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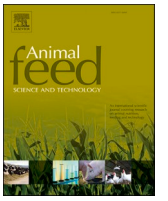

Digestibility of calcium in calcium carbonate varies among origins, but is increased by microbial phytase regardless of origin

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ABSTRACT

The objective of this experiment was to test the hypothesis that there are differences in the apparent total tract digestibility (ATTD) of Ca and in the response to microbial phytase among sources of Ca carbonate produced in different regions of the world. Three hundred and twenty barrows (body weight: 17.47 ± 1.28 kg) were allotted to 40 diets using a completely randomized block design with eight blocks of 40 pigs for a total of eight replicate pigs per diet. All diets were based on corn and potato protein concentrate. Twenty sources of Ca carbonate were obtained from different regions of the world, including the United States, Europe, Asia, and South Africa. Each source of Ca carbonate was used in two diets, one diet without microbial phytase and one diet that contained 1000 phytase units (FYT)/kg of diet. Pigs were housed individually in metabolism crates and were fed experimental diets for 12 days, with the initial five days being the adaptation period. Daily feed allotments were divided into two equal meals and pigs were provided feed at 3.0 times the maintenance requirement for metabolizable energy. Feces were collected for four days following the adaptation period, and at the conclusion of the experiment, fecal samples were dried, ground, and analyzed for Ca and P. Results indicated that there were no interactions between source of Ca carbonate and phytase. Differences in ATTD and standardized total tract digestibility (STTD) of Ca were observed among pigs fed diets containing different sources of Ca carbonate ($P < 0.001$). Pigs fed diets containing 1000 FYT/kg had greater ($P < 0.001$) ATTD and STTD of Ca compared with pigs fed diets containing no phytase (0.809 vs. 0.697 and 0.835 vs. 0.753, respectively). There was a tendency ($P = 0.050$) for source of Ca carbonate to influence ATTD of P, and pigs fed diets containing 1000 FYT/kg had greater ($P < 0.001$) ATTD of P compared with pigs fed diets without phytase (0.793 vs. 0.641). No interactions were observed between region and phytase. The ATTD and STTD of Ca in Ca carbonate from the United States was less ($P < 0.05$) than in Ca carbonate from Europe, Asia, or South Africa. In conclusion, differences in ATTD and STTD of Ca were observed among Ca carbonate obtained from four regions of the world, and inclusion of microbial phytase increased the ATTD and STTD of Ca in Ca carbonate regardless of the region where the Ca carbonate was produced.

Abbreviations: ATTD, apparent total tract digestibility; FYT, phytase unit; STTD, standardized total tract digestibility.

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1. Introduction

Only a small amount of the Ca required by pigs is provided by plant-based ingredients and supplementation of Ca from mineral sources such as Ca carbonate is usually required to meet the Ca requirement of pigs (González-Vega et al., 2015a). The apparent total tract digestibility (ATTD) of Ca in Ca carbonate is not affected by dietary Ca concentration (Stein et al., 2011), but addition of microbial phytase to diets supplemented with Ca carbonate results in an increase in ATTD of Ca (González-Vega et al., 2015a; Lee et al., 2019). Excess dietary Ca can negatively affect growth performance of pigs by reducing absorption and digestibility of P (Stein et al., 2011; González-Vega et al., 2016; Lautrou et al., 2021); therefore, it is important that the digestibility of Ca in Ca carbonate is known because that will allow formulation of diets based on values for digestible Ca rather than total Ca.

The concentration of Ca and micro-minerals in different sources of Ca carbonate may vary due to differences in raw materials, physical structure, geological origin, and processing methods that suppliers utilize (Gilani et al., 2022). Differences in ATTD and standardized total tract digestibility (STTD) of Ca among different sources of Ca carbonate produced in the United States have been observed (Lee et al., 2019), but it is unknown if there are differences in the ATTD and STTD of Ca in Ca carbonate sources produced outside the United States. It is, therefore, not known if the STTD of Ca in Ca carbonate produced in the United States is also representative of Ca carbonate produced in other parts of the world.

Microbial phytase is often included in diets fed to pigs to increase the digestibility of P from plant ingredients (Selle and Ravindran, 2008; She et al., 2015; Lautrou et al., 2021), but microbial phytase also increases digestibility of Ca in plant ingredients, animal protein ingredients, and Ca carbonate produced in the United States (Gonzalez-Vega et al., 2013, 2015a, 2015b; Lee et al., 2019). Because of the detrimental effects of excess dietary Ca on P digestibility, it is important that the increase in STTD of Ca that is a result of including microbial phytase in the diets is accounted for in diet formulation. This is particularly important for Ca carbonate because this ingredient provides up to 700 g/kg of all the Ca in diets that contain phytase (Lee et al., 2023). However, it is not known if microbial phytase also increases the digestibility of Ca in Ca carbonate produced outside the United States. Therefore, the objective of this experiment was to test the null-hypothesis that there are no differences in the ATTD or STTD of Ca and in the response to microbial phytase among sources of Ca carbonate obtained from different regions of the world.

2. Materials and methods

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

2.1. Diets, animals, and experimental design

Twenty sources of Ca carbonate were collected from Europe, Asia, the United States, and South Africa (Table 1). Each source was

Table 1
Analyzed nutrient composition of feed ingredients (as-is basis)[1].

