

## The site of net absorption of Ca from the intestinal tract of growing pigs and effect of phytic acid, Ca level and Ca source on Ca digestibility

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An experiment was conducted to test the hypothesis that the standardised digestibility of Ca in calcium carbonate and *Lithothamnium calcareum* Ca is not different regardless of the level of dietary Ca, and that phytic acid affects the digestibility of Ca in these two ingredients to the same degree. The objectives were to determine where in the intestinal tract Ca absorption takes place and if there are measurable quantities of basal endogenous Ca fluxes in the stomach, small intestine or large intestine. Diets contained calcium carbonate or *L. calcareum* Ca as the sole source of Ca, 0% or 1% phytic acid and 0.4% or 0.8% Ca. A Ca-free diet was also formulated and used to measure endogenous fluxes and losses of Ca. Nine growing pigs (initial body weight  $23.8 \pm 1.3$  kg) were cannulated in the duodenum and in the distal ileum, and faecal, ileal and duodenal samples were collected. Duodenal endogenous fluxes of Ca were greater ( $p < 0.05$ ) than ileal endogenous fluxes and total tract endogenous losses of Ca, but ileal endogenous fluxes were less ( $p < 0.05$ ) than total tract endogenous losses. Standardised digestibility of Ca was not affected by the level of phytic acid, but decreased ( $p < 0.05$ ) as Ca level increased in *L. calcareum* Ca diets, but that was not the case if calcium carbonate was the source of Ca (interaction,  $p < 0.05$ ). The standardised duodenal digestibility (SDD), standardised ileal digestibility (SID) and standardised total tract digestibility (STTD) of Ca were not different if calcium carbonate was the source of dietary Ca. However, the STTD of Ca in *L. calcareum* Ca was greater ( $p < 0.05$ ) than the SID and SDD of Ca. The SDD, SID and STTD of Ca in calcium carbonate were greater ( $p < 0.05$ ) than those of *L. calcareum* Ca. In conclusion, under the conditions of this experiment, standardised digestibility of Ca is not affected by the level of phytic acid, but may be affected by dietary Ca level depending on the Ca source. Calcium from calcium carbonate is mostly absorbed before the duodenum, but Ca from *L. calcareum* Ca is mostly absorbed in the jejunum and ileum.

**Keywords:** calcium absorption; digestibility; endogenous fluxes; endogenous losses; phytic acid; pigs

### 1. Introduction

There is a relatively low concentration of Ca in most feed ingredients produced from plants, and Ca, therefore, has to be supplemented to most diets fed to swine. This is accomplished by adding inorganic Ca, such as calcium carbonate, or organic Ca, such as *Lithothamnium calcareum* (*L. calcareum*), to the diet (Melo and Moura 2009). Although calcium carbonate is the most commonly used source of Ca in swine diets, *L. calcareum*

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may also be used (Fialho et al. 1992). However, the digestibility of Ca in this organic source of Ca has not been reported.

Increasing dietary concentrations of Ca from calcium carbonate does not affect apparent digestibility of Ca, but reduces the digestibility of P (Stein et al. 2011). It is, however, not known if the dietary concentration of *L. calcareum* influences Ca absorption. Phytate is an anti-nutritional factor present in plant ingredients. Phytate not only binds P, but also may bind Ca, zinc, copper (Santos 2012), protein, starch and fatty acids (Kies 2005), which reduces the absorption of these nutrients. However, it is not known how phytate affects the digestibility of Ca from calcium carbonate and *L. calcareum* added at different concentrations to swine diets.

The majority of Ca is absorbed in the small intestine of pigs (Moore and Tyler 1955a, 1955b; Partridge 1978; Liu et al. 2000; Schröder and Breves 2006), but it is not known if Ca also may be absorbed in the stomach or in the large intestine of pigs (Moore and Tyler 1955a, 1955b; Partridge 1978; Liu et al. 2000; Schröder and Breves 2006; Metzler-Zebeli et al. 2010). Endogenous Ca may be lost over the total gastrointestinal tract (Moore and Tyler 1955b; Hansard et al. 1961; Fernández 1995; González-Vega et al. 2013), but there are no data to quantify the flux of endogenous Ca into different segments of the gastrointestinal tract. Therefore, the objective of this experiment was to test the hypothesis that the digestibility of Ca in calcium carbonate and *L. calcareum* is not different regardless of the level of Ca in the diet, and that phytic acid affects the digestibility of Ca in these two ingredients to the same degree. The second objective was to determine if Ca absorption also occurs in the stomach or in the large intestine. The third objective was to determine if there are measurable quantities of basal endogenous Ca fluxes in the stomach, the small intestine or the large intestine.

## 2. Materials and methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol (11098) for the experiment.

### 2.1. Diets, animals and experimental design

The two sources of Ca that were used in this experiment were Vistacal and calcium carbonate. Vistacal was produced by *L. calcareum* algae and contained 32.7% Ca (as-fed basis; Celtic Sea Minerals, Currabinny, Co. Cork, Ireland). Calcium carbonate, which was mined in Iowa (USA) and contained 39% Ca, was procured from ILC Resources, Alden, IA (Table 1). A 50% solution of phytic acid (Sigma-Aldrich, St. Louis, MO) was also used.

A total of nine diets were formulated (Tables 2 and 3). Eight diets were used in a  $2 \times 2 \times 2$  factorial arrangement. There were two sources of Ca (calcium carbonate and *L. calcareum*), two levels of phytic acid (0% and 1%) and two levels of dietary Ca (0.4% and 0.8% Ca), which correspond to 66.7% and 133.3% of the Ca requirement, respectively (NRC 1998). Calcium carbonate or *L. calcareum* Ca was the sole source of Ca in these diets. A Ca-free diet based on sucrose, cornstarch, potato protein isolate, corn gluten meal, vitamins and minerals was formulated and used to measure endogenous fluxes and losses of Ca. Monosodium phosphate was the source of inorganic P. Chromic oxide was included in all diets at 0.4% as an indigestible marker.

