True is more additive than apparent total tract digestibility of calcium in limestone and dicalcium phosphate for twenty-kilogram pigs fed semipurified diets

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ABSTRACT: Two experiments were conducted to determine the Ca digestibility of limestone and dicalcium phosphate (DCP) and if values for Ca digestibility are additive in mixed diets for pigs. In Exp. 1, 48 barrows with an average initial BW of 19.2 ± 1.1 kg were assigned to 1 of 6 dietary treatments in a 2×3 factorial arrangement of 2 Ca sources, including limestone or DCP, and 3 dietary Ca concentrations of 0.54, 0.74, or 0.94%. Diets were fed for a 5-d adjustment period followed by a total collection period of 5 d with chromic oxide and ferric oxide as markers to determine the initiation and termination of fecal collection, respectively. Results indicated that the increased dietary Ca concentration linearly increased ($P < 0.01$) Ca intake, digested Ca, and retained Ca but did not affect the apparent total tract digestibility (ATTD) of Ca or Ca retention of intake (%). The ATTD of P and P retention of intake were linearly increased ($P < 0.05$) as dietary Ca and P increased. In Exp. 2, 72 barrows with an average initial BW of 20.8 ± 1.3 kg were assigned to 1 of 9 dietary treatments in a 3×3 factorial arrangement of 3 Ca sources, including limestone, DCP, or the mixture

of the 2 at a ratio of 1:1, and dietary Ca concentrations of 0.40, 0.50, or 0.60%. Feeding and sample collection procedures were as in Exp. 1. The results also showed that increased Ca concentration linearly increased ($P < 0.001$) Ca intake, fecal Ca output, and Ca absorbed but did not affect the ATTD of Ca within each Ca source. The average ATTD were 66.46, 70.34, and 69.32% for the limestone, DCP, and mixed diets, respectively. By regressing daily digested Ca against daily Ca intake, the true total tract digestibility (TTTD) of Ca was determined to be 70.06, 76.42, and 73.72% for the limestone, DCP, and mixed diets, respectively. The predicted TTTD for Ca in the mixed diets of limestone and DCP was calculated to be 72.67% based on the Ca contribution coefficient of 0.59 for limestone and 0.41 for DCP. The predicted Ca TTTD (72.67%) in the mixed diets was not different from the Ca TTTD (73.72%) determined using the regression method. It is concluded that although the ATTD of limestone and DCP were not affected by the Ca concentration in the diet, TTTD is recommended for evaluation of Ca digestibility because of its additivity in a mixed diet.

Key words: additivity, apparent digestibility, calcium, pigs, true digestibility

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INTRODUCTION

Because Ca sources are inexpensive compared with energy, AA, and P ingredients, oversupply of Ca rather than deficiency is usually an issue. However, studies have shown that a high dietary Ca concentration may inhibit phytase efficiency and reduce P digestibility (Reinhart and Mahan, 1986; Selle et al., 2009; Stein et al., 2011). To avoid the negative effects of Ca oversupply and provide an optimal amount of

Ca into the diet, recent studies have determined digestibility of Ca in feed ingredients (González-Vega et al., 2015a; Zhang et al., 2016) as well as the digestible Ca requirements for pigs (González-Vega et al., 2016).

As it is the case with AA and P, Ca digestibility can be expressed as apparent, standardized, and true digestibility (Kong and Adeola, 2014). Apparent total tract digestibility (ATTD) is the simplest to determine but also creates some challenges. For example, it has been shown for AA or P that apparent digestibility values of ingredients are affected by inclusion rates of AA or P, respectively (Fan et al., 1994; Ajakaiye et al., 2003). It has also been shown that the ATTD of Ca in canola meal were different among different inclusion levels of

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Ca in the diet (González-Vega et al., 2013). As a result, it is important to choose the correct value from these results in a diet formulation. The apparent digestibility in mixed diets are not additive for AA and P, but correcting apparent digestibility values for endogenous losses may yield values that are more additive in mixed diets (Stein et al., 2005; Zhai and Adeola, 2013a; Xue et al., 2014). Therefore, 2 experiments were conducted. The first experiment was conducted to determine the ATTD of Ca in limestone and dicalcium phosphate (DCP) inclusion level and test if the ATTD of Ca in limestone and DCP are affected by the Ca concentration in the diets. The second experiment was conducted to determine the true total tract digestibility (TTTD) of Ca in limestone and DCP using semipurified diets and to test if values for ATTD or TTTD are additive in mixed diets.

