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[1]

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ABSTRACT: Two experiments were conducted to test the hypothesis that standardized total tract digestibility (**STTD**) of Ca and the response to microbial phytase is constant among different sources of Ca carbonate and that the STTD of Ca is constant among different sources of dicalcium phosphate (**DCP**) when fed to growing pigs. In Exp. 1, 80 pigs (initial BW: 19.0 ± 1.9 kg) were randomly allotted to 10 diets and 2 blocks with 4 pigs per diet in each block. Four sources of Ca carbonate were used, and each source was included in a diet without microbial phytase and a diet with microbial phytase (500 units/kg diet). Two Ca-free diets without or with microbial phytase were also formulated. Feed allowance was 2.7 times the maintenance energy requirement for ME and daily feed allotments were divided into 2 equal meals. The initial 4 d of each period were considered the adaptation period to the diets followed by 4 d of fecal collection using the marker-to-marker procedure. Pigs fed diets containing exogenous phytase had lower ($P < 0.05$) basal endogenous loss of Ca compared with pigs fed diets containing no phytase. There were no interactions between phytase and source of Ca carbonate. Values for STTD of Ca were greater ($P < 0.05$) for diets containing microbial phytase (77.3% to 85.4%) compared with diets without exogenous phytase (70.6% to 75.2%), and values for STTD of Ca differed ($P < 0.05$) among the 4 sources of Ca carbonate. In Exp. 2, 40 pigs (initial BW: 14.9 ± 1.3 kg) were allotted to a completely randomized design with 5 diets and 8 replicate pigs per diet. A basal diet in which all Ca was supplied by Ca carbonate was formulated. Three diets were formulated by adding 3 sources of DCP to the basal diet and a Ca-free diet was also used. Feeding and collection methods were as described for Exp. 1. Results indicated that values for STTD of Ca and ATTD of P were not different among diets, indicating that under the conditions of this experiment, the digestibility of Ca and P in DCP appears to be constant regardless of origin of DCP. In conclusion, use of microbial phytase reduces the basal endogenous loss of Ca and increases Ca digestibility in Ca carbonate. The STTD of Ca varies among sources of Ca carbonate, regardless of phytase inclusion, but that appears not to be the case for the STTD of Ca in different sources of DCP.

Key words: calcium, calcium carbonate, dicalcium phosphate, digestibility, phytase, pigs

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INTRODUCTION

The concentration of Ca in most plant feed ingredients is low compared with the requirement for pigs, and Ca carbonate and dicalcium phosphate (**DCP**) are often used in diets for pigs to

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provide additional Ca. Although Ca is relatively inexpensive compared with other nutrients, excess dietary Ca may decrease P digestibility resulting in reduced feed intake and growth performance (Stein et al., 2011; González-Vega et al., 2016; Merriman et al., 2017; Blavi et al., 2018). Reduced digestibility of P may also result in increased excretion of P and possibly increase environmental pollution (Knowlton et al., 2004). Provisions of P and Ca are most correctly assessed as a ratio between digestible Ca and digestible P (González-Vega et al., 2016; Merriman et al., 2017). However, whereas the digestibility of P in most feed ingredients has been reported (NRC, 2012), the number of experiments in which the digestibility by pigs of Ca in feed ingredients was determined is limited (Stein et al., 2016). Therefore, determination of digestibility of Ca in dietary sources of Ca is needed.

Absorption of Ca may be estimated by the total tract digestibility procedure (González-Vega et al., 2014; Zhang et al., 2016), but Ca of endogenous origin is excreted in the feces along with undigested dietary Ca (González-Vega et al., 2013, 2014). Therefore, the concept of standardized total tract digestibility (**STTD**) of Ca that provides values for digestibility of Ca that are corrected for the basal endogenous loss of Ca has been introduced (Stein et al., 2016).

Values for STTD of Ca in one source of Ca carbonate and in one source of DCP were reported by González-Vega et al. (2015a), and it was demonstrated that microbial phytase increased the STTD of Ca in Ca carbonate, but not in DCP. However, different suppliers of inorganic sources of Ca may use different raw materials and different production processes and the concentration of Ca in Ca-containing ingredients may vary among suppliers (Petersen and Stein, 2006). It is, however, not known if differences in raw materials and production procedures among suppliers of inorganic Ca influence the STTD of Ca or the response to microbial phytase. Therefore, the objective of this work was to test the hypotheses that STTD of Ca and the response to microbial phytase are constant among sources of Ca carbonate and that the STTD of Ca is constant among sources of DCP when fed to growing pigs.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for 2 experiments. Pigs used in both experiments were the offspring of Line 359 boars and Camborough females (Pig Improvement Company, Hendersonville, TN).