Nine growing pigs (initial body weight [BW]  $23.8 \pm 1.3$  kg) were surgically equipped with a T-cannula in the duodenum and another T-cannula in the distal ileum (McGinnis et al. 2007). After a 10-d recovery period, pigs were allotted to a  $9 \times 6$  incomplete Latin square design with nine diets and six periods. Pigs used in the experiment were the

Table 1. Composition of ingredients (as-fed basis).

	Potato protein isolate	Corn gluten meal	Calcium carbonate	<i>L. calcareum</i> Ca*	Monosodium phosphate
Gross energy [MJ/kg]	22.0	22.5	–	–	–
DM [%]	91.19	91.46	99.99	98.36	98.99
Crude protein [%]	80.75	63.3	–	–	–
Ash [%]	0.48	2.59	96.08	91.14	90.61
AEE <sup>#</sup> [%]	0.50	4.60	–	–	–
ADF [%]	3.60	6.93	–	–	–
NDF [%]	1.12	5.57	–	–	–
Ca [%]	0.03	0.09	39.11	32.70	0.08
P [%]	0.12	0.54	–	–	29.69
Phytase [FTU/kg] <sup>§</sup>	52	94	–	–	–
Phytic acid [%]	0.33	1.60	–	–	–
Phytate-bound P <sup>†</sup> [%]	0.09	0.45	–	–	–
Non-phytate P <sup>‡</sup> [%]	0.03	0.09	–	–	–
pH	–	–	8.14	7.88	–

Notes: \*Source of Ca produced by *Lithothamnium calcareum* (Vistacal, Celtic Sea Minerals, Currabinny, Co. Cork, Ireland); <sup>#</sup>AEE, Acid-hydrolysed ether extract; <sup>§</sup>FTU, Phytase units; <sup>†</sup>Phytate-bound P was calculated as 28.2% of phytic acid (Tran and Sauvant 2004); <sup>‡</sup>Non-phytate P was calculated as the difference between total P and phytate-bound P.

offspring of G-Performer boars and Fertilis 25 females (Geneticporc, Alexandria, MN, USA).

## 2.2. Feeding and sample collections

Pigs were fed experimental diets during six 10-d periods, and no pigs received any diet more than once. The daily allotments of feed were calculated as three times the daily energy requirement for maintenance (i.e. 443.5 kJ ME/kg BW<sup>0.75</sup>; NRC 1998) and divided into two equal meals that were fed at 07:00 and 19:00 h. Faecal samples were collected from the pigs on days 5 and 6. Ileal samples were collected consecutively for 8 h on days 7 and 8 starting at 07:00 h. Duodenal samples were collected on day 9 from 07:00 to 09:00 h, from 11:00 to 13:00 h and from 15:00 to 17:00 h. On day 10, duodenal samples were collected from 09:00 to 11:00 h, from 13:00 to 15:00 h and from 17:00 to 19:00 h. Faecal, ileal and duodenal samples were stored at –20°C immediately after collection.

## 2.3. Sample analysis

Before chemical analysis, duodenal and ileal samples were thawed at room temperature and mixed within animal and diet, and two subsamples were collected. Duodenal and ileal samples were lyophilised and ground. Faecal samples were dried in a forced-air oven at 65°C and then ground. Ingredients, diets and duodenal, ileal and faecal samples were analysed according to the methods of the Association of Official Analytical Chemists (AOAC 2007). Potato protein isolate, corn gluten meal, calcium carbonate, *L. calcareum* Ca, monosodium phosphate, diets and duodenal, ileal and faecal samples were analysed for Ca by an atomic absorption spectrophotometer procedure (AOAC Method 968.08) after wet digestion sample preparation (AOAC Method 935.13). The concentrations of P

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

Ingredient [%]	Experimental diet									
	Ca from calcium carbonate					Ca from <i>L. calcaireum</i> *				
	0% phytic acid		1% phytic acid			0% phytic acid		1% phytic acid		
	0.40% Ca	0.80% Ca	0.40% Ca	0.80% Ca	10.00	0.40% Ca	0.80% Ca	0.40% Ca	0.80% Ca	Ca free
Corn gluten meal	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Cornstarch	47.94	46.94	45.94	44.94	47.69	46.42	45.69	44.42	44.42	48.94
Sucrose	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Potato protein isolate	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Soybean oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Calcium carbonate	1.00	2.00	1.00	2.00	—	—	—	—	—	—
<i>L. calcaireum</i> Ca	—	—	—	—	1.25	2.52	1.25	2.52	2.52	—
Monosodium phosphate	1.02	1.02	1.02	1.02	1.02	1.02	1.02	1.02	1.02	1.02
Phytic acid <sup>‡</sup>	—	—	2.00	2.00	—	—	2.00	2.00	2.00	—
L-Lysine HCl	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
DL-Methionine	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
L-Tryptophan	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Potassium carbonate	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Magnesium oxide	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Sodium chloride	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Solka flocc <sup>§</sup>	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Vitamin mineral premix <sup>†</sup>	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Chromic oxide	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Notes: \**L. calcaireum* Ca is a source of Ca produced by *Lithothamnium calcaireum* (Vistaal, Celtic Sea Minerals, Currabinnny, Co. Cork, Ireland); <sup>‡</sup>Phytic acid, 50%, Sigma-Aldrich, St. Louis, MO; <sup>§</sup>Fiber Sales and Development Corp., Urbana, OH; <sup>†</sup>Provided the following quantities of vitamins and trace elements minerals per kilogram of complete diet: vitamin A (as retinyl acetate), 11,128 IU; vitamin D<sub>3</sub> (as cholecalciferol), 2204 IU; vitamin E (as DL-alpha tocopheryl acetate), 66 IU; vitamin K (as menadione nicotinamide bisulfite), 1.42 mg; thiamin (as thiamine mononitrate), 0.24 mg; riboflavin, 6.58 mg; pyridoxine (as pyridoxine hydrochloride), 0.24 mg; vitamin B<sub>12</sub>, 0.03 mg; D-pantothenic acid (as D-calcium pantothenate), 23.5 mg; niacin (as nicotinamide and nicotinic acid), 44 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu (as copper sulfate), 10 mg; Fe (as iron sulfate), 125 mg; I (as potassium iodate), 1.26 mg; Mn (as manganese sulfate), 60 mg; Se (as sodium selenite), 0.3 mg; Zn (as zinc oxide), 100 mg.

