Determination of endogenous intestinal losses of calcium and true total tract digestibility of calcium in canola meal fed to growing pigs

J. C. González-Vega, C. L. Walk, Y. Liu and H. H. Stein

J ANIM SCI 2013, 91:4807-4816.
doi: 10.2527/jas.2013-6410 originally published online August 13, 2013

Endogenous intestinal losses of calcium and true total tract digestibility of calcium in canola meal fed to growing pigs[1]

J. C. González-Vega,* C. L. Walk,† Y. Liu,* and H. H. Stein*[2]

* Department of Animal Sciences, University of Illinois, Urbana 61801; and † AB Vista Feed Ingredients, Marlborough, SN8 4AN, United Kingdom

ABSTRACT: An experiment was conducted to test the hypothesis that values for apparent total tract digestibility (ATTD) of Ca in pigs are influenced by endogenous Ca lost from the gastrointestinal tract. The objective was to determine the endogenous loss of Ca, the ATTD of Ca, and the true total tract digestibility (TTTD) of Ca in canola meal without and with microbial phytase. The second objective was to determine the balance of Ca in pigs fed diets based on canola meal without or with microbial phytase. Forty-eight growing barrows (initial BW: 16.72 ± 2.52 kg) were allotted to 8 dietary treatments in a randomized complete block design with 6 pigs per treatment. Diets were based on sucrose, cornstarch, potato protein isolate, corn gluten meal, and canola meal. Diets were formulated to contain 0.08, 0.16, 0.24, or 0.32% Ca from canola meal. All diets were formulated with 0 or 1,500 units/kg of microbial phytase and contained 0.32% digestible P. Feces and urine samples were collected from d 6 to 11. Total endogenous losses of Ca were determined using the regression procedure. Results indicated that ATTD of Ca and Ca retention increased ($P < 0.05$) if dietary Ca increased and also increased ($P < 0.01$) when phytase was added to the diets. The estimated total endogenous loss of Ca was 0.160 and 0.189 g/kg DMI for canola meal without and with microbial phytase, respectively, and these values were not different. The TTTD of Ca increased ($P < 0.01$) if phytase was used but was not affected by the level of dietary Ca. As dietary Ca increased, the amount of Ca absorbed and retained increased ($P < 0.01$) to a greater extent if phytase was used than when no phytase was included in the diet (interaction, $P < 0.05$). Fecal P excretion increased ($P < 0.01$) as dietary Ca increased but was reduced ($P < 0.01$) by the use of phytase. The ATTD of P decreased ($P < 0.01$) with increasing dietary Ca to a lesser extent if phytase was used than if no phytase was used (interaction, $P < 0.01$). In conclusion, endogenous Ca is lost from the gastrointestinal tract of growing pigs, and values for TTTD of Ca are, therefore, different from values for ATTD of Ca. Values for ATTD of Ca are influenced by level of dietary Ca, but that is not the case for values for TTTD of Ca. The ATTD of P decreases as dietary Ca increases, but microbial phytase increases Ca and P digestibility and Ca retention in pigs fed diets based on canola meal whereas it does not influence endogenous losses of Ca.

Key words: apparent digestibility, calcium, endogenous losses, phytase, pigs, true digestibility

 J. Anim. Sci. 2013.91:4807–4816
doi:10.2527/jas2013-6410

INTRODUCTION

In formulating diets for pigs, it is more accurate to use standardized nutrient digestibility than apparent nutrient digestibility because values for standardized digestibility are additive in mixed diets (Stein et al., 2005). Values for the standardized total tract digestibility (**STTD**) of P in pigs have been reported (Petersen and Stein, 2006; Almeida and Stein, 2010; NRC, 2012), but only apparent total tract digestibility (**ATTD**) values have been reported for Ca (Bohlke et al., 2005; Stein et al., 2006, 2008, 2011). The STTD of a nutrient is calculated by correcting the ATTD for basal endogenous losses whereas the true total tract digestibility (**TTTD**) of a nutrient is calculated by correcting ATTD values for total endogenous losses (Stein et al., 2007). Basal endogenous losses may be estimated by using a nutrient-free diet (Petersen and Stein, 2006; Stein et al., 2007) whereas total endogenous losses may be estimat-

[1]Financial support for this research from AB Vista Feed Ingredients (Marlborough, UK) is appreciated.

[2]Corresponding author: hstein@illinois.edu

Received February 25, 2013.
Accepted July 19, 2013.