Animals and Diets

Exp. 1. STTD of Ca in Ca carbonate. Four sources of Ca carbonate (A = Calpro, ILC Resources, Alden, IA; B = Fre-Fro, ILC Resources, Alden, IA; C = GMC-Shelter Limestone, Selter Creek Quarry LLC, Maplehill, NC; and D = Alix-US Lime, US Lime Co-St Clair, Marble City, OK) were procured from commercial companies in the United States (Table 1). Each source was included in 2 diets, i.e., one diet without microbial phytase and one diet with microbial phytase (500 phytase units/kg; Quantum Blue, AB Vista, Marlborough, UK). Eighty growing pigs with an

Table 1. Nutrient composition (as-fed basis) of feed ingredients, and particle size and in vitro solubility of Ca in Ca carbonate, Exp. 1

			Ca carbonate source					
Item, %	Corn	Potato protein concentrate	A	B	C	D	Mean	SD
Nutrient composition[1]								
DM	86.4	91.3	99.8	99.9	99.8	99.9	99.8	0.1
Ash, %	1.1	0.5	94.8	94.5	98.9	97.0	97.2	3.0
Ca, %	< 0.01	0.02	38.9	40.3	40.0	39.7	39.7	0.6
Total P, %	0.22	0.12	<0.01	<0.01	<0.01	<0.01	—	—
Phytate[2], %	0.69	0.32	—	—	—	—	—	—
Phytate P, %	0.20	0.09	—	—	—	—	—	—
Nonphytate P[3], %	0.02	0.03	—	—	—	—	—	—
Particle size, µm	—	—	435	469	407	97	352	172
Solubility, %	—	—	40.0	45.1	43.8	42.0	42.7	2.2

[1]Data for Ca and total P are averages of four samples that were analyzed in duplicate. All other data represent the average of one sample that was analyzed in duplicate.

[2]Phytate was calculated by dividing the analyzed phytate P by 0.282 (Tran and Sauvant, 2004).

[3]Nonphytate P was calculated as the difference between total P and phytate P.

initial BW of 19.0 ± 1.9 kg were randomly allotted to 10 diets and 2 blocks with 4 pigs per diet in each block for a total of 8 replicate pigs per diet. All diets were based on corn and potato protein concentrate (Table 2). Two Ca-free diets without or with microbial phytase (500 phytase units/kg) were also formulated. Amino acids calculated as standardized ileal digestible amino acids, vitamins, and minerals other than Ca in the Ca-free diets were included in all diets to meet current nutrient requirement estimates (NRC, 2012). The vitamin and mineral premix that was used did not contain Ca.

Exp. 2. STTD of Ca in DCP. Forty growing pigs with an average initial body weight of 14.9 ± 1.3 kg were allotted to a randomized complete block design with a total of 8 replicate pigs per diet. Five diets were used and all diets were formulated without exogenous phytase (Table 2). A basal diet in which all Ca was supplied by Ca carbonate was formulated. Three sources of commercial DCP were procured from three commercial suppliers in the United States (A = PCS, PCS Sales USA, Northbrook, IL; B = Ultra-Phos, Kay Dee, LLC, Sioux City, IA; and C = Simphos, J. R. Simplot Company, Boise, ID; Table 3), and 3 diets were formulated using each source of DCP. Calcium carbonate (Source A from Exp. 1) was also included in those diets to obtain a total Ca:STTD P ratio of 2.0:1.0, which is close to the requirement estimates for pigs (NRC, 2012). A Ca-free diet was used to determine the basal endogenous loss of Ca. Amino acids calculated as standardized ileal digestible amino acids, vitamins, and, with the exception of Ca in the Ca-free diet, minerals were included to meet current nutrient requirements (NRC, 2012).

Housing, Feeding, and Sample Collection

Pigs were housed individually in metabolism crates that were equipped with fully slatted floors, a feeder, and a cup waterer. A screen floor was installed below the slatted floor of the crates. Feed allowance was 2.7 times the maintenance energy requirement for ME for pigs (i.e., 197 kcal ME/kg $BW^{0.60}$). Water was available at all times. Daily feed allotments were divided into 2 equal meals that were provided at 0800 and 1600 hours.