MATERIALS AND METHODS

All animal procedures used in these studies were approved by the Purdue Animal Care and Use Committee.

Experiment 1

The experiment was conducted in 2 consecutive periods using the same facility and procedures. Forty-eight Hampshire $\times$ Duroc $\times$ Yorkshire $\times$ Landrace barrows (19.2 ± 1.1 kg initial BW) were used. In each period, 24 barrows were allotted to 6 dietary treatments in a randomized complete block design using initial BW as a blocking factor. Barrows were housed in stainless-steel metabolism crates that allowed for total collection of feces and urine. Pigs were allowed to adapt to the diets and the crates for 5 d followed by a 5-d total collection period of urine and feces. Feed allowance was calculated as 3.5% of the initial average BW of pigs within each block during the experimental period. Pigs were fed 1 of 2 equal portions of a daily feed allowance at 0700 and 1800 h. Chromic oxide and ferric oxide were used as markers to determine the initiation and termination of fecal collection, respectively, as described by Adeola (2001). Urine and fecal samples were collected daily during the collection period. All the feces and a minimum of a 30% subsample of the urine were stored at -18°C until analysis. All samples were pooled within each pig at the end of the experiment.

The 6 dietary treatments consisted of a combination of 2 Ca sources, calcitic limestone (Irving Materials, Inc., Greenfield, IN) and DCP (PCS Sales (USA) Inc., Northbrook, IL), and 3 Ca levels (0.52, 0.72, and 0.92% of Ca; Table 1). Potassium phosphate was supplemented at the expense of cornstarch to adjust the dietary P level and maintain a 1.1:1 total Ca:total P ratio. Potassium carbonate was included to

Table 1. Ingredient composition of experimental diet for pigs in Exp. 1

Dietary Ca concentration, %	Limestone			Dicalcium phosphate		
	0.54	0.74	0.94	0.54	0.74	0.94
Ingredients, %						
Corn	10.00	10.00	10.00	10.00	10.00	10.00
Corn gluten meal	10.00	10.00	10.00	10.00	10.00	10.00
Cornstarch	64.52	63.12	61.72	64.66	63.80	62.96
Sucrose	10.00	10.00	10.00	10.00	10.00	10.00
Soybean oil	1.50	1.50	1.50	1.50	1.50	1.50
Limestone	1.46	2.01	2.56	—	—	—
Dicalcium phosphate	—	—	—	2.13	2.92	3.71
Potassium phosphate	1.82	2.67	3.52	0.00	0.13	0.25
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Potassium carbonate	—	—	—	1.01	0.95	0.88
Vitamin premix ¹	0.15	0.15	0.15	0.15	0.15	0.15
Mineral premix ²	0.10	0.10	0.10	0.10	0.10	0.10
Selenium premix ³	0.05	0.05	0.05	0.05	0.05	0.05
Analyzed composition, %						
DM	88.55	88.28	88.68	88.27	88.40	88.52
CP	7.03	6.91	6.75	7.12	7.06	6.87
GE, kcal/kg	3,846	3,750	3,694	3,751	3,655	3,549
Ca	0.52	0.72	0.88	0.51	0.68	0.90
P	0.48	0.55	0.81	0.43	0.61	0.80
Total Ca:total P ratio	1.08	1.31	1.09	1.20	1.11	1.13

¹Vitamin premix supplied per kilogram of diet, 2,423 IU vitamin A, 242 IU vitamin D₃, 17.6 IU vitamin E, 2.4 mg vitamin K activity, 804 μg menadione, 14.1 μg vitamin B₁₂, 2.8 mg riboflavin, 9 mg D-pantothenic acid, and 13 mg niacin.

²Mineral premix supplied, per kilogram of diet, 9 mg Cu (as copper sulfate), 0.34 mg I (as calcium iodate), 97 mg Fe (as ferrous sulfate), 12 mg Mn (as manganese oxide), and 97 mg Zn (as zinc oxide).

³Supplied 300 μg Se per kilogram of diet.

maintain similar dietary K concentrations and dietary electrolyte balance values among the diets to limit the possible influence of the different dietary electrolyte balances among diets. The inclusion level of corn and gluten meal was set at 10.0% to limit the Ca contribution other than from limestone and DCP.