Table 1. Analyzed composition of ingredients, as-fed basis

Item	Ingredient			
	Potato protein isolate	Corn gluten meal	Canola meal	Monosodium phosphate
GE, kcal/kg	5,268	5,018	4,258	–
DM, %	91.19	90.86	89.65	98.99
CP, %	80.75	59.15	37.69	–
Ash, %	0.48	4.62	7.57	90.61
AEE,[1] %	0.50	4.58	3.27	–
ADF, %	3.60	6.44	19.21	–
NDF, %	1.12	9.25	33.47	–
Ca, %	0.03	0.02	0.66	0.08
P, %	0.12	0.54	1.00	29.69
Phytase,[2] FTU/kg	52	<50	<50	–
Phytic acid, %	0.33	1.64	2.58	–
Phytate-bound P,[3] %	0.09	0.46	0.73	–
Nonphytate P,[4] %	0.03	0.08	0.27	–

[1]AEE = acid-hydrolyzed ether extract.

[2]FTU = phytase units.

[3]Phytate-bound P was calculated as 28.2% of phytate (Tran and Sauvant, 2004).

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

ed using a regression procedure (Fan et al., 2001) or by using radioactively labeled isotopes (Visek et al., 1953). There are endogenous losses of Ca in cattle (Visek et al., 1953; Hansard et al., 1957; Martz et al., 1999) and chickens (Cowieson et al., 2004; Liu et al., 2012), but limited data have been published on endogenous losses of Ca in pigs (Besançon and Guéguen, 1969; Fernández, 1995). It is also not known if microbial phytase can influence endogenous losses of Ca in pigs.

The present experiment was conducted to test the hypothesis that values for the ATTD of Ca in pigs are affected by the endogenous loss of Ca and that values for TTTD of Ca, therefore, are different from values for ATTD of Ca. The second hypothesis was that endogenous losses are not influenced by the presence of microbial phytase in the diet. The objectives were to determine the ATTD and TTTD of Ca in canola meal without and with microbial phytase and to determine the balance of Ca in pigs fed different levels of canola meal without or with microbial phytase.

MATERIALS AND METHODS

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

Animals and Housing

Forty-eight growing barrows with an average initial BW of 16.72 ± 2.52 kg were used (G-Performer boars × Fertilis 25 females; Genetiporc, Alexandria, MN).

Table 2. Ingredient composition of experimental diets, as-fed basis[1]

Ingredient, %	Ca from canola meal, %			
	0.08	0.16	0.24	0.32
Canola meal	12.33	24.66	37.00	50.00
Corn gluten meal	7.50	5.00	2.50	–
Cornstarch	42.48	35.71	28.90	21.42
Sucrose	20.00	20.00	20.00	20.00
Potato protein isolate	10.00	8.00	6.00	4.00
Soybean oil	3.00	3.00	3.00	3.00
Monosodium phosphate	1.08	1.00	0.92	0.84
L-Lys HCl	0.34	0.24	0.13	0.03
DL-Met	0.02	–	–	–
L-Trp	0.03	0.01	–	–
Potassium carbonate	0.30	0.20	0.10	–
Magnesium oxide	0.08	0.05	0.03	–
Sodium chloride	0.29	0.33	0.37	0.41
Solka floc[2]	2.25	1.50	0.75	–
Vitamin mineral premix[3]	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00

[1]Four additional diets that were similar to these diets with the exception that 1,500 units per kilogram of microbial phytase (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK) was included in the diets at the expense of cornstarch were also formulated.

[2]Fiber Sales and Development Corp. (Urbana, OH).

[3]The vitamin–micromineral premix provided the following quantities of vitamins and microminerals per kilogram of complete diet: vitamin A as retinyl acetate, 11,128 IU; vitamin D_3 as cholecalciferol, 2,204 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_{12}, 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; and Zn as zinc oxide, 100 mg.

Pigs were housed individually in metabolism cages that were equipped with a slatted floor, a feeder, and a nipple drinker. A screen floor for fecal collection and a tray for urine collection were placed under each cage. Animals were allotted to 8 dietary treatments in a randomized complete block design with 6 pigs per treatment. The Experimental Animal Allotment Program (Kim and Lindemann, 2007) was used for the allotment.