Table 3. Analysed composition of experimental diets (as-fed basis).

	Experimental diet											
	Ca from calcium carbonate				Ca from <i>L. calcaireum</i> *				Ca free			
	0% phytic acid <sup>#</sup>		1% phytic acid		0% phytic acid		1% phytic acid		0% phytic acid		1% phytic acid	
	0.40% Ca	0.80% Ca	0.40% Ca	0.80% Ca	0.40% Ca	0.80% Ca	0.40% Ca	0.80% Ca	0.40% Ca	0.80% Ca	0.40% Ca	0.80% Ca
Gross energy [MJ/kg]	17.7	17.9	17.9	17.4	17.6	17.4	17.6	17.4	17.6	17.2	17.6	17.2
DM [%]	92.00	92.46	91.43	91.59	92.43	92.50	92.43	92.50	89.97	89.78	89.97	89.78
Crude protein [%]	17.0	16.7	16.4	16.4	16.5	16.9	16.5	16.9	16.8	16.7	16.8	16.7
Ash [%]	3.13	3.95	3.85	4.43	3.03	4.43	3.03	4.43	3.98	5.02	3.98	5.02
AEE <sup>§</sup> [%]	3.69	3.85	3.61	3.83	3.76	3.87	3.76	3.87	3.69	3.50	3.69	3.50
Ca [%]	0.46	0.87	0.47	0.85	0.46	0.83	0.46	0.83	0.48	0.85	0.48	0.85
P [%]	0.39	0.42	0.59	0.64	0.40	0.37	0.40	0.37	0.65	0.66	0.65	0.66
ADF [%]	3.36	3.44	3.19	3.36	3.11	3.60	3.11	3.60	3.31	3.84	3.31	3.84
NDF [%]	3.16	3.25	2.89	2.83	3.39	2.90	3.39	2.90	2.91	3.09	2.91	3.09
Phytase [FTU <sup>†</sup> /kg]	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50	<50
Phytic acid [%]	0.38	0.37	1.25	1.25	0.37	0.36	0.37	0.36	1.24	1.21	1.24	1.21
Phytate-bound P <sup>‡</sup> [%]	0.13	0.14	0.35	0.35	0.14	0.12	0.14	0.12	0.35	0.34	0.35	0.34
Non-phytate P <sup>•</sup> [%]	0.19	0.22	0.24	0.31	0.23	0.24	0.23	0.24	0.28	0.29	0.28	0.29
pH	5.38	6.23	3.79	3.46	5.72	5.71	5.72	5.71	4.28	4.53	4.28	4.53

Notes: \**L. calcaireum* Ca is a source of Ca produced by *Lithothamnium calcaireum* (Vistiagal, Celtic Sea Minerals, Currabimny, Co. Cork, Ireland); <sup>#</sup>Phytic acid, 50%, Sigma-Aldrich, St. Louis, MO; <sup>§</sup>AEE, Acid-hydrolysed ether extract; <sup>†</sup>FTU, Phytase units; <sup>‡</sup>Phytate-bound P was calculated as 28.2% of phytic acid (Tran and Sauvant 2004); <sup>•</sup>Non-phytate P was calculated as the difference between total P and phytate-bound P.

in potato protein isolate, corn gluten meal, monosodium phosphate, diets and duodenal, ileal and faecal samples were analysed using a colorimetric procedure (AOAC Method 931.01). Potato protein isolate, corn gluten meal, calcium carbonate, *L. calcareum* Ca, monosodium phosphate, diets and duodenal, ileal and faecal samples were also analysed for DM by oven drying at 135°C for 2 h (AOAC Method 930.15), and the ingredient and diet samples were also analysed for ash (AOAC Method 942.05). Potato protein isolate, corn gluten meal and diets were analysed for phytase activity (Engelen et al. 2001) and for phytic acid (Megazyme Method; AB Vista, Ystrad Mynach, UK). These samples were also analysed for gross energy using an adiabatic bomb calorimeter (Model 6300, Parr Instruments, Moline, IL, USA), for crude protein using the combustion procedure (AOAC Method 990.03) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ, USA) and for acid-hydrolysed ether extract (AEE) using 3N HCl (Sanderson 1986) followed by crude fat extraction with petroleum ether (AOAC Method 2003.06) on a Soxtec 2050 automated analyser (FOSS North America, Eden Prairie, MN, USA). These samples were also analysed for ADF (AOAC Method 973.18) and NDF (Holst 1973). For diets, calcium carbonate and *L. calcareum* Ca, 5 g of each sample was mixed with 15 ml of distilled water and filtered, and pH was measured in the solution with a pH meter (Accumet Basic, Fisher Scientific, Pittsburgh, PA, USA). For duodenal and ileal samples, pH was measured in the collected samples. Diets and duodenal, ileal, and faecal samples were analysed for chromium by inductively coupled plasma atomic emission spectrometry method (AOAC Method 990.08) after nitric acid–perchloric acid wet ash sample preparation (AOAC Method 968.08D).

#### 2.4. Calculations

Apparent duodenal digestibility (ADD) of Ca was calculated using the following equation (Stein et al. 2007):

$$\text{ADD} [\%] = \left[ 1 - \left( \text{Ca}_{\text{digesta}} / \text{Ca}_{\text{diet}} \right) \cdot \left( \text{M}_{\text{diet}} / \text{M}_{\text{digesta}} \right) \right] \cdot 100, \quad (1)$$

where ADD is the apparent duodenal digestibility [%] of Ca,  $\text{Ca}_{\text{digesta}}$  and  $\text{Ca}_{\text{diet}}$  are the Ca concentration [g/kg DM] in duodenal samples and diets, respectively and  $\text{M}_{\text{diet}}$  and  $\text{M}_{\text{digesta}}$  are the marker concentrations [g/kg DM] in diet and duodenal samples, respectively.