Item	Dry matter, g/kg	Ash, g/kg	Ca, g/kg	P, g/kg	Phytate, g/kg	Phytate-P[3], g/kg	Non-phytate-P[3], g/kg
Corn	859	11.9	0.3	2.3	6.4	1.8	0.5
Potato protein concentrate	910	3.7	0.2	0.5	1.7	0.5	-
Ca carbonate							
A	1000	994	373	-	-	-	-
B	1000	999	390	-	-	-	-
C	1000	996	390	-	-	-	-
D	1000	995	380	-	-	-	-
E	1000	993	377	-	-	-	-
F	1000	994	397	-	-	-	-
G	1000	996	373	-	-	-	-
H	1000	992	359	-	-	-	-
I	1000	984	365	-	-	-	-
J	1000	997	393	-	-	-	-
K	1000	989	375	-	-	-	-
L	999	975	348	-	-	-	-
M	1000	994	409	-	-	-	-
N	999	995	392	-	-	-	-
O	999	993	368	-	-	-	-
P	997	990	377	-	-	-	-
Q	1000	997	376	-	-	-	-
R	999	976	321	-	-	-	-
S	987	954	365	-	-	-	-
T	999	923	366	-	-	-	-

[1]All analyzed data are the average of two duplicate analyses.
[2]Phytate-P was calculated by multiplying analyzed phytate by 0.282 (Lee et al., 2023).
[3]Non-phytate-P was calculated as the difference between total P and phytate-P

shipped to the University of Illinois (Urbana, IL, USA) where sources were labelled and stored until diet mixing. A total of 40 diets were formulated based on corn and potato protein concentrate (Tables 2 and 3). Each source of Ca carbonate was used in two diets, one diet without microbial phytase and one diet that contained 1000 phytase units/kg of diet (FYT/kg; HiPhorius, dsm-firmenich, Kaiseraugst, Switzerland). Crystalline amino acids, vitamins, and minerals were included in all diets to meet the requirements for 11–25 kg pigs (NRC, 2012).

Three hundred and twenty barrows (body weight: 17.47 ± 1.28 kg) were allotted to the 40 diets using a completely randomized block design with eight blocks of 40 pigs for a total of eight replicate pigs per diet. Pigs were housed individually in metabolism crates (0.71 × 0.84 m) that were equipped with a fully slatted tri-bar floor, a nipple waterer, and a feeder. A mesh screen and a pan were installed under the slatted floor during the collection period to allow for separate collection of feces and orts.

2.2. Feeding and sample collection

Daily feed allotments were divided into two equal meals that were provided at 0800 and 1600 h, and pigs were provided feed in the amount of 3.0 times the maintenance requirement for metabolizable energy (i.e., 0.82 MJ metabolizable energy per kg body weight$^{0.60}$; NRC, 2012). Pigs had free access to water throughout the experiment. Experimental diets were fed for 12 days, with the initial five days being the adaptation period to the diets followed by four days of collection using the marker-to-marker procedure (Adeola, 2001). Fecal collection commenced when the first marker (i.e., indigo carmine), which was supplemented in the morning of day 6, appeared in the feces, and ceased when the second marker (i.e., ferric oxide), which was supplemented in the morning of day 10, appeared in the feces (Adeola, 2001). Feces were stored at −20 °C immediately after collection.

2.3. Chemical analysis

At the conclusion of the experiment, fecal samples were thawed, dried at 65 °C in a forced air oven, and finely ground using a 500 G swing type grain mill (model RRH-500, Zhejiang Winki Plastic Industry Co., Ltd., Zhejiang, China) prior to analyses. Main ingredients,

Table 2
Ingredient composition of diets (as-is basis).

Item, g/kg	Diet[1]
Potato protein concentrate	180.0
Corn	768.7
Calcium carbonate	17.5
Soybean oil	12.0
$_{L}$-Lys·HCl, 780 g/kg Lys	1.4
$_{DL}$-Met, 980 g/kg Met	0.3
$_{L}$-Trp, 980 g/kg Trp	0.1
Monosodium phosphate	10.9
Salt	4.0
Vitamin-mineral premix[2]	5.0
Phytase concentrate[3]	0.1
Calculated nutrient composition[4]	
Metabolizable energy, MJ/kg	14.02
Crude protein, g/kg	208.6
Ca, g/kg	7.0
Total P, g/kg	5.0
Standardized total tract digestible P, g/kg	3.3

[1]Twenty sources of Ca carbonate were included in one diet without microbial phytase and in one diet with microbial phytase (1000 phytase units/kg of diet).
[2]The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 10,622 IU; vitamin D_3 as cholecalciferol, 1660 IU; vitamin E as $_{DL}$-alpha-tocopheryl acetate, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.40 mg; thiamin asthiamine mononitrate,1.08 mg; riboflavin, 6.49 mg; pyridoxine as pyridoxine hydrochloride, 0.98 mg; vitamin B_{12}, 0.03 mg; $_{D}$-pantothenic 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.
[3]The phytase concentrate contained 10,000 units of phytase (HiPhorius; dsm-firmenich Animal Nutrition and Health, Kaiseraugst, Switzerland) per g. At 0.1 g/kg inclusion, the expected concentration of phytase was 1000 units per kg of complete diet.
[4]Calculated from NRC (2012)

Table 3
Analyzed nutrient composition of experimental diets (as-fed basis)[1,2].