Experiment 2

The experiment was conducted in 4 consecutive periods using the same facility and procedures as Exp. 1. Seventy-two Hampshire $\times$ Duroc $\times$ Yorkshire $\times$ Landrace barrows with an initial BW of 20.8 ± 1.3 kg were used. In each period, 18 pigs were blocked by weight and randomly assigned to 9 dietary treatments in a 3×3 factorial arrangement of 3 Ca sources, including calcitic limestone, DCP, and the mixture of the 2 at a ratio of 1:1, and dietary Ca concentrations of 0.40, 0.50, and 0.60% (Table 2). The same experimental procedure was followed as described in Exp. 1. Feed allowance was calculated as 3.0% of the initial average BW of the pigs within each block during the experimental period. Potassium phosphate

was supplemented at the expense of cornstarch to adjust the dietary P level and maintain a 1.0:1 total Ca:total P ratio. Potassium carbonate was included for the same reason described in Exp. 1. All housing, feeding, collection, and sampling procedures were as in Exp. 1.

Chemical Analyses

All feces were oven-dried at 55°C to a constant weight. Diets and feces were finely ground (< 0.75 mm) using a centrifugal grinder (ZM 200; Retsch GmbH, Haan, Germany) before analyses. All samples were dried at 105°C in a drying oven (Precision Scientific Co., Chicago, IL) for 24 h to determine DM content (method 934.01; AOAC, 2006). Urine samples for each pig were thawed and thoroughly mixed, after which approximately 500-mL subsamples were filtered in 3 steps using glass wool. Samples of ingredients, diets, urine, and feces were digested in concentrated nitric acid and 70% perchloric acid (Fenton and Fenton, 1979) and analyzed for Ca and P after wet digestion. Calcium concentration in wet digested samples was determined using an atomic absorption spectrometer (AAnalyst 300 [PerkinElmer, Inc., Norwalk, CT]; method 985.01 [AOAC, 2006]). Concentration of P was determined using spectrophotometry at 620 nm (Spectra Count, model AS1000 [Packard Instrument Company, Inc., Meriden, CT]; method 985.01 (A, B, D) [AOAC, 2006]). For feed samples, nitrogen content was determined using the combustion method (TruMac N analyzer; LECO Corp., St. Joseph, MI), and GE was determined using isoperibol bomb calorimetry using a Parr 1261 calorimeter (Parr Instrument Co., Moline, IL).

Calculations and Statistical Analysis

The ATTD (%) of Ca and Ca retention of intake (CaR; %) were calculated using the following equations described by Adeola (2001):

$$\text{ATTD} = 100 \times (\text{Ca}_i - \text{Ca}_f) / \text{Ca}_i \quad \text{and}$$

$$\text{CaR} = 100 \times (\text{Ca}_i - \text{Ca}_f - \text{Ca}_u) / \text{Ca}_i,$$

in which Ca_i represents the dietary Ca intake (g/d), Ca_f is the fecal Ca output (g/d), and Ca_u is the urine output of Ca (g/d). The equations above were also used for P digestibility and retention calculation, with P replacing Ca.

In Exp. 2, the TTTD of Ca in limestone and DCP was determined by regressing digested Ca (CaD ; g/d, calculated as $\text{Ca}_i - \text{Ca}_f$) against Ca_i in grams per day for diets within each Ca source using the following model described by Akinmusire and Adeola (2009):

$$\text{CaD} = (\text{TTTD} \times \text{Ca}_i) - \text{ECaL},$$

in which TTTD (%) is the slope of the regression $\times 100$, endogenous losses of Ca (ECaL ; g/d) is the negative intercept of the regression, and CaD and Ca_i represent the dependent and independent variables, respectively.

The predicted TTTD of Ca in the mixed diets of limestone and DCP were derived using the following equation described by Zhai and Adeola (2013b):

$$\text{TTTD}_{\text{PM}} = \text{TTTD}_{\text{L}} \times \text{C}_{\text{L}} + \text{TTTD}_{\text{D}} \times \text{C}_{\text{D}},$$

in which C_{L} and C_{D} are the Ca contributed from limestone and DCP, respectively, in the mixed diets; TTTD_{PM} (%) stands for the predicted TTTD of Ca in the mixed diets of limestone and DCP; and TTTD_{L} and TTTD_{D} represent the determined TTTD of Ca in limestone and DCP, respectively, using the regression method.