The initial 4 d of each period were considered the adaptation period to the diets followed by 4 d 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 (Adeola, 2001). Fecal samples were stored at −20 °C as soon as collected.

Table 2. Ingredient composition of experimental diets (as-is basis), Exps. 1 and 2

	Exp. 1		Exp. 2		
Ingredient, %	Ca carbonate[1]	Ca-free[1]	Basal	DCP[2]	Ca-free
Corn	80.19	82.39	80.15	80.45	81.85
Potato protein concentrate	15.00	15.00	15.00	15.00	15.00
Ca carbonate	1.70	—	1.70	0.70	—
Dicalcium phosphate	—	—	—	1.80	—
Soybean oil	1.00	0.50	1.00	1.00	1.00
$_{L}$-Lys·HCl	0.35	0.35	0.35	0.35	0.35
$_{DL}$-Met	0.05	0.05	0.10	0.10	0.10
$_{L}$-Trp	0.05	0.05	0.05	0.05	0.05
Monosodium phosphate	1.10	1.10	1.10	—	1.10
Salt	0.40	0.40	0.40	0.40	0.40
Vitamin–mineral premix[3]	0.15	0.15	0.15	0.15	0.15
Phytase premix[4]	0.01	0.01	—	—	—

[1]Four sources of commercial Ca carbonate were each included in one diet without microbial phytase and in one diet with microbial phytase (500 phytase units/kg; Quantum Blue, AB Vista); the Ca-free diet was formulated without or with microbial phytase (500 phytase units/kg; Quantum Blue, AB Vista).

[2]Three sources of commercial DCP were used, and each source was included in one diet.

[3]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 D_3 as cholecalciferol, 2,210 IU; vitamin E as selenium yeast, 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 B_{12}, 0.03 mg; $_{D}$-pantothenic acid as $_{D}$-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26mg 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.

[4]The phytase premix (Quantum Blue, AB Vista) contained 5,000 units of phytase per gram; corn starch was used at the expense of the phytase premix in diets without microbial phytase.

Table 3. Nutrient composition of ingredients and particle size of dicalcium phosphate (as-is basis), Exp. 2

				Dicalcium phosphate source				
Item, %	Corn	Potato protein concentrate	Ca carbonate	A	B	C	Mean	SD
Nutrient composition[1]								
DM	86.6	91.0	100.0	94.9	94.0	95.1	94.7	0.6
Ash	1.2	0.4	94.2	84.1	81.5	83.3	83.0	1.3
Ca	<0.01	0.02	39.7	20.5	18.9	18.9	19.5	1.7
Total P	0.23	0.13	0.13	18.5	19.8	19.1	19.1	0.7
Phytate[2]	0.64	0.28	—	—	—	—	—	—
Phytate P	0.18	0.08	—	—	—	—	—	—
Nonphytate P[3]	0.05	0.05	0.13	—	—	—	—	—
Particle size, µm	—	—	—	508	1,079	465	684	343

[1]All samples were analyzed in duplicate except Ca and total P, which was analyzed in duplicate in four separate samples.

[2]Phytate was calculated by dividing the analyzed phytate P by 0.282 (Tran and Sauvant, 2004).

[3]Nonphytate P was calculated as the difference between total P and phytate P.

Chemical Analyses

At the conclusion of the experiments, fecal samples were dried at 65 °C in a forced air oven in Exp. 1, but fecal samples were lyophilized in Exp. 2. The dried fecal samples then were finely ground through a 1-mm screen using a Wiley Mill (Model 4; Thomas Scientific, Swedesboro, NJ). Based on the methods described in AOAC International (2007), Ca and P in feed ingredients, diets, and fecal samples were analyzed by inductively coupled plasma spectroscopy (Method 985.01 A, B, and C) after wet ash sample preparation [Method 975.03 B(b)]. Concentrations of Ca and P in all samples were analyzed in duplicate at University of Missouri Experiment Station Chemical Laboratory (Colombia, MO). Four separate samples of diets and ingredients and one sample of feces were analyzed in duplicate. Diets were analyzed for phytase activity (ESC, Ystrad Mynach, UK) by the ELISA method using Quantiplate Kits for Quantum Blue and feed ingredients were also analyzed for phytate P (Megazyme method; ESC, Ystrad Mynach, UK). Feed ingredients, diets, and fecal samples were analyzed for dry matter (DM; AOAC International, 2007; method 930.15). Feed ingredient and diet samples were also analyzed for ash (AOAC International, 2007; method 942.05). Particle size of Ca carbonate and DCP was measured (ASABE, 2008), and the in vitro solubility of the 4 sources of Ca carbonate was determined using the procedure described by Zhang and Coon (1997a).