Item	Dry matter, g/kg	Ash, g/kg	Ca, g/kg	P, g/kg	Phytase, FYT/kg	Phytate[3], g/kg	Phytate-P[4], g/kg	Non-phytate-P[5], g/kg
Ca carbonate (0 FYT/kg)								
A	879.3	36.0	6.9	5.4	< 70	5.2	1.5	3.9
B	879.7	36.2	7.3	5.1	< 70	5.2	1.5	3.6
C	879.7	36.6	6.9	5.5	< 70	5.2	1.5	4.0
D	878.6	37.2	6.8	4.8	< 70	5.2	1.5	3.3
E	879.2	35.8	7.0	5.4	< 70	5.2	1.5	3.9
F	880.2	35.1	6.4	4.9	< 70	5.2	1.5	3.4
G	882.0	34.0	7.2	4.9	< 70	5.2	1.5	3.4
H	880.0	35.7	6.3	5.5	< 70	5.2	1.5	4.0
I	879.4	35.3	6.8	4.9	< 70	5.2	1.5	3.4
J	879.6	37.2	7.1	5.0	< 70	5.2	1.5	3.5
K	875.9	33.7	6.9	4.7	< 70	5.2	1.5	3.2
L	877.2	35.9	6.5	5.2	< 70	5.2	1.5	3.7
M	875.6	36.2	7.1	5.3	< 70	5.2	1.5	3.8
N	871.7	36.5	6.9	5.6	< 70	5.2	1.5	4.1
O	873.6	39.6	6.4	5.1	< 70	5.2	1.5	3.6
P	873.7	40.5	6.9	5.1	< 70	5.2	1.5	3.6
Q	873.3	34.7	6.5	4.8	< 70	5.2	1.5	3.3
R	874.2	33.3	5.4	4.8	< 70	5.2	1.5	3.3
S	873.6	39.2	6.7	5.2	< 70	5.2	1.5	3.7
T	872.9	31.7	6.8	4.7	< 70	5.2	1.5	3.2
Ca carbonate (1000 FYT/kg)								
A	869.5	40.8	6.4	4.9	995	5.2	1.5	3.4
B	870.9	42.6	7.1	5.0	1550	5.2	1.5	3.5
C	871.6	42.3	7.4	5.4	1150	5.2	1.5	3.9
D	873.1	34.3	6.4	5.5	1135	5.2	1.5	4.0
E	873.3	40.4	7.2	5.2	895	5.2	1.5	3.7
F	873.0	40.4	7.3	5.4	1200	5.2	1.5	3.9
G	872.0	42.6	7.1	5.3	1350	5.2	1.5	3.8
H	872.3	34.3	6.6	4.7	1250	5.2	1.5	3.2
I	881.7	38.9	6.8	5.2	1400	5.2	1.5	3.7
J	871.2	38.2	7.1	5.4	1450	5.2	1.5	3.9
K	868.5	43.4	6.9	4.9	1075	5.2	1.5	3.4
L	873.0	40.5	6.0	5.4	1200	5.2	1.5	3.9
M	868.0	34.2	7.0	5.2	1230	5.2	1.5	3.7
N	869.5	33.6	7.2	4.9	1200	5.2	1.5	3.4
O	872.1	40.7	7.1	5.0	1400	5.2	1.5	3.5
P	871.6	36.3	6.5	4.8	1365	5.2	1.5	3.3
Q	872.4	33.9	6.4	5.1	1200	5.2	1.5	3.6
R	872.3	34.8	5.2	4.8	1400	5.2	1.5	3.3
S	872.1	43.5	6.6	4.8	1300	5.2	1.5	3.3
T	871.3	34.0	6.9	4.7	1245	5.2	1.5	3.2

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

diets, and fecal samples were analyzed in duplicate for dry matter (method 930.15; AOAC Int, 2019), and ash (method 942.05; AOAC Int, 2019). These ingredients were also analyzed for Ca and P (method 985.01 A, B and C; AOAC Int, 2019) using inductively coupled plasma-optical emission spectroscopy (ICP-OES; Avio 200, PerkinElmer, Waltham, MA, USA). Sample preparation included dry ashing at 600 °C for 4 h (method 942.05; AOAC Int, 2019) and wet digestion with nitric acid (method 3050 B; U.S. Environmental Protection Agency, 2000). Diets were analyzed for phytase activity (method 2000.12; AOAC Int, 2019) and corn and potato protein concentrate were analyzed for phytic acid (Ellis et al., 1977).

2.4. Calculations and statistical analysis

Phytate in the diets was calculated as the sum of the analyzed phytate in corn and potato protein concentrate multiplied by the inclusion rate of each ingredient in the diet. 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 ATTD of Ca and P in each experimental diet was calculated according to the following equation (Almeida and Stein, 2010):

$$\text{ATTD} = \frac{\text{intake} - \text{output}}{\text{intake}}$$

where both intake and output are expressed in grams per day. The STTD of Ca in each experimental diet without phytase was determined by correcting the ATTD of Ca for an average basal endogenous loss of Ca (i.e., 433 mg/kg dry matter intake) according to Lee and Stein (2023), whereas it was assumed that the basal endogenous loss of Ca was 211 mg/kg dry matter intake in diets containing phytase (Nelson et al., 2022). Digestible Ca in each source of Ca carbonate was calculated by multiplying the concentration of Ca in each source by the STTD of Ca.