In both Exp. 1 and Exp. 2, data were analyzed using the GLM procedures of SAS (SAS Inst, Inc., Cary, NC). In addition to the factorial arrangement for treatments, period was treated as the random variable, and period and blocks nested within period were treated as the random variables. Linear contrasts were used to test the effects of dietary Ca level on the intake, output, digestibility, and retention of Ca and P of each Ca source. The individual pig was considered the experimental unit. Least squares means were calculated, and an α level of 0.05 was considered significant.

In Exp. 2, regression coefficients were compared between Ca sources using the confidence intervals derived from SE of the respective regression coefficients. To test whether the TTTD of Ca determined using the regression method in the mixed diets (TTTD_{DM}) of limestone and DCP is different from the TTTD_{PM} , a 2-tailed, 1-sample *t*-test was performed (Zhai and Adeola, 2013b), with the null hypothesis being H_0 : $\text{TTTD}_{\text{DM}} = \text{TTTD}_{\text{PM}}$ vs. the alternative hypothesis H_a : $\text{TTTD}_{\text{DM}} \neq \text{TTTD}_{\text{PM}}$ as follows:

$$t = (\bar{x} - \mu) / \text{SE},$$

in which the TTTD_{DM} of Ca was taken as the sample mean ($\bar{x}$) and TTTD_{PM} of Ca as the population mean (μ). The equations above were also used to test for additivity of the ATTD of Ca in mixed diet with ATTD replacing TTTD.

RESULTS

Analyzed chemical composition of the experimental diets used in Exp. 1 and 2 are shown in Tables 1 and 2, respectively. The analyzed dietary Ca concentrations in both experiments were close to the calcu-

Table 2. Ingredient composition of experimental diet for pigs in Exp. 2

Dietary Ca concentration, %	Limestone			Dicalcium phosphate			Mixed diets		
	0.40	0.50	0.60	0.40	0.50	0.60	0.40	0.50	0.60
Ingredients, %									
Corn	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Corn gluten meal	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Cornstarch	45.01	44.58	44.05	45.12	44.68	44.25	45.04	44.62	44.20
Sucrose	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Soybean oil	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Limestone	1.09	1.37	1.65	—	—	—	0.65	0.81	0.97
Dicalcium phosphate	—	—	—	1.58	1.98	2.38	0.65	0.81	0.97
Potassium phosphate	1.30	1.70	2.10	0.00	0.07	0.14	0.76	1.06	1.26
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Potassium carbonate	0.40	0.15	0.00	1.10	1.07	1.03	0.70	0.50	0.40
Vitamin premix ¹	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Mineral premix ²	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Selenium premix ³	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Analyzed composition, g/kg									
DM	91.00	91.11	91.15	90.70	91.02	91.08	91.03	91.46	91.58
CP	15.32	14.68	14.87	15.45	15.03	14.59	15.37	15.32	15.11
GE, kcal/kg	4,089	4,028	4,021	4,072	4,058	4,015	4,095	4,060	4,033
Ca	0.41	0.47	0.61	0.42	0.47	0.60	0.39	0.49	0.61
P	0.40	0.51	0.62	0.43	0.50	0.59	0.43	0.51	0.62
Total Ca:total P ratio	1.03	0.93	0.99	0.97	0.94	1.00	0.91	0.97	0.98

¹Vitamin premix supplied, per kilogram of diet, 2,423 IU vitamin A, 242 IU vitamin D₃, 17.6 IU vitamin E, 2.4 mg vitamin K activity, 804 µg menadione, 14.1 µg vitamin B₁₂, 2.8 mg riboflavin, 9 mg D-pantothenic acid, and 13 mg niacin.

²Mineral premix supplied, per kilogram of diet, 9 mg Cu (as copper sulfate), 0.34 mg I (as calcium iodate), 97 mg Fe (as ferrous sulfate), 12 mg Mn (as manganese oxide), and 97 mg Zn (as zinc oxide).

³Supplied 300 µg Se per kilogram of diet.

lated values. The total Ca:total P ratios were similar among diets for each experiment, except for the diet containing limestone and 0.74% Ca in Exp. 1.