Diets and Feeding

Eight diets were formulated to contain 0.32% standardized total tract digestible P. Monosodium phosphate was used as the source of inorganic P. Diets were based on sucrose, cornstarch, potato protein isolate, corn gluten meal, vitamins, minerals, and canola meal (Table 1). All minerals, except Ca, were included at recommended levels (NRC, 1998). Four diets containing 12.3, 24.7, 37.0, or 50.0% canola meal were formulated to contain 0.08, 0.16, 0.24, or 0.32% Ca, respectively (Tables 2 and 3). Canola meal was used in this experiment because it is one of the

Table 3. Analyzed composition of experimental diets, as-fed basis

	Diet							
	Ca from canola meal, %				Ca from canola meal + phytase,[1] %			
Item	0.08	0.16	0.24	0.32	0.08	0.16	0.24	0.32
GE, kcal/kg	4,156	4,167	4,176	4,198	4,187	4,156	4,183	4,212
DM, %	92.82	91.68	91.82	91.06	92.01	92.07	91.96	91.45
CP, %	18.48	20.23	21.94	23.71	19.26	20.97	22.47	23.05
Ash, %	2.74	3.46	4.46	5.04	2.90	3.68	4.44	5.38
AEE,[2] %	2.91	4.36	4.64	4.97	4.10	4.11	4.56	5.11
NDF, %	8.80	10.89	13.90	16.94	8.04	12.36	13.69	17.06
ADF, %	5.86	6.68	8.34	10.08	5.61	7.55	8.48	9.72
Ca, %	0.15	0.21	0.29	0.36	0.15	0.21	0.29	0.38
P, %	0.51	0.60	0.63	0.75	0.50	0.57	0.63	0.76
Phytase,[3] FTU/kg	<50	<50	<50	<50	1,990	1,300	1,440	1,290
Phytic acid, %	0.65	0.88	1.14	1.40	0.53	0.85	0.93	1.29
Phytate-bound P,[4] %	0.18	0.25	0.32	0.40	0.15	0.24	0.26	0.36
Nonphytate P,[5] %	0.33	0.35	0.31	0.35	0.35	0.33	0.37	0.40
pH	5.30	5.10	5.05	5.04	5.21	5.21	5.09	5.09

[1]These diets contained 1,500 units of phytase/kg (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK).

[2]AEE = acid-hydrolyzed ether extract.

[3]FTU = phytase units.

[4]Phytate-bound P was calculated as 28.2% of phytate (Tran and Sauvant, 2004).

[5]Nonphytate P was calculated as the difference between total P and phytate-bound P.

few ingredients that contain both phytate and appreciable amounts of Ca. Four additional diets, which were similar to the initial 4 diets with the exception that they also contained 1,500 units per kilogram of microbial phytase (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK), were also formulated. Canola meal and microbial phytase were included in these diets at the expense of cornstarch. Canola meal provided all Ca in the diets.

Experimental diets were fed for 12 d. Pigs were fed 3 times the daily maintenance energy requirement (i.e., 106 kcal of ME/kg $BW^{0.75}$; NRC, 1998), and the daily allotments were divided into 2 equal meals that were provided at 0700 and 1700 h. The initial 5 d was an adaptation period to the diets. On d 6, an indigestible marker (ferric oxide) was added to the morning meal to mark the beginning of fecal collection and on d 11, Indigo carmine was added to the morning meal to mark the conclusion of fecal collection. Feces were collected quantitatively using the marker-to-marker approach (Adeola, 2001). Urine collection started on d 6 and ceased on d 11. Fecal samples and 20% of the collected urine were stored at –20°C immediately after collection. Orts collected during the collection period were dried in a forced-air oven at 65°C, and the weight was subtracted from the total feed intake. Pigs had free access to water throughout the experiment.