Calculations

The apparent total tract digestibility (**ATTD**) of Ca and P in experimental diets was calculated using the following equation (Almeida and Stein, 2010):

$$\text{ATTD} = \frac{\text{intake} - \text{output}}{\text{intake}} \times 100, \quad (1)$$

where ATTD is in % and intake and output in feces are expressed as gram per day. Because all Ca in the Ca carbonate-containing diets was from Ca carbonate in Exp. 1, the ATTD of Ca in the Ca carbonate-containing diets was considered the ATTD of Ca in Ca carbonate.

The basal endogenous loss of Ca that was estimated as the total tract flow of Ca from pigs fed the Ca-free diets was expressed as mg/kg of DM intake (DMI) and was calculated using DMI in kilogram per day and Ca output in feces that was expressed as gram per day. The daily basal endogenous loss of Ca was calculated by multiplying the basal endogenous loss by the DMI of each pig.

Values for STTD of Ca (%) were calculated using the following equation (Almeida and Stein, 2010):

$$\text{STTD} = \frac{\text{intake} - (\text{output} - \text{daily basal endogenous loss})}{\text{intake}} \times 100, \quad (2)$$

where intake, output, and daily basal endogenous loss are in grams per day.

To calculate the ATTD and STTD of Ca in three sources of DCP used in Exp. 2, the contributions of Ca from Ca carbonate to the DCP-containing diets were calculated and the ATTD and STTD of Ca in each source of DCP were calculated using the difference procedure (Adeola, 2001).

Statistical Analysis

Normality of data was verified using the UNIVARIATE procedure (SAS Inst. Inc., Cary, NC), and outliers were identified as values that deviated from the first or third quartiles by more

than 3 times the interquartile range (Tukey, 1977). One outlier was identified in each experiment, and these outliers were excluded from the final statistical analysis. The pig was the experimental unit for all analyses. Data were analyzed using the PROC MIXED of SAS (SAS Institute Inc., Cary, NC). In Exp. 1, the statistical model included Ca source, phytase, and the Ca-source × phytase interaction as fixed effects and block and replicate within block as random effects. Mean separation was conducted by the PDIFF option with the Tukey's adjustment if an interaction was significant. In Exp. 2, the statistical model included diet or source of DCP as fixed effects and replicate as random effect. Mean separation was conducted by the PDIFF option with the Tukey's adjustment. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

RESULTS

Pigs remained healthy during both experiments and very little feed refusals were observed. Analyzed Ca and P in some diets in Exp. 1 varied slightly from calculated values, but analyzed Ca and P in the DCP-containing diets used in Exp. 2 were close to calculated values (Tables 4 and 5). To avoid confounding effects due to analytical discrepancies, calculated values for all diets were used for calculations of Ca and P digestibility values.

Exp. 1. STTD of Ca in Ca carbonate

Values for ATTD of DM did not differ between pigs fed Ca-free diets without or with microbial phytase (Table 6). However, fecal Ca excretion from pigs fed the Ca-free diet with microbial phytase was less ($P < 0.05$) than from pigs fed the Ca-free diet

Table 4. Analyzed composition of experimental diets (as-fed basis), Exp. 1

Item[1], %	0 phytase units/kg					500 phytase units/kg				
Ca carbonate source	A	B	C	D	Ca-free	A	B	C	D	Ca-free
DM	88.1	87.9	88.3	88.3	86.8	87.5	87.4	87.4	87.3	87.3
Ash	3.65	4.11	3.74	4.21	2.44	4.18	3.84	4.55	3.87	2.74
Ca	0.68	0.69	0.62	0.69	0.02	0.68	0.67	0.56	0.64	0.01
Total P	0.49	0.51	0.51	0.46	0.46	0.46	0.48	0.48	0.48	0.49
Phytate[2]	0.60	0.60	0.60	0.60	0.61	0.60	0.60	0.60	0.60	0.61
Phytate P[3]	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Nonphytate P[4]	0.32	0.34	0.34	0.29	0.29	0.29	0.31	0.31	0.31	0.32
Phytase activity[5]	<50	<50	<50	<50	<50	572	644	601	546	744

[1]Data are the average of duplicate analyses of one sample with the exception that data for Ca and total P are the average of four separate samples that were analyzed in duplicate.