Experiment 1

Calcium balance and utilization are presented in Table 3. The daily Ca intake and fecal Ca output (g/d) linearly increased ($P < 0.001$) as dietary Ca concentration increased in both limestone and DCP diets. The dietary Ca concentration linearly increased ($P < 0.001$) urine Ca output (g/d) for the DCP treatment but not for the limestone treatment. Digested and retained Ca linearly increased ($P < 0.001$) as dietary Ca concentration increased. However, the ATTD of Ca and retention of Ca calculated as a percentage of Ca intake (%) of Ca were not affected by Ca concentration.

Daily P intake, fecal P output, and urine P output (g/d) linearly increased ($P < 0.001$) as dietary Ca increased (Table 4). Digested and retained P (g/d) increased ($P < 0.001$) as dietary Ca concentration increased. However, the ATTD and retention of P (%) linearly increased ($P < 0.05$) with increasing concentration of dietary Ca

Experiment 2

One barrow from the DCP treatment with 0.60% Ca was removed from the trial because of feed refusal during the collection period. The increasing Ca concentration of each Ca source linearly increased ($P < 0.001$) Ca intake, Ca fecal output, and digested Ca (g/d). The ATTD of Ca was not affected by the Ca concentration. The average values for ATTD of Ca in the limestone, DCP, and mixed diets were 66.46, 70.34, and 69.32%, respectively, and these values were not different.

The estimates of ECaL are presented in Table 5. None of the endogenous Ca estimates was different from 0. The overall ECaL was 134 mg/d. The regression method-determined TTTD of Ca for the limestone, DCP, and mixed diets were 70.06, 76.42, and 73.72%, respectively, and no difference in the TTTD of Ca between Ca sources was observed. The Ca contribution coefficient for the mixed diets was 0.59 from limestone and 0.41 from DCP. Hence, the expected TTTD and ATTD of Ca in the mixed diets were predicted to be 72.67 and 68.05%, respectively (Table 5). Based on the *t*-test, the predicted Ca TTTD in the mixed diets, which was 72.67%, was not statistically different from the regression method-determined Ca TTTD, which was 73.72% ($P = 0.80$). However, predicted Ca ATTD in

Table 3. Dietary Ca and P balance and apparent total tract digestibility (ATTD) of Ca and P in Exp. 1¹

Item	Limestone			Dicalcium phosphate				P-value				
	0.54	0.74	0.94	0.54	0.74	0.94	SEM	Source	Level	Source × level	Linear effect of limestone	Linear effect of dicalcium phosphate
DMI, g/d	531	529	535	523	528	530	4.2	0.53	0.24	0.73	0.55	0.29
Ca utilization												
Ca intake, g/d	3.13	4.34	5.32	3.34	4.06	5.4	0.13	0.587	<0.001	0.581	<0.001	<0.001
Fecal Ca output, g/d	0.80	1.09	1.23	0.91	1.15	1.28	0.084	0.587	<0.001	0.757	0.001	0.004
Digested Ca, g/d	2.33	3.25	4.1	2.43	2.91	4.11	0.12	0.847	<0.001	0.718	<0.001	<0.001
Urine Ca output, g/d	0.29	0.19	0.22	0.19	0.33	0.45	0.047	0.007	0.037	0.001	0.38	0.001
Retained Ca, g/d	2.04	3.06	3.87	2.25	2.58	3.66	0.14	0.262	<0.001	0.144	<0.001	<0.001
ATTD of Ca, %	74.43	74.81	76.67	72.63	71.88	76.27	1.82	0.534	0.12	0.704	0.39	0.17
Ca retention, % of intake	65.10	70.40	72.50	66.65	63.85	67.92	2.26	0.346	0.059	0.177	0.02	0.69
P utilization												
P intake, g/d	2.90	3.31	4.89	2.54	3.67	4.77	0.084	0.15	<0.001	0.183	<0.001	<0.001
Fecal P output, g/d	0.80	0.65	0.91	0.78	1.05	1.11	0.090	0.54	0.021	0.225	0.39	0.011
Digested P, g/d	2.10	2.66	3.99	1.76	2.61	3.66	0.10	0.52	<0.001	0.978	<0.001	<0.001
Urine P output, g/d	0.15	0.22	0.25	0.15	0.16	0.19	0.004	<0.001	<0.001	<0.001	<0.001	<0.001
Retained P, g/d	1.94	2.44	3.73	1.61	2.45	3.47	0.11	0.43	<0.001	0.761	<0.001	<0.001
ATTD of P, %	72.26	80.32	81.52	69.87	70.89	76.66	2.50	0.90	0.003	0.625	0.013	0.064
P retention, % of intake	66.92	73.55	76.32	63.99	66.50	72.71	2.45	0.72	0.001	0.890	0.011	0.018