Normality and homogeneity of variances were verified (SAS Institute, 2016) and outliers were identified as values that deviated from the first or third quartiles by more than 3 times the interquartile range using Internally Studentized Residuals (Tukey, 1977). Pig was the experimental unit for all analyses. Seven outliers were identified and removed from the final statistical analysis and the average SEM is indicated in results tables if the number of observations was not identical among dietary treatments. Data were analyzed using the PROC GLM in SAS (SAS Institute, 2016). The initial model included Ca carbonate source, phytase, and the Ca source × phytase interaction as fixed effects, but no interactions between Ca source and phytase were observed, and the final model, therefore, included only Ca source and phytase as fixed effects. A second analysis was used to compare digestibility of Ca carbonate obtained from different regions of the world (i.e., Europe, Asia, the United States, and South Africa). In this model, region, phytase, and the region × phytase interaction were fixed effects. However, no interactions between region and phytase were observed and therefore, the final model included only region and phytase as fixed effects. Means were calculated, and least significant differences were used to separate means. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Table 4
Main effects of Ca source and phytase on apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) of Ca in diets containing 20 different sources of calcium carbonate[1].

Item	Feed intake, kg/d	Dried fecal excretion, g/d	ATTD of dry matter	Ca intake, g/d	Ca in feces, g/kg	Fecal Ca output, g/d	ATTD of Ca	STTD of Ca[4]	Digestible Ca in source[5], g/kg
Ca carbonate[2]									
A	0.82	81.92	0.891	5.62	14.0	1.14	0.794	0.836	311.7
B	0.85	89.33	0.886	6.09	16.6	1.50	0.754	0.794	309.6
C	0.87	88.10	0.889	6.22	14.2	1.24	0.799	0.838	326.6
D	0.84	87.50	0.883	5.81	13.3	1.18	0.798	0.839	318.8
E	0.86	86.33	0.890	5.90	15.3	1.33	0.776	0.817	307.9
F	0.89	88.33	0.892	6.46	16.6	1.47	0.773	0.812	322.3
G	0.86	83.45	0.893	5.87	24.1	1.98	0.659	0.701	261.3
H	0.89	91.44	0.888	5.88	14.9	1.35	0.769	0.812	291.5
I	0.83	90.14	0.879	5.53	19.5	1.78	0.680	0.722	263.5
J	0.84	86.15	0.887	6.03	20.2	1.75	0.711	0.749	294.3
K	0.84	82.08	0.892	5.75	18.5	1.49	0.740	0.781	293.0
L	0.85	85.95	0.888	5.41	15.5	1.33	0.755	0.801	278.6
M	0.86	81.68	0.894	6.38	16.0	1.32	0.795	0.832	340.2
N	0.84	87.27	0.886	5.99	16.4	1.39	0.767	0.806	316.1
O	0.78	86.45	0.876	5.29	16.3	1.41	0.730	0.772	284.1
P	0.85	85.98	0.889	5.88	15.8	1.37	0.768	0.809	305.0
Q	0.84	92.00	0.880	5.81	15.0	1.39	0.762	0.803	302.0
R	0.88	93.95	0.883	5.20	14.0	1.29	0.750	0.798	256.1
S	0.85	86.75	0.888	5.71	14.5	1.26	0.779	0.820	299.3
T	0.89	93.07	0.884	5.94	18.0	1.71	0.711	0.752	275.2
SEM	0.03	4.53	0.005	0.23	1.1	0.12	0.018	0.017	6.3
LSD	0.09	12.6	0.013	0.62	2.6	0.33	0.047	0.047	17.6
P-value	0.890	0.912	0.391	0.007	< 0.001	0.002	< 0.001	< 0.001	< 0.001
Phytase[3], FYT/kg									
0	0.85	87.41	0.887	5.79	20.1	1.75	0.697	0.753	282.1
1000	0.86	87.40	0.887	5.89	12.9	1.12	0.809	0.835	313.3
SEM	0.01	1.43	0.002	0.07	0.3	0.04	0.006	0.005	2.0
LSD	0.03	4.00	0.004	0.20	0.8	0.10	0.015	0.015	5.6
P-value	0.391	1.00	0.996	0.402	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

[1]Each mean represents 155 observations for diets containing no phytase and 158 observations for diets containing 1000 FYT/kg.
[2]Each mean represents 16 observations per Ca carbonate source with the exception that there were 15 observations for diets containing Ca carbonate sources C, J, L, M, O, S, and T.
[3]FYT = phytase unit.
[4]STTD of Ca without phytase was calculated by correcting the ATTD of Ca with an average value for basal endogenous loss of Ca (i.e., 433 mg/kg dry matter intake) obtained from 7 experiments (Lee and Stein, 2023). To calculate the STTD of Ca in diets containing microbial phytase, a basal endogenous loss of Ca of 211 mg/kg dry matter intake was used (Nelson et al., 2022).
[5]Digestible Ca in source was calculated by multiplying the concentration of Ca in each source of Ca carbonate by the STTD of Ca and dividing by 100

3. Results

Feed intake, fecal excretion, and ATTD of dry matter were not different among pigs fed diets containing different sources of Ca carbonate (Table 4). There were differences in Ca intake among pigs fed different Ca carbonate sources ($P = 0.007$) and values ranged from 5.20 to 6.46 g/day. Differences ($P < 0.01$) were also observed in Ca concentration in feces (13.3–24.1 g/kg) and fecal Ca output (1.14–1.98 g/day). There were also differences ($P < 0.001$) in ATTD (0.659–0.799) and STTD (0.701–0.839) of Ca among pigs fed diets containing different sources of Ca carbonate ($P < 0.001$). The concentration of standardized total tract digestible Ca ranged from 256.1 to 340.2 g/kg among the 20 sources of Ca carbonate and these values were different ($P < 0.001$).