The apparent ileal digestibility (AID) and the apparent total tract digestibility (ATTD) of Ca [%] were calculated as ADD except that Ca and marker concentrations in ileal or faecal samples were used rather than in duodenal samples.

Basal endogenous duodenal fluxes of Ca (ECaF) were calculated from pigs fed the Ca-free diet according to the following equation (Stein et al. 2007):

$$\text{Duodenal ECaF [g/kg DMI]} = \text{Ca}_{\text{digesta}} \cdot \left( \text{M}_{\text{diet}} / \text{M}_{\text{digesta}} \right), \quad (2)$$

where ECaF is the basal duodenal flow of Ca [g/kg DMI]. The basal ileal ECaF and the basal total tract endogenous losses of Ca were calculated as duodenal ECaF except that Ca and marker concentrations in ileal digesta or faecal samples were used rather than in duodenal samples.

The standardised duodenal digestibility (SDD) of Ca was calculated according to the following equation (Stein et al. 2007):

$$\text{SDD} [\%] = \text{ADD} + \left[ \left( \frac{\text{duodenal ECaF}}{\text{Ca}_{\text{diet}}} \right) \cdot 100 \right], \quad (3)$$

where SDD is the standardised duodenal digestibility [%] of Ca. The standardised ileal digestibility (SID) and standardised total tract digestibility (STTD) of Ca [%] were calculated as the SDD of Ca, with the exception that values for the AID or ATTD were used instead of ADD and values for ileal ECaF or total tract ECaL were used rather than the duodenal ECaF. Apparent and standardised duodenal, ileal and total tract fluxes [g/d] were calculated by multiplying the Ca intake [g/d] with the percentage of non-digestible Ca, which was calculated by subtracting the digestibility values [%] from 100.

### 2.5. Statistical analyses

Data for Ca and P digestibility and fluxes were analysed as a  $2 \times 2 \times 2 \times 3$  factorial and digesta pH data were analysed as a  $2 \times 2 \times 2 \times 2$  factorial using the MIXED procedure (SAS Inst. Inc., Cary, NC, USA) with a randomised complete block design. The model included the fixed effects of Ca level, phytic acid level, Ca source, collection site and all possible interactions and the random effects of period and pig. Means were calculated using the LSMeans statement, and if significant differences were observed, means were separated using the PDIF option. For endogenous fluxes and losses of Ca, data were analysed using the MIXED procedure, the model included collection site as fixed effect and period as random effect and means were calculated using the LSMeans statement. The UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC, USA) was used to determine the normality of residuals, and this procedure was also used to identify outliers. Pig was the experimental unit for all analyses. An  $\alpha$ -value of 0.05 was used to assess significance among treatments.

## 3. Results

Pigs remained healthy and consumed their respective diets. Values of analysed concentration of Ca in diets were up to 0.08% greater than expected and values for analysed P in the diets were from 0.06% less to 0.05% greater than expected (Table 3). However, it was assumed that results of the experiment were not affected by the differences between analysed and expected values because the expected differences among diets were obtained. Analysed values for phytic acid were up to 0.18% greater than expected for the diets that contained 0% phytic acid, but for diets containing 1% phytic acid, all analysed values were close to expected values. The pH of diets that contained 0% phytic acid ranged from 5.38 to 6.23 and the pH of diets that contained 1% phytic acid ranged from 3.46 to 4.53 and the pH of the Ca-free diet was 5.27.

Duodenal endogenous fluxes of Ca (1.03 g/kg DMI) were greater ( $p < 0.05$ ) than that of ileal endogenous fluxes (0.42 g/kg DMI) and total tract endogenous losses of Ca (0.67 g/kg DMI), but ileal endogenous fluxes were less ( $p < 0.05$ ) than total tract endogenous losses of Ca. Mean values for ADD, AID and ATTD of Ca and P; SDD, SID and STTD of Ca and digesta pH in the experimental diets are shown in Table 4. If calcium carbonate was the source of Ca, the ADD, AID and ATTD of Ca were not different, and this was also the case for SDD, SID and STTD of Ca (Table 5). If *L. calcareum* Ca was the source of Ca, the AID and ATTD of Ca were not different, but were greater ( $p < 0.05$ ) than the ADD of Ca (Ca source  $\times$  site,  $p <$

Table 4. Apparent and standardised digestibility of Ca, apparent digestibility of P and digesta pH in pigs fed experimental diets.

	Experimental diet										Pooled SEM <sup>†</sup>
	Ca from calcium carbonate					Ca from <i>L. calcareum</i>					
	0% phytic acid		1% phytic acid		0.80% Ca	0% phytic acid		1% phytic acid		0.80% Ca	
0.40% Ca	0.80% Ca	0.40% Ca	0.80% Ca	0.40% Ca		0.80% Ca	0.40% Ca	0.80% Ca			
Apparent digestibility of Ca [%]											
Duodenal	37.60	47.69	40.92	45.65	13.54	13.44	19.82	17.26	6.78		
Ileal	43.82	50.27	38.91	53.09	45.12	32.53	35.64	29.57			
Total tract	55.67	43.79	45.29	60.08	48.29	40.93	36.80	33.57			
Standardised digestibility of Ca [%]											
Duodenal	58.14	58.60	60.90	56.75	34.22	24.86	39.11	28.06	6.78		
Ileal	52.18	54.71	47.05	57.60	53.54	37.18	43.50	33.96			
Total tract	68.98	50.86	58.24	67.27	61.70	48.33	49.30	40.57			
Apparent digestibility of P [%]											
Duodenal	26.07	38.98	22.20	25.13	28.06	17.56	30.06	20.60	4.98		
Ileal	74.70	74.01	46.37	49.99	63.37	40.69	49.84	33.06			
Total tract	76.82	74.69	65.87	64.38	69.01	47.43	58.30	45.55			
Digesta pH											
Duodenal	5.13	5.43	4.69	4.63	5.29	5.37	5.06	4.94	0.11		
Ileal	7.32	7.24	7.43	7.20	7.08	7.32	7.43	7.37			

Note: <sup>†</sup>SEM, Standard error of the mean.



