup>	7.94	0.775	0.694	0.023
Ca in feces, %	2.36ª	1.77 <sup>b</sup>	1.58 <sup>b</sup>	1.51 <sup>b</sup>	0.126	0.013	< 0.001	0.042
Ca output in feces, g/d	5.07ª	3.45 <sup>b</sup>	3.06 <sup>b</sup>	3.16 <sup>b</sup>	0.249	0.004	< 0.001	0.001
ATTD of Ca, %	60.77	66.88	69.16	69.34	2.089	0.141	0.014	0.164
Ca absorbed, g/d	7.84ª	6.97 <sup>b</sup>	6.81 <sup>b</sup>	7.13 <sup>b</sup>	0.226	0.241	0.062	0.012
Urine output, g/d	3,983	4,180	4,469	4,456	819.2	0.909	0.638	0.896
Ca in urine, %	0.014	0.005	0.008	0.005	0.0030	0.035	0.262	0.352
Ca in urine, g/d	0.41	0.15	0.30	0.18	0.059	0.003	0.521	0.231
Ca retention, % of intake	57.58	65.46	66.14	67.58	1.997	0.025	0.011	0.116
Ca retained, g/d	7.43ª	6.82 <sup>ab</sup>	6.51 <sup>b</sup>	6.95 <sup>ab</sup>	0.224	0.707	0.084	0.025

<sup>a-c</sup>Means within a row lacking a common superscript letter are different (P < 0.05).

<sup>1</sup>Each least squares mean represents 10 observations.

<sup>2</sup>Values for daily intake and output are the average of a 4-d collection.

Item <sup>3</sup> Phytase:	0 FTU		500 FTU			P-value		
Requirement: Total Ca	Total Ca	STTD Ca	Total Ca	STTD Ca	SEM	Req.	Phytase	Req. × phytase
Daily DMI, g/d	1,908	1,905	1,904	1,905	27.6	0.970	0.939	0.936
P intake, g/d	11.10	11.46	8.24	8.46	0.150	0.058	< 0.001	0.642
P in feces, %	2.33	2.34	1.49	1.47	0.075	0.910	< 0.001	0.837
P output in feces, g/d	4.99	4.56	2.91	3.09	0.176	0.426	< 0.001	0.101
ATTD of P, %	55.06°	60.17 <sup>b</sup>	64.66ª	63.51 <sup>ab</sup>	1.616	0.170	< 0.001	0.034
EPL4, mg/d	362.6	362.0	361.7	362.0	5.25	0.975	0.940	0.936
STTD of P, %	58.33°	63.33 <sup>b</sup>	69.05ª	67.79ª	1.616	0.195	< 0.001	0.034
P absorbed, g/d	6.11 <sup>b</sup>	6.90ª	5.32°	5.37°	0.198	0.017	< 0.001	0.033
P in urine, %	0.006 <sup>b</sup>	0.025ª	0.006 <sup>b</sup>	0.006 <sup>b</sup>	0.0042	0.048	0.030	0.030
P in urine, g/d	0.16 <sup>b</sup>	0.62ª	0.17 <sup>b</sup>	0.19 <sup>b</sup>	0.070	< 0.001	< 0.001	< 0.001
P retention, % of intake	53.65	54.79	62.54	61.22	1.397	0.949	< 0.001	0.385
P retained, g/d	5.95	6.28	5.15	5.18	0.162	0.268	< 0.001	0.354

Table 4. Phosphorus balance for pigs fed diets formulated based on requirements for total Ca or standardized total tract digestible (STTD) Ca, without or with 500 units of microbial phytase per kilogram of feed (FTU)<sup>1,2</sup>

<sup>a-c</sup>Means within a row lacking a common superscript letter are different (P < 0.05).

<sup>1</sup>Each least squares mean represents 10 observations.

<sup>2</sup>Values for daily intake and output are the average of a 4-d collection.

<sup>3</sup>DMI = dry matter intake; EPL = endogenous loss of P.