No effects of phytase were observed for feed intake, fecal excretion, ATTD of dry matter, or Ca intake. Pigs fed diets containing 1000 FYT/kg had less ($P < 0.001$) Ca in feces, expressed as g/kg feces or g/day, compared with pigs fed diets without phytase. Apparent total tract digestibility and STTD of Ca were greater ($P < 0.001$) in pigs fed diets containing 1000 FYT/kg compared with pigs fed diets without phytase. The concentration of digestible Ca in Ca carbonate increased ($P < 0.001$) when 1000 FYT/kg was included in the diet compared with diets without phytase.

The calculated ATTD, STTD, and concentration of standardized total tract digestible Ca for each individual source of Ca carbonate both without and with microbial phytase also demonstrated some differences among sources (Supplemental Table 1).

No differences among diets were observed for P intake, but differences ($P < 0.05$) for P concentration in feces (12.6–14.8 g/kg) and fecal P output (1.01–1.35 g/day) were observed (Table 5). The ATTD of P ranged from 0.682 to 0.757 and there was a tendency ($P = 0.05$) for diets containing different sources of Ca carbonate to have different ATTD of P.

No differences were observed for P intake between pigs fed diets with 1000 FYT/kg and pigs fed diets without phytase, but pigs fed diets containing 1000 FYT/kg had less ($P < 0.001$) P in feces (10.0 vs. 17.2 g/kg) and less ($P < 0.001$) daily fecal output of P (0.87 vs. 1.49 g/day), compared with pigs fed diets without phytase. Pigs fed diets containing 1000 FYT/kg also had greater ($P < 0.001$) ATTD of P (0.793 vs. 0.641) compared with pigs fed diets without phytase.

Calcium intake was not different among pigs fed diets containing Ca carbonate sourced from Europe, Asia, the United States, or South Africa (Table 6). However, pigs fed diets containing Ca carbonate from the United States had greater ($P < 0.05$) concentration of Ca in feces, expressed as g/kg or g/day, than pigs fed Ca carbonate from other regions. As a consequence, the ATTD and STTD of Ca in Ca carbonate from the United States was less ($P < 0.05$) than in Ca carbonate from other regions of the world, but no differences among Europe, Asia, and South Africa were observed. Calcium carbonate from Europe contained more ($P < 0.05$) digestible Ca than Ca

Table 5
Main effects of Ca source and phytase on apparent total tract digestibility (ATTD) of P in diets containing 20 different sources of Ca carbonate[1].

Item	P intake, g/d	P in feces, g/kg	Fecal P output, g/d	ATTD of P
Ca carbonate[2]				
A	4.07	12.9	1.04	0.738
B	4.23	14.8	1.35	0.682
C	4.31	12.7	1.12	0.739
D	4.14	14.5	1.27	0.688
E	4.23	14.1	1.23	0.711
F	4.40	14.3	1.27	0.713
G	4.25	12.6	1.01	0.757
H	4.42	14.5	1.34	0.696
I	4.09	12.8	1.11	0.718
J	4.16	12.9	1.11	0.729
K	4.14	14.8	1.17	0.711
L	4.18	13.3	1.16	0.723
M	4.23	13.5	1.09	0.741
N	4.13	14.3	1.20	0.706
O	3.88	13.6	1.17	0.684
P	4.21	13.9	1.17	0.720
Q	4.18	13.0	1.17	0.718
R	4.35	13.2	1.21	0.718
S	4.23	13.0	1.13	0.731
T	4.38	12.6	1.18	0.731
SEM	0.16	0.5	0.07	0.016
LSD	0.45	1.4	0.19	0.043
P-value	0.890	0.009	0.029	0.050
Phytase[3], FYT/kg				
0	4.18	17.2	1.49	0.641
1000	4.24	10.0	0.87	0.793
SEM	0.05	0.2	0.02	0.005
LSD	0.14	0.5	0.06	0.014
P-value	0.391	< 0.001	< 0.001	< 0.001

[1]Each mean represents 155 observations for diets containing no phytase and 158 observations for diets containing 1000 FYT/kg.
[2]Each mean represents 16 observations per Ca carbonate source with the exception that there were 15 observations for diets containing Ca carbonate source C, J, L, M, O, S, and T.
[3]FYT = phytase unit

carbonate from the other three regions in the world, and Ca carbonate from Asia contained more ($P < 0.05$) digestible Ca than Ca carbonate from South Africa or the United States. There was no effect of phytase on Ca intake, but pigs fed diets containing 1000 FYT/kg had less ($P < 0.001$) Ca excreted in feces, expressed as g/kg or g/day, compared with pigs fed diets containing no phytase and consequently, pigs fed diets containing 1000 FYT/kg had greater ($P < 0.001$) ATTD and STTD of Ca compared with pigs fed diets without phytase. Concentration of digestible Ca in Ca carbonate increased ($P < 0.001$) when 1000 FYT/kg was included in diets compared with diets containing no phytase.