¹Data are the least squares mean of 8 observations.

the mixed diets, which was 68.05%, showed a trend to be different from the determined Ca ATTD, which was 69.32% at $P = 0.09$. Therefore, the values of the ATTD of Ca may not be additive in the mixed diets ($P = 0.09$).

DISCUSSION

The calcitic limestone and DCP used in Exp. 1 and Exp. 2 were from the same batch. The analyzed concentration of Ca in limestone used in these experiments was 36.4%, which is in the range of previous published data, between 33.5 and 42.0% (Wang et al., 2014; Anwar et al., 2016, 2017). The concentration of Ca in DCP used in this study was 25.3%, which is higher than recently published data, which ranged from 20.88 to 22.72% (Baker et al., 2013; González-Vega et al., 2015a; Kwon and Kim, 2017). The variation in Ca concentration arises from commercially available DCP being a blend of monocalcium phosphate, DCP, and unreacted calcium carbonate (González-Vega et al., 2015a).

Experiment 1

The Ca concentration was analyzed to be 0.23 and 0.33 g/kg for corn and corn gluten meal, respectively. Hence, the majority of Ca in the pig's diet is from the limestone or DCP, with less than 2% of dietary Ca contributed by corn and corn gluten meal in each diet. The ATTD of Ca in DCP, between 71.88 and 76.27%, are close to the value previously reported (González-Vega et al., 2015a). However, the ATTD of Ca limestone,

between 74.43 and 76.67%, are higher than previously reported for calcium carbonate (Stein et al., 2011; González-Vega et al., 2015a; Zhang et al., 2016), which is inconsistent with a previous observation that the bio-availability of Ca in calcitic limestone is similar to that of calcium carbonate (Ross et al., 1984). This may be due to different basal diets used in various studies. A corn–corn gluten meal–cornstarch diet was used in the current study. Compared with corn- or corn–soybean meal–based diets used in other studies mentioned above, the dietary phytate concentration in the current experiment was lower than that of other studies. The poly-anionic phytate molecule from plant ingredients could chelate up to 6 atoms of Ca and form Ca–phytate complex, which could reduce Ca digestibility (Selle et al., 2009; González-Vega et al., 2015a). This inference was also indicated in a recent study from Liu et al. (2014), which indicated that pigs fed corn grits diets had a higher ATTD of Ca than that of pigs fed a corn–soybean meal diet, due to a higher level of phytate in the corn–soybean meal diet than in the corn grits diet. However, in the study of González-Vega et al. (2015b), the determined digestibility of Ca in fish meal was greater in a corn-based diet with higher phytate concentration than in a cornstarch-based diet. It was explained that the lack of fiber in cornstarch-based diets may result in precipitation of Ca in the intestinal tract and reduced the Ca digestibility (González-Vega et al., 2015b).

In the current experiment, the dietary Ca concentration ranged from 0.51 to 0.90, which is between 73 and 129% of the requirement for 11- to 25-kg pigs (NRC,

Table 4. Dietary Ca and P intake, fecal Ca and P output, and apparent total tract digestibility (ATTD) of Ca and P in Exp. 2