<sup>4</sup>Calculated by multiplying the EPL (190 mg/kg DMI; NRC, 2012) by the daily DMI.

lower when phytase was included in diets formulated based on total Ca, but if diets were formulated based on a ratio between STTD Ca and STTD P, there was no effect of phytase on the ATTD of P, and the quantity of P absorbed was lower if 500 FTU of phytase were included in the diet than if no phytase was used (interaction, P < 0.05). Pigs fed diets formulated based on a ratio between STTD Ca and STTD P had reduced percentage and quantity (grams per day) of P in urine if phytase was included in the diet compared with pigs fed no phytase, but no differences between the two inclusion levels of phytase were observed in pigs fed diets based on total Ca (interaction, P < 0.05). Pigs fed diets with phytase retained less (P < 0.05) P (grams per day) daily, but had a greater (P < 0.05) retention of P as a percentage of intake, than pigs fed diets without phytase.

### Discussion

Diets for growing-finishing pigs may be formulated based on STTD Ca values because data for the digestibility of Ca in Ca-containing feed ingredients have been generated from diets without or with microbial phytase (Stein et al., 2016). This also allowed for the estimation of requirements for a ratio between STTD Ca and STTD P by growing pigs, which is believed to be a more appropriate way to express Ca requirements (NRC, 2012). Therefore, STTD Ca:STTD P requirements to optimize growth performance of 11- to 25-kg (Lagos et al., 2019a), 25- to 50-kg (González-Vega et al., 2016c), 50- to 85-kg (Lagos et al., 2019b), and 100- to 130-kg pigs (Merriman et al., 2017) have been determined. These values were recently validated in diets without microbial phytase or with 500 FTU of microbial phytase when fed to pigs from 11 to 130 kg (Lagos et al., 2021).

The four diets used in this experiment were formulated using NRC (2012) values for Ca and P in corn, soybean meal, calcium carbonate, and monocalcium phosphate. The analyzed values for Ca and P in these feed ingredients were slightly greater than those used in diet formulation, which is likely the reason for the differences between calculated and analyzed values in diets. However, the expected differences or similarities in concentrations of Ca and P among the four diets were obtained. The reason the two diets with phytase turned out to be practically identical regardless of the diet formulation principle used, is that in formulation of the diet based on total Ca, it was assumed that phytase would release 0.16% total Ca, and the provision of Ca was, therefore, reduced by that amount (i.e., from 0.59 to 0.43). Taken the digestibility of Ca in the four ingredients into account (in the presence of phytase) the calculated STTD Ca in this diet was 0.35%. Using actual STTD values for the four ingredients in diet formulation, instead of using values for total Ca, and assuming the requirement for STTD Ca was 1.25 times the requirement for STTD P, resulted in a calculated requirement of 0.34% STTD Ca and a provision of 0.42% total Ca. As a consequence, the provisions of total Ca and STTD Ca in the two diets with phytase turned out to be almost the same although calculated in very different ways. This indicates that the assumed release of total Ca by phytase likely was accurate. However, because of the similarities between the two diets with phytase, it was not surprising that no differences between these diets were observed.

The ability of microbial phytase to release P from phytate in ingredients of plant origin and to increase P digestibility in diets fed to pigs (She et al., 2017) was evidenced in this experiment by the increase in the ATTD and STTD of P in diets with 500 FTU of microbial phytase compared with diets without phytase. Values for the digestibility of P in diets obtained in this experiment concur with data from Zeng et al. (2016) and Archs Toledo et al. (2020) where corn-soybean mealbased diets with 500 FTU of microbial phytase were fed to 19 and 40 kg-pigs, respectively. The fact that phytase also releases Ca bound to phytate in plant feed ingredients (González-Vega et al., 2013) and from limestone (González-Vega et al., 2015; Lee et al., 2019) was demonstrated in this experiment by the increased ATTD of Ca in diets containing phytase compared with diets without phytase. Similar observations have been reported in the past (Almeida et al., 2013; Arredondo et al., 2019; Archs Toledo et al., 2020).