4. Discussion

Analyzed concentrations of Ca and P in corn and potato protein concentrate were consistent with reported values (NRC, 2012; Nelson et al., 2022). Based on the molecular weight, Ca carbonate should contain 400 g/kg Ca; however, due to impurities in raw materials, the analyzed concentration of Ca in Ca carbonate is typically lower and has an average value of 385 g/kg (NRC, 2012). The fact that Ca concentrations in the 20 sources of Ca carbonate used in this experiment ranged from 321 to 409 g/kg demonstrates that there is considerable variation in Ca concentration among sources of Ca carbonate obtained from different regions of the world.

The observation that ATTD and STTD of Ca were different among the 20 sources of Ca carbonate is consistent with previous results where differences in Ca digestibility were observed among four sources of Ca carbonate from the United States (Lee et al., 2019). The observation that Ca carbonate from the United States had lower ATTD and STTD of Ca compared with Ca carbonate from Asia, Europe, and South Africa, demonstrates that origin does impact digestibility of Ca in Ca carbonate. These differences may be the result of differences in the composition of the raw materials that are mined, but differences in processing practices used by producers of Ca carbonate may also influence ATTD and STTD of Ca. Values for ATTD and STTD of Ca among Ca carbonate sources from the United States agree with previous data (González-Vega et al., 2015a; Merriman and Stein, 2016; Lee et al., 2019). The observation that inclusion of 1000 FYT/kg in diets reduced Ca excretion in feces and increased ATTD and STTD of Ca also concurs with results of experiments in which increases in ATTD and STTD of Ca from Ca carbonate were observed when 500 phytase units/kg were added to diets (González-Vega et al., 2015a; Lee et al., 2019). In the present experiment, a dose of 1000 FYT/kg were used to reflect the tendency in recent years to use greater doses of microbial phytase in commercial diets fed to pigs during the initial six to eight weeks post-weaning (Lagos et al., 2021). In the experiments in which 500 phytase units per kg was used, the STTD of Ca in Ca carbonate increased by four to 12 percentage units as a result of phytase usage with an average increase of eight percentage units (Gonzalez-Vega et al., 2015a; Lee et al., 2019; Lee et al., 2023). The observation that the average increase in STTD of Ca in Ca carbonate that was observed in the current experiment was around eight percentage units would seem to suggest that there is no advantage to STTD of Ca of providing more than 500 phytase units per kg to the diets. However, in the current experiment, STTD values for diets containing phytase were calculated using a reduced value for basal endogenous losses of Ca compared with diets containing no microbial phytase, which results in a reduced calculated value for STTD of Ca compared with previous values. Indeed, as is indicated from the differences in ATTD values, the increased digestibility caused by microbial phytase is around 10 percentage units, which is also the increase that will be calculated for STTD of Ca if an identical value for basal endogenous losses is used for diets without and with phytase. The implication of these observations is that if 1000 phytase units per kg are used, the digestibility of calcium is increased more than if 500 phytase units per kg are used, which has also previously been demonstrated (Almeida et al., 2013).

Phytate has the capacity to chelate up to five Ca cations resulting in the formation of indigestible Ca-phytate complexes (Selle et al., 2009). Observations from this experiment further demonstrate that Ca in Ca carbonate may be chelated by phytate, but addition of phytase to diets reduces chelation and increases digestibility of Ca in Ca carbonate. However, the increase in ATTD and STTD of Ca caused by phytase is likely partly a result of reduced endogenous loss of Ca because phytase not only prevents binding of dietary Ca to phytate, but also reduces binding of endogenous Ca to phytate (Nelson et al., 2022). The STTD values for Ca that were calculated for

Table 6
Main effects of region and phytase on apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) of Ca in diets containing 20 different sources of Ca carbonate.

Item	Region[1]							Phytase[2], units/kg				
	Europe	Asia	United States	South Africa	SEM	LSD	P-value	0	1000	SEM	LSD	P-value
Ca												
Ca intake, g/d	5.92	5.89	5.81	5.57	0.11	0.30	0.153	5.79	5.89	0.04	0.20	0.304
Ca in feces, g/kg	15.6^{cb}	16.6^{b}	19.0^{a}	14.5^{c}	0.5	1.4	< 0.001	20.1	12.9	0.2	0.9	< 0.001
Fecal Ca output, g/d	1.34^{b}	1.44^{b}	1.68^{a}	1.32^{b}	0.06	0.16	< 0.001	1.75	1.12	0.02	0.11	< 0.001
ATTD of Ca	0.773^{a}	0.755^{a}	0.710^{b}	0.764^{a}	0.008	0.024	< 0.001	0.697	0.809	0.003	0.016	< 0.001
STTD[3] of Ca	0.812^{a}	0.797^{a}	0.751^{b}	0.807^{a}	0.008	0.024	< 0.001	0.753	0.835	0.006	0.016	< 0.001
Digestible Ca in source[4], g/kg	313.2^{a}	296.9^{b}	281.4^{c}	285.5^{c}	3.4	10.1	< 0.001	282.1	313.3	2.4	6.8	< 0.001