Item	Limestone			Dicalcium phosphate			Mixed diets			SD	<i>P</i> -value		
	0.4	0.5	0.6	0.4	0.5	0.6	0.4	0.5	0.6		Source	Linear effect ¹	Source × level
No. of observations	8	8	8	8	8	7	8	8	8				
DMI, g/d	505	506	476	511	486	521	500	511	489	29.0	0.34	0.20	0.25
Ca digestibility													
Ca intake, g/d	2.55	3.11	3.44	2.34	2.52	3.40	2.58	3.19	3.58	0.20	0.20	<0.001	0.59
Fecal Ca output, g/d	0.86	1.05	1.13	0.69	0.77	0.98	0.82	0.97	1.06	0.11	0.33	<0.001	0.84
Digested Ca, g/d	1.69	2.06	2.32	1.65	1.75	2.41	1.76	2.22	2.52	0.18	0.52	<0.001	0.55
ATTD of Ca, %	66.0	66.1	67.3	70.5	69.4	71.1	67.9	69.7	70.3	3.37	0.50	0.17	0.68
P digestibility													
P intake, g/d	2.20	2.84	3.22	2.40	2.69	3.39	2.33	2.82	3.32	0.17	0.84	<0.001	0.95
Fecal P output, g/d	0.56	0.66	0.66	0.78	0.80	0.93	0.69	0.68	0.76	0.09	0.64	<0.001	0.43
Digested P, g/d	1.64	2.18	2.56	1.62	1.89	2.46	1.64	2.14	2.56	0.16	0.92	<0.001	0.65
ATTD of P, %	74.3	76.7	79.6	67.6	70.2	72.5	70.2	75.7	77.1	3.24	0.45	<0.001	0.65

¹Linear effect of dietary Ca concentration

2012). The values of the ATTD of Ca are not affected by the concentration of Ca. This observation agrees with previous data that the ATTD of Ca in calcium carbonate is not affected by the concentration of Ca, which may be due to the compensation between active Ca transport and passive Ca transport during Ca absorption (Stein et al., 2011). To maintain a constant total Ca:total P ratio among all diets, potassium phosphate was added to the diets to adjust the P level. The fact that the ATTD of Ca was not different among the diets also indicated that there is no significant effects of P concentration on the ATTD of Ca when P is below the requirement (5.43 g/d total P; NRC, 2012), which is consistent with a previous observation that a P concentration that ranged between 26 and 147% of requirement had minimal effect on the ATTD of Ca of growing pigs (Stein et al., 2008).

The retention of Ca was expected to increase when the Ca intake was below the requirement and decrease when the Ca intake above the requirement. The highest Ca concentration used in this experiment was 29% higher than the recommended requirement of total Ca for 11- to 25-kg pigs (NRC, 2012). However, due to the restricted feed intake, the total Ca intake per day ranged from 3.1 to 5.3 g/d but was still less than the recommendation (6.34 g/d total Ca; NRC, 2012). In the limestone treatments, the retained Ca linearly increased whereas the urinary Ca remains unchanged. However, for DCP, due to the increased urinary Ca output, although the retained Ca linearly increased, the Ca retention of intake remained the same as the Ca concentration increased.

For P digestibility and balance, the ATTD of P linearly increased as Ca concentration increased, which is in agreement with our previous observation (Zhang et al., 2016). One reason for the observation is that the inclusion level of inorganic P increased as the Ca concentra-

tion increased. As a result, a greater proportion of P in the diet was of inorganic origin, which increased from 83 to 98%. Due to a higher P digestibility in inorganic sources compared with corn and corn gluten meal, the dietary ATTD of P increased. Besides, it is possible that the increased dietary inclusion level of P reduced the relative proportion of endogenous P to the total P digesta flow, which increased the determined apparent digestibility.

Experiment 2

The concentration of corn and corn gluten meal in Exp. 2 were higher than those in Exp. 1, which led to a higher phytate concentration. As described above, by forming a Ca-phytate complex with Ca, phytate could reduce the Ca digestibility (Selle et al., 2009). This may partly explain why the determined ATTD of Ca in limestone and DCP were lower than the determined values for limestone and DCP in Exp. 1, even though the ingredients were from the same batch.

Consistent with Exp. 1, there was no difference in the ATTD of Ca between limestone and DCP. This observation is different from the results of González-Vega et al. (2015a), which showed that pigs fed monocalcium phosphate and DCP had a greater ATTD of Ca compared with pigs fed calcium carbonate. Consistently, the concentration of Ca had no effects on the ATTD of Ca in each Ca source, when all dietary Ca concentrations were lower than the Ca requirement (57–86% of requirement; NRC, 2012), whereas González-Vega et al. (2013) showed that the ATTD of Ca values in canola meal were influenced by the inclusion level of canola meal, which was used as the sole dietary Ca source. This indicated that the reduced contribution of endogenous Ca to digesta flow could significantly affect the ATTD of Ca of ingredients

Table 5. Linear relationship between total tract Ca digested (g/d) and dietary Ca intake (g/d), as-fed basis