Maximizing bone mineralization requires more Ca than maximizing growth performance of pigs (Crenshaw, 2001), but the amount of dietary Ca needed to optimize Ca retention in pigs is greater than the amount needed to optimize bone mineralization (González-Vega et al., 2016a, 2016c). Therefore, it was hypothesized that feeding diets based on a ratio between STTD Ca and STTD P that contained less Ca than diets based on total Ca, especially when microbial phytase was not included, would not affect the retention of Ca by pigs. As expected, regardless of the inclusion of phytase there were no differences in Ca retention between pigs fed diets based on total Ca and pigs fed diets based on a STTD Ca to STTD P ratio. The decreased concentration (percentage and grams per day) of Ca in urine from pigs fed diets formulated based on a ratio between STTD Ca and STTD P compared with pigs fed diets based on total Ca is the reason there was no difference between the two diet formulation principles for retention of Ca despite the greater Ca concentration in diets formulated based on total Ca. This observation indicates that if diets are formulated on calculated ratios between STTD Ca and STTD P, less Ca is needed in the diet. The reason there was no difference in Ca retention between the two diets containing phytase likely is that although formulated based on different principles, the two diets contained similar quantities of Ca.

The observation that the ATTD of P, STTD of P, and the quantity of P absorbed by pigs fed diets formulated on a ratio between STTD Ca and STTD P was greater than by pigs fed diets based on total Ca if microbial phytase was not used, concurs with data indicating that increasing concentration of dietary Ca reduces P digestibility (Stein et al., 2011; González-Vega et al., 2016b; Velayudhan et al., 2019). This is likely a result of formation of indigestible complexes between Ca and P in the gastrointestinal tract of pigs. Therefore, by reducing the concentration of dietary Ca (0.35 percentage units less calcium carbonate), as is the case for the non-phytase diets based on a ratio between STTD Ca and STTD P compared with total Ca based diets, less P is bound by Ca in the digestive system of pigs, which results in increased ATTD and STTD of P. However, data from this experiment also indicate that pigs fed diets without phytase and based on a STTD Ca to STTD P ratio absorbed more P than needed because these pigs excreted more P in urine than pigs fed total Ca based diets. Because both Ca and P are needed in adequate concentrations in the diet for bone mineralization to occur (Crenshaw, 2001), pigs fed diets with reduced concentration of Ca absorbed more P, but did not synthesize more bone tissue, which is likely because they already met the requirement. As a consequence, there were no differences in P retention between pigs fed diets formulated on the two diet principles. This indicates that less P may be needed in diets formulated on the basis of a STTD Ca to STTD P ratio, but research needs to be conducted to validate this hypothesis.

The negative effect of the inclusion of microbial phytase on retention of P regardless of the diet formulation principle indicates that the assumed value of 0.11% STTD P release by phytase may have been overestimated. However, the observation that there are no differences in Ca and P balance between pigs fed diets with phytase based on total Ca and a STTD Ca to STTD P ratio was expected because these diets only had 0.03 percentage units difference in calcium carbonate. This observation also indicates that the STTD values for Ca used in this experiment for diet formulation were accurate. The current data also indicate that ratios between STTD Ca and STTD P can be used to formulate diets without or with microbial phytase, as demonstrated by the lack of differences in the Ca balance of pigs.

#### **Conclusions**

Calcium retention was not affected by diet formulation principle because pigs fed diets based on a ratio between STTD Ca and STTD P ingest and absorb less Ca than pigs fed total Ca based diets, and also excrete less Ca in urine. In diets without microbial phytase, formulating diets based on a ratio between STTD Ca and STTD P improved the ATTD of P and the quantity of P absorbed by pigs, which confirms the negative effect of excess Ca on P digestibility. However, P in urine was increased and P retention was not improved in these pigs, which indicates that less P is needed in diets formulated on a ratio between STTD Ca and STTD P. A value of 0.16% total Ca released by phytase or STTD Ca values for each ingredient can be used for diet formulation to account for the Ca-releasing effect of phytase.