[1]Each mean represents 109, 95, 62, and 47 observations for Europe, Asia, United States, and South Africa, respectively.
[2]Each mean represents 155 and 158 observations for diets containing no phytase and 1000 FYT/kg, respectively.
[3]STTD of Ca in diets without phytase was calculated by correcting the ATTD of Ca with an average basal endogenous loss of Ca (i.e., 433 mg/kg dry matter intake) obtained from 7 experiments (Lee and Stein, 2023). To calculate the STTD of Ca in diets containing microbial phytase, a basal endogenous loss of Ca of 211 mg/kg dry matter intake was used (Nelson et al#, 2022).
[4]Digestible Ca was calculated by multiplying the concentration of Ca in each source by the STTD of Ca and dividing by 100

this experiment were calculated using an assumed constant value for the basal endogenous loss of Ca of 433 mg per kg dry matter intake (Lee and Stein, 2023) for diets without phytase and 211 mg/kg dry matter intake for diets with phytase (Nelson et al., 2022). Because our facility only has space for 40 metabolism crates, we were not able to determine the endogenous loss of Ca in this particular experiment and that is why the average values were used. However, whereas it is theoretically correct to use two different values for the basal endogenous losses of Ca depending on the presence or not of microbial phytase, as was done in this experiment, it is recognized that this approach results in a bias in the calculated STTD values against microbial phytase because the STTD of Ca in reality is increased more by phytase than what is indicated in the calculated data. This bias is a result of the fact that the reduced basal endogenous loss caused by microbial phytase is not captured in the STTD values calculated using this approach although this is a gain in absorbed Ca when phytase is used in the diets. From a diet formulation standpoint, it may, therefore, be argued that it would be more correct to use the basal endogenous loss of Ca obtained in pigs fed no phytase for all calculations.

The observation that inclusion of microbial phytase increased ATTD of P has been documented in previous experiments (Poulsen et al., 2010; Almeida et al., 2013; Arredondo et al., 2019). Phytase hydrolyzes the phytate molecule, thereby liberating phytate-bound P and increasing P absorption, resulting in less P excreted in the feces (Selle and Ravindran, 2008). Differences observed for P excretion among diets containing different sources of Ca carbonate may be due to the differences observed in Ca concentration of diets, which ranged from 5.9 to 7.5 g/kg because of the differences in concentrations of Ca in the different sources of Ca carbonate. Increases in the Ca to P ratio negatively affect P digestibility by formation of indigestible Ca-P and Ca-phytate complexes that precipitate in the small intestine and results in greater excretion of P in the feces (Selle et al., 2009; Stein et al., 2011). However, in the current experiment, no significant correlations between diet Ca concentration and ATTD of P was observed, which indicates that differences among diets were too small to affect ATTD of P.

Calcium is relatively inexpensive to supplement to diets and, as a result, is often oversupplied in commercial pig diets in the United States and Europe (Walk, 2016; Lagos et al., 2023). Calcium carbonate is used most often to supplement Ca in diets, but dietary Ca is also provided by monocalcium phosphate and dicalcium phosphate. Unlike Ca carbonate, microbial phytases have no effect on digestibility of Ca in monocalcium phosphate or dicalcium phosphate in pigs (González-Vega et al., 2015a; Lee et al., 2019). Calcium in monocalcium phosphate and dicalcium phosphate is bound to phosphates in phosphoric acid, resulting in Ca cations being less available to bind to phytate in the intestines (Walk, 2016), and thus, if phytase is included in the diet, there is no observed increase in Ca digestibility in these ingredients. With the possibility of having STTD of P being up to around 0.800 in corn-soybean meal diets if newer phytases are used (Espinosa et al., 2022; Lagos et al., 2022), the need to supplement P from monocalcium phosphate and dicalcium phosphate is decreasing, and consequently, more Ca needs to be supplemented by Ca carbonate, which increases the need to accurately predict the digestibility of Ca in calcium carbonate both without and with microbial phytase.

5. Conclusion

Calcium concentration varied considerably among sources of Ca carbonate used in this experiment. Differences in apparent total tract digestibility and standardized total tract digestibility of Ca were observed among sources of Ca carbonate obtained from four regions of the world and inclusion of microbial phytase increased apparent total tract digestibility and standardized total tract digestibility of Ca in Ca carbonate. Therefore, consideration should be given to differences in analyzed concentrations of Ca in Ca carbonate, values for standardized total tract digestibility of Ca, and response to microbial phytase to ensure Ca is not oversupplied when formulating diets.

CRediT authorship contribution statement

April Zhang: Writing – review & editing. **Carrie L. Walk:** Writing – review & editing, Funding acquisition, Conceptualization. **Hans H Stein:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Heng-Xiao Zhai:** Writing – review & editing. **Megan E. Nelson:** Writing – original draft, Methodology, Investigation. **Su A A Lee:** Writing – review & editing, Writing – original draft, Resources, Investigation, Conceptualization.

Declaration of Competing Interest

At the time the experiment was conducted, CLW, AZ, and HXZ were employees at dsm-firmenich Animal Nutrition and Health, Kaiseraughst, Switzerland, which is a global supplier of microbial phytase. MEN, SAL, and HHS have no conflicts of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.anifeedsci.2025.116230.

References

Adeola, O., 2001. Swine nutrition. In: Lewis and, A.J., Southern, L.L. (Eds.), Digestion and balance techniques in pigs. CRC Press, Washington, D.C, pp. 903–916.

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

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

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

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

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

Espinosa, C.D., Torres, L.J., Velayudhan, D.E., Dersjant-Li, D., Stein, H.H., 2022. Ileal and total tract digestibility of energy and nutrients in pig diets supplemented with a novel consensus bacterial 6-phytase variant. J. Anim. Sci. 100, skac364. https://doi.org/10.1093/jas/skac364.