[2]Phytate was calculated by dividing the analyzed phytate P by 0.282 (Tran and Sauvant, 2004).

[3]Phytate P values were calculated from analyzed phytate P in the ingredients.

[4]Nonphytate P was calculated as the difference between total P and phytate P.

[5]Phytase activity = phytase units/kg of diet.

Table 5. Analyzed composition of experimental diets (as-fed basis), Exp. 2

		Dicalcium phosphate source			
Item[1], %	Basal	A	B	C	Ca-free
DM	88.3	87.7	87.5	87.3	87.6
Ash	4.53	4.06	3.56	3.49	3.42
Ca	0.68	0.69	0.62	0.61	0.03
Total P	0.58	0.58	0.58	0.57	0.61
Phytate[2]	0.55	0.55	0.55	0.55	0.56
Phytate P[3]	0.16	0.16	0.16	0.16	0.16
Nonphytate P[4]	0.42	0.42	0.42	0.41	0.45
Phytase activity, unit/kg	<50	<50	<50	<50	<50

[1]Data are the average of duplicate analyses of one sample with the exception that data for Ca and total P are the average of four separate samples that were analyzed in duplicate.

[2]Phytate was calculated by dividing the analyzed phytate P by 0.282 (Tran and Sauvant, 2004).

[3]Phytate P values were calculated from analyzed phytate P in the ingredients.

[4]Nonphytate P was calculated as the difference between total P and phytate P.

Table 6. Basal endogenous loss (BEL) of Ca and ATTD of DM and P in Ca-free diets without or with microbial phytase fed to growing pigs[1], Exp. 1

	Phytase, unit/kg			
Item	0	500	SEM	*P*-value
Feed intake, g/d	688	645	44	0.506
Fecal excretion, g/d	60	66	6	0.539
ATTD of DM, %	90.5	88.8	0.7	0.103
Fecal Ca excretion, mg/d	273	165	42	0.013
BEL of Ca, mg/kg DMI	463	304	64	0.037
P intake, g/d	3.2	3.0	0.2	0.521
Fecal P excretion, g/d	1.0	0.8	0.1	0.021
Absorbed P, g/d	2.2	2.2	0.2	0.890
ATTD of P, %	68.4	73.4	2.5	0.061

[1]Each least squares mean represents eight observations.

without phytase, which resulted in lower ($P < 0.05$) basal endogenous loss of Ca from pigs fed the diet containing phytase. The ATTD of P tended ($P = 0.061$) to be greater in the Ca-free diet with phytase than in the Ca-free diet without phytase.

There were no interactions between phytase and source of Ca carbonate (Table 7). Feed intake and Ca and P intakes were not affected by use of microbial phytase or by the source of Ca carbonate included in the diet. However, fecal Ca excretion, daily basal endogenous Ca loss, and fecal P excretion were less ($P < 0.001$) from pigs fed diets containing Ca carbonate with microbial phytase compared with pigs fed the diets without microbial phytase. Therefore, absorbed Ca, ATTD of Ca, STTD of Ca, absorbed P, and ATTD of P increased ($P < 0.01$) if microbial phytase was included in the diets compared with diets without microbial phytase, regardless of the source of Ca carbonate. Fecal Ca excretion from pigs fed diet containing Ca carbonate source D was less ($P < 0.05$; data not shown) compared with Ca carbonate sources A, B, and C. Values for ATTD and STTD of Ca in Ca carbonate source A was greater ($P < 0.05$) than in Ca carbonate source D, but there was no difference among Ca carbonate source A and Ca carbonate sources B and C or among sources B and C and source D (data not shown). The ATTD and STTD of Ca did not differ among Ca carbonate sources B, C, and D (data not shown). The ATTD of P was not influenced by the source of Ca carbonate in the diet.

Exp. 2. STTD of Ca in DCP

Feed intake, basal endogenous Ca loss, and P intake tended to be less ($P < 0.10$) for pigs fed the basal diet compared with pigs fed the DCP-containing diets (Table 8). Fecal excretion from pigs fed the diet containing DCP source B was greater ($P < 0.05$) than from pigs fed the basal diet. Values for the ATTD of Ca and STTD of Ca did not differ among the three sources of DCP (Table 9). Apparent total tract digestible Ca and standardized total tract digestible Ca were also not affected by source of DCP.