Table 5. Apparent and standardised duodenal, ileal and total tract digestibility of Ca; apparent duodenal, ileal and total tract digestibility of P and duodenal and ileal digesta pH in pigs fed diets containing calcium carbonate or *L. calcaireum* Ca.

	Digestibility of Ca [%]		Apparent digestibility of P [%]	Digesta pH
	Apparent	Standardised		
Ca from calcium carbonate				
Duodenal	42.97 <sup>abc</sup>	58.60 <sup>ab</sup>	28.10 <sup>e</sup>	4.97 <sup>c</sup>
Ileal	46.52 <sup>ab</sup>	52.89 <sup>ab</sup>	61.27 <sup>b</sup>	7.30 <sup>a</sup>
Total tract	51.21 <sup>a</sup>	61.34 <sup>a</sup>	70.44 <sup>a</sup>	–
Ca from <i>L. calcaireum</i>				
Duodenal	16.02 <sup>d</sup>	31.56 <sup>d</sup>	24.07 <sup>e</sup>	5.17 <sup>b</sup>
Ileal	35.71 <sup>c</sup>	42.05 <sup>c</sup>	46.74 <sup>d</sup>	7.30 <sup>a</sup>
Total tract	39.90 <sup>bc</sup>	49.98 <sup>bc</sup>	55.07 <sup>c</sup>	–
Pooled SEM <sup>†</sup>	4.01	4.01	3.43	0.06
<i>p</i> -values				
Source × site	0.017	0.017	0.010	0.068
Site	<0.001	0.002	<0.001	<0.001

Notes: <sup>†</sup>SEM, Standard error of the mean; <sup>a-c</sup>Means within a row lacking a common superscript letter are different ( $p < 0.05$ ).

0.05), and this was also the case for SDD, SID and STTD of Ca. The ADD, AID and ATTD of Ca were greater ( $p < 0.05$ ) if calcium carbonate was the source of Ca than when *L. calcaireum* Ca was used, and this was also the case for SDD, SID and STTD of Ca. For both calcium carbonate and *L. calcaireum* Ca, the ATTD of P was greater ( $p < 0.05$ ) than the AID and ADD of P, but AID was greater ( $p < 0.05$ ) than ADD of P. The ADD of P was not different between calcium carbonate and *L. calcaireum* Ca, but AID and ATTD of P were greater ( $p < 0.05$ ) in calcium carbonate than that in *L. calcaireum* Ca (Ca source × site,  $p < 0.05$ ). The ileal digesta pH was greater ( $p < 0.05$ ) than duodenal digesta pH for both Ca sources.

If calcium carbonate was the source of Ca, the apparent digestibility of Ca was not different between diets containing 0.4% and 0.8% Ca, and this was also the case for the standardised digestibility of Ca (Table 6). If *L. calcaireum* Ca was the source of Ca, the apparent digestibility of Ca was not different between diets containing 0.4% and 0.8% Ca, but the standardised digestibility of Ca was greater ( $p < 0.05$ ) if diets contained 0.4% Ca than 0.8% Ca (Ca level × Ca source,  $p < 0.05$ ). For both levels of Ca, values for apparent digestibility of Ca in calcium carbonate were greater ( $p < 0.05$ ) than in *L. calcaireum* Ca, and this was also the case for standardised digestibility of Ca. If calcium carbonate was the source of Ca, the apparent digestibility of P was not different between diets containing 0.4% and 0.8% Ca, but if *L. calcaireum* Ca was the source of Ca, the apparent digestibility of P was greater ( $p < 0.05$ ) if diets contained 0.4% Ca than 0.8% Ca (Ca level × Ca source,  $p < 0.001$ ). If diets contained 0.4% Ca, no differences were observed in apparent digestibility of P between both Ca sources, but if diets contained 0.8% Ca the apparent digestibility of P was greater ( $p < 0.05$ ) in diets containing calcium carbonate than in diets containing *L. calcaireum* Ca. Calcium source and Ca level did not affect the digesta pH.

There was no effect of phytic acid on apparent and standardised digestibility of Ca (Table 7). If calcium carbonate was the source of Ca, the apparent digestibility of P was greater ( $p < 0.05$ ) if diets contained 0% phytic acid than 1% phytic acid. But if *L. calcaireum* Ca was the source of Ca, the apparent digestibility of P was not different between diets with 0% phytic acid and diets with 1% phytic acid (phytic acid × Ca source,

Table 6. Apparent and standardised digestibility of Ca, apparent digestibility of P and digesta pH in pigs fed diets containing calcium carbonate or *L. calcaireum* Ca at 0.4% or 0.8% Ca.

	Digestibility of Ca [%]		Apparent digestibility of P [%]	Digesta pH
	Apparent	Standardised		
Ca from calcium carbonate				
0.4% Ca	43.70 <sup>a</sup>	57.58 <sup>a</sup>	52.01 <sup>a</sup>	6.14
0.8% Ca	50.10 <sup>a</sup>	57.63 <sup>a</sup>	54.53 <sup>a</sup>	6.12
Ca from <i>L. calcaireum</i>				
0.4% Ca	33.20 <sup>b</sup>	46.90 <sup>b</sup>	49.77 <sup>a</sup>	6.22
0.8% Ca	27.88 <sup>b</sup>	35.49 <sup>c</sup>	34.15 <sup>b</sup>	6.25
Pooled SEM <sup>†</sup>	3.63	3.63	3.24	0.06
<i>p</i> -values				
Ca level × source	0.028	0.032	<0.001	0.608
Source	<0.001	<0.001	<0.001	0.063
Ca level	0.840	0.035	<0.001	0.882

Notes: <sup>†</sup>SEM, standard error of the mean; <sup>a-c</sup>Means within a row lacking a common superscript letter are different ( $p < 0.05$ ).