Gilani, S., Mereu, A., Li, W., Plumstead, P.W., Angel, R., Wilks, G., Dersjant-Li, Y., 2022. Global survey of limestone used in poultry diets: calcium content, particle size, and solubility. J. Appl. Anim. Nutr. 10, 19–30. https://doi.org/10.3920/JAAN2021.0015.

Gonzalez-Vega, J.C., Walk, C.L., Liu, Y., Stein, H.H., 2013. Determination of endogenous intestinal losses of calcium and true total tract digestibility of calcium in canola meal fed to growing pigs. J. Anim. Sci. 92, 4807–4816. https://doi.org/10.2527/jas.2013-6410.

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

González-Vega, J.C., Walk, C.L., Stein, H.H., 2015a. 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. https://doi.org/10.2527/jas.2014–8215.

González-Vega, J.C., Liu, Y., McCann, J.C., Walk, C.L., Loor, J.J., Stein, H.H., 2016. 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. https://doi.org/10.2527/jas.2016-0444.

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

Lagos, L.V., Bedford, M.R., Stein, H.H., 2021. Increased microbial phytase increased phytate destruction, plasma inositol, and feed efficiency of weanling pigs, but reduced dietary calcium and phosphorus did not affect gastric pH or fecal score and reduced growth performance and bone ash. J. Anim. Sci. 99, skab333. https://doi.org/10.1093/jas/skab333.

Lagos, L.V., Bedford, M.R., Stein, H.H., 2022. Apparent digestibility of energy and nutrients and efficiency of microbial phytase is influenced by body weight of pigs. J. Anim. Sci. 100, skac269. https://doi.org/10.1093/jas/skac269.

Lagos, L.V., Woodworth, J.C., Kim, S.W., Stein, H.H., 2023. Short communication: commerical diets for pigs in the united states contain more calcium than formulated. J. Anim. Sci. 101, skad102. https://doi.org/10.1093/jas/skad102.

Lautrou, M., Nancy, A., Dourmad, J.Y., Pomar, C., Schmidely, P., Letourneau-Montminy, M.P., 2021. Dietary phosphorus and calcium utilization in growing pigs: requirements and improvements. Front. Vet. Sci. 8, 734365. https://doi.org/10.3389/fvets.2021.734365.

Lee, S.A., Stein, H.H., 2023. Digestibility and availability of nutrients in feed ingredients. In: Chiba, L.I. (Ed.), Sustainable Swine Nutrition. 2nd rev. ed. https://doi.org/10.1002/9781119583998.ch19.

Lee, S.A., Lagos, L.V., Walk, C.L., Stein, H.H., 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. https://doi.org/10.1093/jas/skz176.

Lee, S.A., Lagos, L.V., Merriman, L.A., Stein, H.H., 2023. Digestibility of calcium in calcium-containing ingredients and requirements for digestible calcium by growing pigs. J. Anim. Sci. 101, skad328. https://doi.org/10.1093/jas/skad328.

Merriman, L.A., Stein, H.H., 2016. Particle size of calcium carbonate does not affect apparent and standardized total tract digestibility of calcium, retention of calcium, or growth performance of growing pigs. J. Anim. Sci. 94, 3844–3850. https://doi.org/10.2527/jas.2015-0252.

Nelson, M.E., Lee, S.A., Dersjant-Li, Y., Remus, J., Stein, H.H., 2022. Microbial phytase reduces basal endogenous loss of calcium in pigs fed diets containing phytate phosphorus at commercial levels. J. Anim. Sci. 100, skac280. https://doi.org/10.1093/jas/skac280.

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC, USA.

Poulsen, H.D., Carlson, D., Nørgaard, J.V., Blaabjerg, K., 2010. Phosphorus digestibility is highly influenced by phytase but slightly by calcium in growing pigs. Livest. Sci. 134, 100–102. https://doi.org/10.1016/j.livsci.2010.06.110.

Selle, P.H., Ravindran, V., 2008. Phytate-degrading enzymes in pig nutrition. Livest. Sci. 113, 99–122. https://doi.org/10.1016/j.livsci.2007.05.014.

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

She, Y., Yongb, S., Ling, L., Chengfei, H., Juntao, L., Ping, L., Defa, L., Xiangshu, P., 2015. Effects of microbial phytase on coefficient of standardized total tract digestibility of phosphorus in growing pigs fed corn and corn co-products, wheat and wheat co-products and oilseed meals. Anim. Feed Sci. Technol. 208, 132–144. https://doi.org/10.1016/j.anifeedsci.2015.07.011.

Stein, H.H., Adeola, O., Cromwell, G.L., Kim, S.W., Mahan, D.C., Miller, P.S., 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. https://doi.org/10.2527/jas.2010-3522.

Tran, G., Sauvant, D., 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. second ed. Wageningen, The Netherlands. Wageningen Acad. Publ. pp. 17–24.

Tukey, J.W., 1977. Exploratory data analysis. Addison-Wesley Pub. Co, Boston, MA, USA.

U.S. Environmental Protection Agency. 2000. Acid digestion of sediments, sludges, and soils, U.S. EPA, Washington, DC, USA. https://www.epa.gov/sites/production/files/2015-12/documents/3050b.pdf (accessed April, 2023).

Walk, C.L., 2016. The influence of calcium on phytase efficacy in non-ruminant animals. Anim. Prod. Sci. 56, 1345–1349. https://doi.org/10.1071/AN15341.