DISCUSSION

Based on the total molecular mass, analytical grade Ca carbonate ($CaCO_3$) contains 40.0% Ca. The average analyzed concentration of Ca in the 4 commercial sources of Ca carbonate used in Exp. 1 was 39.7%, which indicates very little impurity in the Ca carbonate sources used. For DCP ($CaHPO_4$), based on the total molecular mass, the expected Ca concentration is 29.5%, and the expected P concentration is 22.8%. However, the 3 sources of DCP used in Exp. 2 had an average concentration of 19.1% P, and the average concentration of Ca was 19.5%, which indicates some impurity in these sources of DCP. The P concentration in a commercial DCP is usually lower than 22.8% because all sources of feed grade DCP in reality are mixtures of DCP, monocalcium phosphate, and unreacted Ca carbonate (Baker, 1989; Petersen and Stein, 2006). In addition, Ca fluoride, silica, Mg oxide, Mg phosphate, and Fe phosphate are often present in commercial sources of feed grade DCP (Baker, 1989), which is the reason feed grade DCP does not contain 22.8% P and 29.5% Ca.

The calculated concentration of Ca in the 8 Ca carbonate-containing diets used in Exp. 1 was between 0.67% and 0.69%, depending on the source of Ca carbonate used. Six of the 8 diets with Ca carbonate were within 0.03 percentage units of this value, but 2 diets analyzed only 0.62% and 0.56% Ca. Those values were the average of 4 separate diet samples that were analyzed for Ca. It has been demonstrated that analyzed dietary Ca values often vary greatly (Wu et al., 2018), but because calculated Ca values were used in all calculations, these analytical discrepancies did not impact digestibility data that were calculated for Exp. 1.

Values for the ATTD of P in the Ca-free diets that were formulated based on corn and monosodium phosphate without and with phytase were in agreement with expected values (NRC, 2012; González-Vega et al., 2015a; Blavi et al., 2017). However, the ATTD of P in the Ca carbonate-containing diets was less than the ATTD of P in the Ca-free diets. This indicates binding of Ca

Table 7. ATTD and STTD of Ca and ATTD of DM and P in experimental diets containing four sources of Ca carbonate fed to growing pigs[1], Exp. 1

Item, %	0 phytase units/kg				500 phytase units/kg					*P*-value		
Ca carbonate source	A	B	C	D	A	B	C	D	SEM	Phytase	Source	Interaction
Feed intake, g/d	835	778	824	850	840	756	810	898	43	0.886	0.090	0.846
Fecal excretion, g/d	83	76	85	85	83	76	87	90	6	0.665	0.128	0.972
ATTD of DM, %	89.5	89.5	89.1	89.4	89.1	89.0	88.4	89.1	0.5	0.206	0.698	0.973
Calcium												
Ca intake, g/d	5.6	5.4	5.7	5.8	5.6	5.2	5.6	6.1	0.3	0.909	0.154	0.853
Fecal Ca excretion, g/d	1.8	1.7	1.9	2.1	1.0	1.3	1.4	1.6	0.1	<0.001	0.001	0.422
Absorbed Ca, g/d	3.8	3.7	3.7	3.7	4.6	3.9	4.2	4.5	0.3	0.003	0.441	0.663
ATTD of Ca, %	68.7	69.4	66.1	64.6	81.4	74.9	75.0	73.4	1.8	<0.001	0.007	0.227
Basal endogenous Ca loss[2], mg/d	340	316	336	347	223	200	215	238	15	<0.001	0.120	0.984
STTD of Ca[3], %	74.8	75.2	72.0	70.6	85.4	78.7	78.8	77.3	1.8	<0.001	0.006	0.235
Phosphorus												
P intake, g/d	3.8	3.6	3.8	3.9	3.9	3.5	3.7	4.1	0.2	0.908	0.081	0.852
Fecal P excretion, g/d	1.9	1.7	2.0	1.9	1.2	1.3	1.4	1.5	0.1	<0.001	0.064	0.351
Absorbed P, g/d	1.9	1.9	1.8	2.0	2.6	2.2	2.3	2.6	0.2	<0.001	0.127	0.618
ATTD of P, %	49.5	51.9	47.0	51.6	67.8	62.9	62.1	64.0	2.3	<0.001	0.230	0.295

[1]Each least squares mean for experimental diets from growing pigs represents eight observations, respectively, with the exception for the diet containing source C with 500 phytase units/kg diet (n = 7); the outlier deviated from first- and third-quartile by 3.5 times the interquartile range within the treatment.

[2]The daily basal endogenous Ca loss (mg/d) was calculated by multiplying the basal endogenous Ca loss (mg/kg DMI) by the daily DM feed intake (kg/d) of each diet.