Table 7. Apparent and standardised digestibility of Ca, apparent digestibility of P and digesta pH in pigs fed diets containing calcium carbonate or *L. calcaireum* Ca without or with phytic acid.

	Digestibility of Ca [%]		Apparent digestibility of P [%]	Digesta pH
	Apparent	Standardised		
Ca from calcium carbonate				
0% phytic acid	46.47	57.25	60.88 <sup>a</sup>	6.28 <sup>a</sup>
1% phytic acid	47.32	57.97	45.66 <sup>b</sup>	5.99 <sup>b</sup>
Ca from <i>L. calcaireum</i>				
0% phytic acid	32.31	43.30	44.35 <sup>bc</sup>	6.26 <sup>a</sup>
1% phytic acid	28.78	39.08	39.57 <sup>c</sup>	6.20 <sup>a</sup>
Pooled SEM <sup>†</sup>	3.65	3.65	3.26	0.06
<i>p</i> -values				
Phytic acid × source	0.423	0.366	0.005	0.040
Phytic acid	0.622	0.521	<0.001	0.002

Notes: <sup>†</sup>SEM, standard error of the mean; <sup>a-c</sup>Means within a row lacking a common superscript letter are different ( $p < 0.05$ ).

$p < 0.01$ ). The apparent digestibility of P was greater ( $p < 0.05$ ) in calcium carbonate diets than in *L. calcaireum* Ca diets. If diets contained 0% phytic acid, no differences were observed in digesta pH between the two sources of Ca, but if diets contained 1% phytic acid, the digesta pH was greater ( $p < 0.05$ ) in *L. calcaireum* Ca diets than in calcium carbonate diets (phytic acid × Ca source,  $p < 0.05$ ).

If diets contained 0% phytic acid, the AID and ATTD of P were not different, but were greater ( $p < 0.05$ ) than the ADD of P (Table 8). If diets contained 1% phytic acid, the ATTD of P was greater ( $p < 0.05$ ) than AID and ADD of P, and AID was greater ( $p < 0.05$ ) than ADD of P (Phytic acid × site,  $p < 0.05$ ). The ADD of P was not different between diets containing 0% or 1% phytic acid, but the AID and ATTD of P were greater ( $p < 0.05$ ) if diets contained 0% phytic acid than 1% phytic acid. For both diets containing 0% or 1% phytic acid, the ileal digesta pH was greater ( $p < 0.05$ ) than the duodenal

Table 8. Apparent and standardised duodenal, ileal and total tract digestibility of Ca; apparent duodenal, ileal and total tract digestibility of P and duodenal and ileal digesta pH in pigs fed diets without or with phytic acid.

	Digestibility of Ca [%]		Apparent digestibility of P [%]	Digesta pH
	Apparent	Standardised		
0% phytic acid				
Duodenal	28.07	43.95	27.67 <sup>d</sup>	5.31 <sup>b</sup>
Ileal	42.93	49.40	63.19 <sup>ab</sup>	7.24 <sup>a</sup>
Total tract	47.17	57.47	66.99 <sup>a</sup>	–
1% phytic acid				
Duodenal	30.91	46.21	24.50 <sup>d</sup>	4.83 <sup>c</sup>
Ileal	39.30	45.53	44.81 <sup>c</sup>	7.36 <sup>a</sup>
Total tract	43.93	53.84	58.52 <sup>b</sup>	–
Pooled SEM <sup>†</sup>	4.02	4.02	3.43	0.06
<i>p</i> -values				
Phytic acid × site	0.512	0.543	0.001	<0.001

Notes: <sup>†</sup>SEM, standard error of the mean; <sup>a-d</sup>Means within a row lacking a common superscript letter are different ( $p < 0.05$ ).

digesta pH. The ileal digesta pH was not different between diets containing 0% or 1% phytic acid, but the duodenal digesta pH was greater ( $p < 0.05$ ) if diets contained 0% phytic acid than 1% phytic acid (phytic acid × site,  $p < 0.05$ ).

Calcium level did not affect the digesta pH regardless of the level of phytic acid. If diets contained 0.4% Ca, the digesta pH was not different between diets containing 0% or 1% phytic acid (Table 9). But if diets contained 0.8% Ca, digesta pH was greater ( $p < 0.05$ ) if diets contained 0% phytic acid than 1% phytic acid (phytic acid × Ca level,  $p < 0.05$ ).

If calcium carbonate was the source of Ca, the apparent duodenal, ileal and total tract fluxes of Ca were not different, and this was also the case for standardised duodenal, ileal and total tract fluxes of Ca (Table 10). If *L. calcareum* Ca was the source of Ca, the apparent ileal and total tract fluxes were not different, but were less ( $p < 0.05$ ) than the duodenal fluxes of Ca (Ca source × site,  $p < 0.05$ ), and this was also the case for

Table 9. Apparent and standardised digestibility of Ca, apparent digestibility of P and digesta pH in pigs fed diets without or with phytic acid at 0.4% or 0.8% Ca.

	Digestibility of Ca [%]		Apparent digestibility of P [%]	Digesta pH
	Apparent	Standardised		
0% phytic acid				
0.4% Ca	40.67	54.79	56.34	6.20 <sup>ab</sup>
0.8% Ca	38.11	45.76	48.90	6.34 <sup>a</sup>
1% phytic acid				
0.4% Ca	36.23	49.68	45.44	6.15 <sup>bc</sup>
0.8% Ca	39.87	47.37	39.78	6.03 <sup>c</sup>
Pooled SEM <sup>†</sup>	3.65	3.65	3.26	0.06
<i>p</i> -values				
Phytic acid × Ca level	0.254	0.216	0.618	0.018

Notes: <sup>†</sup>SEM, standard error of the mean; <sup>a-c</sup>Means within a row lacking a common superscript letter are different ( $p < 0.05$ ).