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### **Conflict of Interest Statement**

MRB is an employee at AB Vista, a global supplier of microbial phytase. However, LVL, SAL, and HHS have no conflicts of interest.

#### **Literature Cited**

- Almeida, F. N., and H. H. Stein. 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. doi:10.2527/jas.2009-2285
- Almeida, F. N., R. C. Sulabo, and H. H. Stein. 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. doi:10.1186/2049-1891-4-8
- AOAC Int. 2007. Official methods of analysis of AOAC int. 18th Rev. 2nd ed. Gaithersburg, MD: AOAC Int.
- Archs Toledo, J. L., S. A. Lee, M. L. McGhee, G. G. Mateos, and H. H. Stein. 2020. Intrinsic phytase in hybrid rye increases the digestibility of phosphorus in corn and soybean meal in diets fed to growing pigs. J. Anim. Sci. 98. doi:10.1093/jas/skaa295
- Arredondo, M. A., G. A. Casas, and H. H. Stein. 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. doi:10.1016/j.anifeedsci.2019.01.001
- Crenshaw, T. D. 2001. Calcium, phosphorus, vitamin D, and vitamin K in swine nutrition. In: A. J. Lewis, and L. L. Southern, editors. *Swine nutrition*. 2nd ed. CRC Press, Boca Raton, FL. p. 187–212.
- González-Vega, J. C., Y. Liu, J. C. McCann, C. L. Walk, J. J. Loor, and H. H. Stein. 2016a. Requirement for digestible calcium by eleven- to twenty-five-kilogram pigs as determined by growth performance, bone ash concentration, calcium and phosphorus balances, and expression of genes involved in transport of calcium in intestinal and kidney cells. J. Anim. Sci. 94:3321–3334. doi:10.2527/jas.2016-0444
- González-Vega, J. C., C. L. Walk, Y. Liu, and H. H. Stein. 2013. Endogenous intestinal losses of calcium and true total tract

digestibility of calcium in canola meal fed to growing pigs. J. Anim. Sci. **91**:4807–4816. doi:10.2527/jas.2013-6410

- González-Vega, J. C., C. L. Walk, M. R. Murphy, and H. H. Stein. 2016b. Effect of increasing concentrations of digestible calcium and digestible phosphorus on apparent total tract digestibility of calcium and phosphorus by pigs. J. Anim. Sci. 94(E-Suppl. 5):459 (Abstr.).
- González-Vega, J. C., C. L. Walk, M. R. Murphy, and H. H. Stein. 2016c. Requirement for digestible calcium by 25 to 50 kg pigs at different dietary concentrations of phosphorus as indicated by growth performance, bone ash concentration, and calcium and phosphorus balances. J. Anim. Sci. **94**:5272– 5285. doi:10.2527/jas.2016-0751
- González-Vega, J. C., C. L. Walk, and H. H. Stein. 2015. Effects of microbial phytase on apparent and standardized total tract digestibility of calcium in calcium supplements fed to growing pigs. J. Anim. Sci. **93**:2255–2264. doi:10.2527/jas.2014-8215
- Kong, C., and O. Adeola. 2014. Evaluation of amino acid and energy utilization in feedstuff for Swine and poultry diets. Asian-Australas. J. Anim. Sci. 27:917–925. doi:10.5713/ ajas.2014.r.02
- Lagos, L. V., M. R. Bedford, and H. H. Stein. 2021. Formulating diets based on digestible calcium instead of total calcium does not affect growth performance or carcass characteristics, but microbial phytase ameliorates bone resorption caused by low calcium in diets fed to pigs from 11 to 130 kg. J. Anim. Sci. 99:1–11. doi:10.2527/jas/skab057
- Lagos, L. V., S. A. Lee, G. Fondevila, C. L. Walk, M. R. Murphy, J. J. Loor, and H. H. Stein. 2019a. Influence of the concentration of dietary digestible calcium on growth performance, bone mineralization, plasma calcium, and abundance of genes involved in intestinal absorption of calcium in pigs from 11 to 22 kg fed diets with different concentrations of digestible phosphorus. J. Anim. Sci. Biotechnol. 10:47. doi:10.1186/ s40104-019-0349-2
- Lagos, L. V., C. L. Walk, M. R. Murphy, and H. H. Stein. 2019b. Effects of dietary digestible calcium on growth performance and bone ash concentration in 50- to 85-kg growing pigs fed diets with different concentrations of digestible phosphorus. Anim. Feed Sci. Technol. 247:262–272. doi:10.1016/j. anifeedsci.2018.11.019
- Lee, S. A., L. V. Lagos, C. L. Walk, and H. H. Stein. 2019. Standardized total tract digestibility of calcium varies among sources of calcium carbonate, but not among sources of dicalcium phosphate, but microbial phytase increases