[3]The STTD of Ca in diets was calculated by correcting the ATTD of Ca for basal endogenous Ca loss that was obtained from pigs fed the Ca-free diets; basal endogenous Ca loss from pigs fed the Ca-free diet without microbial phytase = 463 mg/kg DMI; basal endogenous Ca loss from pigs fed the Ca-free diet with microbial phytase = 304 mg/kg DMI.

Table 8. ATTD and STTD of Ca and ATTD of DM and P in experimental diets fed to growing pigs[1], Exp. 2

	Basal	DCP source[2]				
Item		A	B	C	SEM	*P*-value
Feed intake, g/d	565	625	739	660	43	0.063
Fecal excretion, g/d	60[b]	74[ab]	89[a]	80[ab]	7	0.037
ATTD of DM, %	88.8	86.8	87.1	87.0	1.0	0.448
Ca intake, g/d	3.8	4.0	4.6	4.1	0.3	0.318
Fecal Ca excretion, g/d	1.1	1.3	1.3	1.1	0.1	0.395
Absorbed Ca, g/d	2.7	2.8	3.3	3.0	0.2	0.398
ATTD of Ca, %	71.5	68.4	71.2	72.9	2.2	0.551
Basal endogenous Ca loss[2], mg/d	389	428	506	451	30	0.070
STTD of Ca[3], %	81.7	79.0	82.3	83.9	2.2	0.473
P intake, g/d	3.3	3.6	4.3	3.7	0.2	0.079
Fecal P excretion, g/d	1.5	1.7	1.9	1.7	0.1	0.194
Absorbed P, g/d	1.8	2.0	2.4	2.1	0.2	0.215
ATTD of P, %	55.7	53.1	56.1	55.1	3.0	0.893

[1]Each least squares mean for experimental diets from growing pigs represents eight observations, respectively, with the exception for the diet containing DCP source B diet (n = 7); the outlier deviated from first- and third-quartile by 3.2 times the interquartile range within the treatment.

[2]The daily basal endogenous Ca loss (mg/d) was calculated by multiplying the basal endogenous Ca loss (mg/kg DMI) by the daily DM feed intake (kg/d) of each diet.

[3]The STTD of Ca in diets was calculated by correcting the ATTD of Ca for basal endogenous Ca loss that was obtained from pigs fed the Ca-free diet (basal endogenous loss Ca loss = 782 mg/kg DMI).

Table 9. ATTD and STTD of Ca in three different sources of DCP fed to growing pigs[1], Exp. 2

	DCP source				
Item	A	B	C	SEM	*P*-value
ATTD					
Digestibility, %	66.1	71.0	74.0	4.1	0.393
Digestible Ca, %	14.5	14.1	13.7	0.8	0.811
STTD					
Digestibility, %	77.0	82.8	85.8	4.1	0.314
Digestible Ca, %	16.9	16.4	15.9	0.8	0.716

[1]Each least squares mean for experimental diets from growing pigs represents eight observations, respectively, with the exception that the diet containing DCP source B only had seven observations.

and P in the intestinal tract of pigs if both minerals are included in the diet, which has also been reported in the past (Stein et al., 2011). However, the observation that the ATTD of P in the Ca-free diet is greater than in a diet adequate in Ca indicates that the digestibility of P in growing pigs is not downregulated by the lack of Ca in the diet although the lack of Ca likely prevented the use of absorbed P for bone tissue synthesis. This observation is in agreement with recent data from gestating sows that also indicated that the ATTD of P is greater in a Ca-free diet than in a diet containing Ca carbonate (Lee et al., 2019).

The basal endogenous loss of Ca that was determined in the two experiments was within the range of reported values (González-Vega et al., 2015b; Merriman, 2016; Merriman and Stein, 2016; Blavi et al., 2017). The observation that use of microbial phytase decreased the basal endogenous loss of Ca is different from data for total endogenous loss of Ca determined in pigs fed diets based on canola meal (González-Vega et al., 2013). However, if phytase was added to a Ca-free corn-based diet fed to gestating sows, there was also a decrease in the basal endogenous loss of Ca (Lee et al., 2019). This indicates that a Ca-phytate complex is formed in the intestinal tract between phytate in corn and endogenous Ca. Phytase addition to the diet results in release of this endogenous Ca from the complex, which in turn decreases the excretion of endogenous Ca in feces. Likewise, the negatively charged phytate may chelate Ca ions from feed ingredients in the intestine of pigs, resulting in formation of nondigestible Ca-phytate complexes (Nelson and Kirby, 1987; Selle and Ravindran, 2008). As a consequence, if phytase is used in diets, both Ca and P are released from the complex and the digestibility of Ca and P will increase (Almeida et al., 2013; Rodríguez et al., 2013; González-Vega et al., 2015a). The observation that the ATTD and STTD of Ca and the ATTD of P in the corn–Ca carbonate diets increased if phytase was added to the diets concur with previous data (González-Vega et al., 2015a). These observations support the hypothesis that the Ca from Ca carbonate may bind to phytate in corn and that phytase reduces chelation, resulting in an increase in the digestibility of Ca and P.