Table 10. Apparent and standardised duodenal, ileal and total tract fluxes of Ca in pigs fed diets containing calcium carbonate or *L. calcareum* Ca.

	Fluxes of Ca [g/d]	
	Apparent	Standardised
Ca from calcium carbonate		
Duodenal	3.94 <sup>bcd</sup>	2.86 <sup>cd</sup>
Ileal	3.69 <sup>cd</sup>	3.25 <sup>cd</sup>
Total tract	3.37 <sup>d</sup>	2.67 <sup>d</sup>
Ca from <i>L. calcareum</i>		
Duodenal	5.79 <sup>a</sup>	4.72 <sup>a</sup>
Ileal	4.44 <sup>b</sup>	4.00 <sup>b</sup>
Total tract	4.15 <sup>bc</sup>	3.45 <sup>bc</sup>
Pooled SEM <sup>†</sup>	0.28	0.28
<i>p</i> -values		
Source × site	0.017	0.017
Site	<0.001	0.002

Notes: <sup>†</sup>SEM, standard error of the mean; <sup>a-d</sup>Means within a row lacking a common superscript letter are different ( $p < 0.05$ ).

standardised duodenal, ileal and total tract fluxes of Ca. The apparent duodenal, ileal and total tract fluxes of Ca were greater ( $p < 0.05$ ) if *L. calcareum* Ca was the source of Ca than if calcium carbonate was used, and this was also the case for standardised duodenal, ileal and total tract fluxes of Ca.

If calcium carbonate was the source of Ca, the apparent flux of Ca was not different between diets containing 0.4% and 0.8% Ca, and this was also the case for the standardised digestibility of Ca (Table 11). If *L. calcareum* Ca was the source of Ca, the apparent flux of Ca was not different between diets containing 0.4% and 0.8% Ca, but the standardised flux of Ca was greater ( $p < 0.05$ ) if diets contained 0.8% Ca than 0.4% Ca (Ca level × Ca source,  $p < 0.05$ ). For both levels of Ca, values for apparent flux of Ca in

Table 11. Apparent and standardised fluxes of Ca in pigs fed diets containing calcium carbonate or *L. calcareum* Ca at 0.4% or 0.8% Ca.

	Fluxes of Ca [g/d]	
	Apparent	Standardised
Ca from calcium carbonate		
0.4% Ca	3.88 <sup>b</sup>	2.93 <sup>c</sup>
0.8% Ca	3.44 <sup>b</sup>	2.92 <sup>c</sup>
Ca from <i>L. calcareum</i>		
0.4% Ca	4.61 <sup>a</sup>	3.66 <sup>b</sup>
0.8% Ca	4.98 <sup>a</sup>	4.45 <sup>a</sup>
Pooled SEM <sup>†</sup>	0.25	0.25
<i>p</i> -values		
Ca level × source	0.028	0.032
Source	<0.001	<0.001
Ca level	0.840	0.035

Notes: <sup>†</sup>SEM, standard error of the mean; <sup>a-c</sup>Means within a row lacking a common superscript letter are different ( $p < 0.05$ ).

*L. calcareum* Ca were greater ( $p < 0.05$ ) than that in calcium carbonate, and this was also the case for the standardised flux of Ca.

#### 4. Discussion

The concentrations of Ca in calcium carbonate and in *L. calcareum* that were analysed in this experiment are in agreement with reported values (Sauvant et al. 2004; Melo and Moura 2009; Stein et al. 2011; NRC 2012). However, the concentration of P in the monosodium phosphate used in this experiment was slightly greater than previous values (Sauvant et al. 2004; NRC 2012).

The relatively high duodenal endogenous flux of Ca is likely caused by Ca secreted in saliva (Tryon and Bibby 1966), gastric juice (Trautmann and Kirchhof 1937; Moore and Tyler 1955b), pancreatic juice (Gamble and McIver 1928; Partridge et al. 1982; Fernández 1995; Bronner 1997) and bile (Sullivan et al. 1981; Allen 1982; Bronner 1997). It is likely that a portion of these endogenous secretions of Ca was reabsorbed in the small intestine (Allen 1982), which is the reason the ileal endogenous flux was less than the duodenal flux. The observation that ileal endogenous fluxes were not different from total tract endogenous losses of Ca indicates that there may be no secretion or absorption of Ca in the large intestine.

It was surprising that Ca digestibility was not affected by inclusion of phytic acid to the diets, because phytic acid may bind Ca, which is believed to reduce the digestibility of Ca (Selle et al. 2009). Phytate in plant ingredients is often bound to magnesium and potassium ions and has greater affinity for zinc and copper than for Ca, but because Ca is included in high amounts in mixed diets, Ca–phytate complexes may be formed (Selle et al. 2009). It is, however, possible that there are differences in the properties of phytate between natural phytate in plant ingredients and synthetic phytate, which was used in this experiment (Onyango et al. 2009; Santos 2012). Solubility of free synthetic phytate is greater than magnesium–potassium phytate, which indicates that synthetic phytate is more susceptible to be hydrolysed than magnesium–potassium phytate (Onyango et al. 2009). Factors such as pH, temperature and ionic strength also influence the binding between phytate and Ca (Graft 1983).