Item	Limestone	Dicalcium phosphate	Mixed diets
Regression equation	$y = 0.7003x - 0.1034$	$y = 0.7642x - 0.1603$	$y = 0.7372x - 0.1324$
SE of the slope	0.0517	0.0398	0.0424
SE of the intercept	0.1586	0.1103	0.1338
r^2	0.89	0.95	0.93
Estimate of endogenous Ca loss, ¹ g/d	0.103	0.160	0.132
Estimate of true total tract Ca digestibility, ² %	70.06	76.42	73.72
Estimate of apparent total tract Ca digestibility, %	66.46	70.34	69.32
No. of observations	24	23	24
Determined true total tract digestibility of Ca in mixed diets, %			73.72
Predicted true total tract P digestibility of Ca in mixed diets, ³ %			72.67
Determined apparent total tract digestibility of Ca in mixed diets, %			69.32
Predicted apparent total tract P digestibility of Ca in mixed diets, ⁴ %			68.05

¹The endogenous losses of Ca were 0.207, 0.316, and 0.264 g/kg DMI for the limestone, dicalcium phosphate, and mixed diets, respectively, by dividing the average kilograms per day DMI of each Ca source.

²The estimates of true total tract Ca digestibility compared using the confidence interval were not different among the limestone, dicalcium phosphate, and mixed diets.

³Predicted true total tract Ca digestibility was not significantly different from the determined (P -value = 0.80).

⁴Predicted apparent total tract Ca digestibility was not significantly different from the determined (P -value = 0.09).

with lower digestible Ca. But for inorganic sources of Ca, they could provide a considerable amount of digestible Ca, which may limit the contribution of ECaL as well as its effect on ATTD values. Although the no effect of Ca level on the ATTD of Ca was observed in either Exp. 1 or Exp. 2, the P -values (0.11 and 0.17, respectively) indicated that there is a risk that the ATTD may have different values when the inclusion of Ca ingredients varies, and correction of ECaL is still recommended.

The regression method has been widely used to estimate the true digestibility of AA and P as well as the endogenous losses of AA and P (Fan and Sauer, 1997; Dilger and Adeola, 2006; Zhai and Adeola, 2013b). The regression method determined that daily ECaL in the current experiment were between 0.11 to 0.159 g. These are lower compared with the data reported by Fernández (1995), which varied from 0.14 to 0.8 g/d determined using isotope dilution technique, and slightly higher than the values of 0.11 to 0.13 g reported by González-Vega et al. (2013) using the regression method. Considering that the pigs in our experiment had a greater BW than those used by González-Vega et al. (2013) and different experimental diets were used, the difference appears plausible. The endogenous losses in the current experiment were between 0.207 to 0.316 g/kg DM intake. These values are also close to the basal ECaL determined using the Ca-free diets. In the study of González-Vega et al. (2015b), the basal ECaL were determined to be 0.22 and 0.396 g/kg DMI with a cornstarch–potato protein isolate–sucrose diet and a corn–potato protein isolate diet, respectively.

No difference was observed between the values of TTTD and ATTD for each ingredient. This result is different than the previous report that TTTD values

of Ca in canola meal are different from ATTD values (González-Vega et al., 2013). Perhaps the difference is due to the different Ca contents of the ingredients used in these 2 experiments. Compared with inorganic Ca sources, canola meal is lower in Ca. As a result, the endogenous Ca accounts for a larger proportion of Ca in the digesta flow for canola meal as well as a greater difference between the ATTD and the TTTD. The additivity results of the TTTD and ATTD of Ca are consistent with previous reports that apparent digestibility for complete diets predicted from the apparent digestibility of each ingredient underestimates of apparent digestibility of CP, AA, and P for growing pigs (Stein et al., 2005; Zhai and Adeola, 2013b; Xue et al., 2014). This underestimation could be due to the contribution of ECaL to the total flow of Ca in the intestinal tract. It is reasonable to speculate that lower predicted values for the ATTD of Ca in diets could be obtained from low-Ca ingredients that may reduce additivity.

In summary, even though the results imply that the dietary Ca level from limestone and DCP did not affect the ATTD of Ca in each ingredient, the TTTD of Ca was shown to be more additive than the ATTD of Ca in a semipurified mixed diet. Hence, TTTD is recommended for expressing digestible Ca in diets. Furthermore, the TTTD of Ca in limestone and DCP were determined at 70.06 and 76.42%, respectively, using the regression method.

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