calcium digestibility in calcium carbonate. J. Anim. Sci. 97:3440–3450. doi:10.1093/jas/skz176

- Merriman, L. A., C. L. Walk, M. R. Murphy, C. M. Parsons, and H. H. Stein. 2017. Inclusion of excess dietary calcium in diets for 100- to 130-kg growing pigs reduces feed intake and daily gain if dietary phosphorus is at or below the requirement. J. Anim. Sci. 95:5439–5446. doi:10.2527/jas2017.1995
- NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Washington, D.C.: Natl. Acad. Press.
- She, Y., D. Li, and S. Zhang. 2017. Methodological aspects of determining phosphorus digestibility in swine: a review. *Anim. Nutr.* 3:97–102. doi:10.1016/j.aninu.2017.02.003
- Sørensen, K. U., A. H. Tauson, and H. D. Poulsen. 2018. Long term differentiated phosphorus supply from below to above requirement affects nutrient balance and retention, body weight gain and bone growth in growing-finishing pigs. *Livest.* Sci. 211:14–20. doi:10.1016/j.livsci.2018.03.002
- Stein, H. H., O. Adeola, G. L. Cromwell, S. W. Kim, D. C. Mahan, and P. S. Miller; North Central Coordinating Committee on Swine Nutrition (NCCC-42). 2011. Concentration of dietary calcium supplied by calcium carbonate does not affect the apparent total tract digestibility of calcium, but decreases digestibility of phosphorus by growing pigs. J. Anim. Sci. 89:2139–2144. doi:10.2527/jas.2010-3522.
- Stein, H. H., L. A. Merriman, and J. C. González-Vega. 2016. Establishing a digestible calcium requirement for pigs. In: C. L. Walk, I. Kühn, H. H. Stein, M. T. Kidd, and M. Rodehutscord, editors. Phytate destruction - consequences for precision animal nutrition. Wageningen, The Netherlands: Wageningen Academic Publishers. p. 207–216.
- Tran, G., and D. Sauvant. 2004. Chemical data and nutritional value. In: D. Sauvant, J.-M. Perez, and G. Tran, editors. Tables of composition and nutritional value of feed materials: pigs, poultry, cattle, sheep, goats, rabbits, horses and fish. Wageningen, The Netherlands: Wageningen Academic Publishers. p. 17–24.
- Velayudhan, D. E., L. Marchal, and Y. Dersjant-Li. 2019. Metaanalysis: effect of increasing dietary calcium and phytase dose on phosphorus digestibility in weaning piglets and growing pigs. J. Anim. Sci. 97 (Suppl. 3):353–354 (Abstr.). doi:10.1093/jas/skz258.704
- Zeng, Z. K., Q. Y. Li, P. F. Zhao, X. Xu, Q. Y. Tian, H. L. Wang, L. Pan, S. Yu, and X. S. Piao. 2016. A new phytase continuously hydrolyzes phytate and improves amino acid digestibility and mineral balance in growing pigs fed phosphorous-deficient diet. J. Anim. Sci. 94:629–638. doi:10.2527/jas.2015-9143