The observation that the ATTD of Ca was not affected by the level of dietary Ca is in agreement with the observation that increasing dietary Ca levels from 55% to 173% of the requirement of growing pigs does not influence ATTD of Ca (NRC 1998; Stein et al. 2011). Although Ca absorption from the intestines may be down-regulated by increased Ca intake (Bronner 1987), results of previous experiments have indicated that the main regulatory site for Ca homeostasis is not the intestines. Instead, Ca homeostasis is mainly regulated at the renal level (Stein et al. 2011; González-Vega et al. 2013), and results of the present experiment support the previous results. The fact that the STTD and standardised total tract fluxes of Ca in *L. calcareum* Ca, but not in calcium carbonate, were negatively affected by increasing dietary Ca level may be explained by possible formations of Ca–P complexes (Clark 1969; Brink et al. 1992; Stein et al. 2011). The greater solubility of Ca in *L. calcareum* Ca than of Ca in calcium carbonate may result in increased formation of Ca–P complexes in the intestinal tract of pigs if *L. calcareum* Ca is used (Walk et al. 2012), which may result in reduced digestibility and increased Ca fluxes. Another reason for the reduced formation of Ca–P complexes if calcium carbonate is used may be that most Ca in calcium carbonate was absorbed before the duodenum, and only a small amount of Ca was available in the remaining portion of the small intestine to form Ca–P complexes. In contrast, absorption prior to the duodenal cannula of Ca from

*L. calcareum* Ca was relatively minor; so more Ca entered the distal small intestine, where complexes could be formed.

Although most Ca is absorbed in the small intestine (Moore and Tyler 1955a, 1955b; Partridge 1978; Liu et al. 2000), absorption of Ca in the colon has also been observed (Liu et al. 2000), and Ca absorption is affected by the type of diet that is fed (Partridge 1978). In the present experiment, Ca from calcium carbonate diets was mainly absorbed in the region before the duodenal cannula, which was placed approximately 10 cm distal to the pancreatic duct. This observation indicates that Ca from calcium carbonate is absorbed in the stomach or the early part of the duodenum. However, most of the Ca from *L. calcareum* Ca was absorbed between the duodenal and the ileal cannula, indicating that Ca in *L. calcareum* Ca is released later in the small intestine. Calcium-binding proteins transport the Ca through the enterocyte in the small intestine (Bronner 1998), but they have also been identified in the stomach of pigs (Raeymaekers et al. 1993), indicating that Ca may be absorbed before the duodenum. Secretion of gastric acids and enzymes contribute to the release of Ca from the diet to a soluble or ionic form, which is required for Ca to be absorbed, and greater absorption of Ca in the proximal small intestine than in the jejunum is promoted by the greater concentration of Ca-binding proteins in the enterocytes in the duodenum than in the jejunum (Allen 1982). Results of this experiment support this hypothesis, but also indicate that some variation among feed ingredients exists, which results in differences in the site where Ca is absorbed. However, the present results do not support the hypothesis that Ca is also absorbed in the large intestine because no differences between ileal and total tract digestibility and between ileal and total tract fluxes were observed. This conclusion is in agreement with data from Bohlke et al. (2005) who also reported that there is no absorption of Ca in the large intestine of pigs.

The reason Ca in calcium carbonate had greater digestibility than Ca in *L. calcareum* Ca may be that there are differences in solubility between the two ingredients, which may result in the formation of more Ca–P complexes in the intestinal tract of pigs fed *L. calcareum* Ca compared with pigs fed calcium carbonate. The fact that the digestibility of P was also less if *L. calcareum* Ca was used supports this hypothesis. To our knowledge, there are no other data for the digestibility of Ca in *L. calcareum* Ca fed to pigs, but the digestibility of Ca in *L. calcareum* Ca fed to broilers is not less than that in calcium carbonate (Walk et al. 2012). Differences in pH, transit time and enzyme properties between pigs and chickens (Créviu-Gabriel et al. 1999) may explain the differences observed between pigs and broilers fed *L. calcareum* Ca.

One of the factors that may affect P digestibility is the dietary level of Ca, and increasing dietary Ca may reduce P digestibility (Stein et al. 2011). In this experiment, this was true only for *L. calcareum* Ca diets, but not for calcium carbonate diets. The reduction of P digestibility in *L. calcareum* Ca diets that were observed as Ca in the diets increased is likely a result of increased formation of Ca–P complexes in the small intestine, which reduced the availability of P for absorption (Clark 1969; Stein et al. 2011). However, the reason P digestibility was not affected by Ca level in the calcium carbonate diets may be the low concentration of Ca in the small intestine, because most Ca was absorbed before the early part of duodenum. This hypothesis explains the fact that no differences were observed between the calcium carbonate and *L. calcareum* Ca diets in the ADD of P, but the AID and ATTD of P were greater in calcium carbonate diets than in *L. calcareum* Ca diets if no phytic acid was added to the diets. In a recent experiment with broilers, it was also observed that the digestibility of P was less in birds fed *L. calcareum* Ca diets than in birds fed limestone diets (Walk et al. 2012).

It is believed that most P is absorbed in the small intestine, and neither secretion nor absorption of P occurs in the large intestine (Crenshaw 2001; Bohlke et al. 2005). Results from this experiment obtained for diets that contained no phytic acid support this hypothesis, but data obtained for diets containing 1% phytic acid indicate that P may be absorbed in the large intestine. We do not have an explanation for this observation.

It has been reported that the pH in the stomach, duodenum, jejunum and ileum of a weaned pig is 3.2, 5.7, 5.9 and 6.9, respectively (Li et al. 2008) and the pH in the stomach, duodenum, jejunum and ileum of a finishing pig is 4.5, 6.3, 6.4 and 6.6, respectively (Merchant et al. 2011). Although the values obtained in this experiment for duodenal pH are less and the values for ileal digesta are greater than the reported values, the digesta pH may vary depending on the diet and the feed intake. Inclusion of phytic acid decreased the pH of the diet, which is likely a result of the added acids from phytic acid. The fact that the digesta pH was greater in pigs fed diets containing *L. calcareum* Ca than in pigs fed calcium carbonate may be a result of the reduced absorption of Ca and P from *L. calcareum* Ca compared with calcium carbonate.

## 5. Conclusions

Due to endogenous fluxes of Ca along the gastrointestinal tract, values for apparent digestibility need to be corrected for the endogenous Ca to obtain standardised digestibility values. The site where Ca is absorbed from the intestinal tract may vary among Ca sources, which may be a result of differences among Ca sources in formation of Ca–P complexes in the intestinal tract. The fact that there is no net absorption of Ca in the large intestine indicates that total tract digestibility of Ca may be used to estimate ileal digestibility.

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