Values for ATTD and STTD of Ca in Ca carbonate were in line with previous data (González-Vega et al., 2015a; Merriman and Stein, 2016; Blavi et al., 2017; Kwon and Kim, 2017). The small, but significant, difference in the digestibility of Ca among the 4 sources of Ca carbonate demonstrates that differences among commercial sources of Ca carbonate exist. In this experiment, Ca carbonate source D had a lower digestibility than Ca carbonate source A although the particle size was greater in source A. Differences in the digestibility of Ca are not likely a result of differences in particle size because there appears to be no influence of particle size on digestibility of Ca in Ca carbonate (Merriman and Stein, 2016) or on bioavailability of Ca in Ca carbonate (Ross et al., 1984). Solubility of Ca carbonate may affect Ca utilization in poultry (Zhang and Coon, 1997b; Kim et al., 2018), but ATTD and STTD of Ca in the 4 sources of Ca carbonate used in this experiment were not correlated with solubility (data not shown).

Values for the ATTD and STTD of Ca in the 3 sources of DCP that were used in this experiment are in agreement with previous data (González-Vega et al., 2015a; Zhang and Adeola, 2017). The observation that there was no difference in the digestibility of Ca and P among the 3 sources indicates that production processes and origins of the 3 sources of DCP used in this experiment did not influence digestibility of Ca and P.

Values for the STTD of Ca in DCP were greater than the STTD of Ca in Ca carbonate. This may seem surprising because Ca in DCP originates from Ca carbonate (Baker, 1989), but previous data also demonstrated a greater digestibility of Ca in DCP compared with Ca from Ca carbonate (González-Vega et al., 2015a; Zhang and Adeola, 2017). The reason for this observation may be that some of the Ca from dietary Ca carbonate binds to the phytate that is supplied by corn in the diet when the 2 ingredients reach the aqueous environment in the stomach of pigs, which reduces the digestibility of Ca from Ca carbonate. In contrast, it appears that Ca in DCP, which is bound to P in the phosphoric acid, is less likely to solubilize in the stomach and becomes less available for chelation with phytate (Walk, 2016). These possible effects explain why phytase, as demonstrated in Exp. 1 and in previous experiments (González-Vega et al., 2015a; Blavi et al., 2017), results in increased digestibility of Ca from Ca carbonate because phytase may release the Ca that was bound to phytate. In contrast, there is no effect of phytase on the digestibility of Ca in DCP (González-Vega et al., 2015a), which is consistent with the hypothesis that Ca from DCP is not chelated by phytate.

The small, but significant, differences in STTD of Ca in calcium carbonate that was observed in this experiment (up to 8 percentage units) will result in a difference in STTD Ca in a diet containing 1% limestone of ~0.03%. Considering analytical inaccuracies and variabilities in Ca concentrations in raw materials, it is unlikely this difference would make a measurable difference in pig growth performance. The difference in STTD of Ca among the 3 sources of DCP that was similar to the difference among the 4 sources of calcium carbonate, but because of the greater standard error that is usually associated with using the difference procedure, the differences among the 3 sources of DCP were not significant. However, because the concentration of Ca in DCP is much less than in calcium carbonate, and because inclusion of DCP in phytase containing diets is relatively low, it is unlikely these differences will result in measurable differences in pig performance. As a consequence, using an average value for the STTD of Ca in calcium carbonate and an average value for the STTD of Ca in DCP in feed formulation may be the most practical approach.

In conclusion, use of microbial phytase reduces the basal endogenous loss of Ca and increases Ca digestibility in Ca carbonate. The STTD of Ca varies among sources of Ca carbonate, but that appears not to be the case for the STTD of Ca in different sources of DCP. The STTD of Ca in DCP is greater than the STTD of Ca in Ca carbonate.

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