Sample Analysis

Before analysis, fecal samples were dried in a forced-air oven at 65°C and ground through a 2-mm screen in a Wiley mill (Model 4; Thomas Scientific, Swedesboro, NJ). Urine samples were thawed at room temperature and a subsample of 10 mL was collected. Potato protein isolate, corn gluten meal, canola meal, monosodium phosphate, feces, and urine samples were analyzed for Ca and P by inductively coupled plasma spectroscopy (Method 985.01 A, B, and C; AOAC Int., 2007) after wet ash sample preparation [Method 975.03 B(b); AOAC Int., 2007], and diets were analyzed for Ca by an atomic absorption spectrophotometer procedure (Method 968.08; AOAC Int., 2007) after wet digestion sample preparation (Method 935.13; AOAC Int., 2007) and the concentration of P in the diets was analyzed using a colorimetric procedure (Method 931.01; AOAC Int., 2007). Potato protein isolate, corn gluten meal, canola meal, monosodium phosphate, diets, and fecal samples were also analyzed for DM by oven drying at 135°C for 2 h (Method 930.15; AOAC Int., 2007), and ingredients and diets were analyzed for ash (Method 942.05; AOAC Int., 2007). Potato protein isolate, corn gluten meal, canola meal, and diets were analyzed for phytase activity (Engelen et al., 2001) and for phytic acid (Megazyme method; AB Vista Feed Ingredients, Ystrad Mynach, UK). These samples were also analyzed for GE using an adiabatic bomb calorimeter (Model 6300; Parr Instruments, Moline, IL) with benzoic acid as the standard for calibration and for CP using the combustion procedure (Method 990.03; AOAC Int., 2007) and an apparatus (Elementar Rapid N-cube Protein/Nitrogen Apparatus; Elementar Americas Inc., Mt. Laurel, NJ). Aspartic acid was used as a calibration standard and CP was calculated as N × 6.25. Potato

protein isolate, corn gluten meal, canola meal, and diets were also analyzed for acid-hydrolyzed ether extract using 3 *N* HCl (Sanderson, 1986) followed by crude fat extraction using petroleum ether (Method 2003.06, AOAC Int., 2007) on a Soxtec 2050 automated analyzer (FOSS North America, Eden Prairie, MN). Potato protein isolate, corn gluten meal, and canola meal were also analyzed for ADF (Method 973.18; AOAC Int., 2007) and NDF (Holst, 1973). Five grams of each diet and 15 mL of distilled water were mixed and filtered, and diet pH was measured in the solution with a pH meter (Accumet Basic; Fisher Scientific, Pittsburgh, PA).

Calculations and Statistical Analysis

The concentration of phytate-bound P in potato protein isolate, corn gluten meal, canola meal, and diets was calculated as 28.2% of phytate (Tran and Sauvant, 2004) and the concentration of non-phytate-bound P was calculated as the difference between total P and phytate-bound P. Values for ATTD (%) of Ca were calculated according to the following equation (Petersen and Stein, 2006):

$$ATTD = [(Ca_{intake} - Ca_{fecal})/Ca_{intake}] \times 100, \quad [1]$$

where ATTD is the apparent tract total digestibility (%) of Ca, Ca_{intake} is the total Ca intake (g), and Ca_{fecal} is the total fecal Ca output (g). The ATTD of P was calculated using the same equation.

Total endogenous losses of Ca were estimated using the regression procedure (Fan et al., 2001). The dependent variable, apparent total tract digested Ca (Ca_D) expressed as grams per kilogram DMI, was regressed against the independent variable, dietary Ca intake (g/kg DM). Separate regressions were conducted using the 4 diets without microbial phytase and for the 4 diets with microbial phytase. Because there was a linear relationship between the graded levels of Ca intake and the digested Ca, the following equation was used (Akinmusire and Adeola, 2009):

$$Ca_D = (TTTD \times Ca_{intake}) - ECaL. \quad [2]$$

The slope of the regression line represents the estimate for TTTD of Ca, and ECaL is the negative *Y*-intercept and represents the estimate for total endogenous loss of Ca (g/kg DMI). The TTTD (%) of Ca in each diet was also calculated by correcting the ATTD of Ca for total endogenous losses of Ca according to Eq. [3], which was adopted from Stein et al., 2007:

$$TTTD = ATTD + [(ECaL_{total}/Ca_{diet}) \times 100], \quad [3]$$

where Ca_{diet} is the concentration of Ca in the diet (g/kg DM).

Retention of Ca was calculated using Eq. [4] (Petersen and Stein, 2006):

$$Ca_R = [(Ca_{intake} - Ca_{fecal})/Ca_{intake}] \times 100, \quad [4]$$

in which Ca_R is Ca retention (%) and Ca_{urine} is the total Ca output in the urine (g). The retention of P was calculated using the same equation.

Endogenous losses of Ca expressed in grams per day were calculated by using the average of the 2 estimates of endogenous losses obtained from the linear regression and it was multiplied by DMI expressed in kilograms per day for each pig. For the partitioning of Ca output, dietary Ca in feces was calculated by subtracting endogenous losses of Ca from total fecal output of Ca. Dietary Ca absorbed was calculated by subtracting dietary Ca in feces from Ca intake, and dietary Ca retained was calculated by subtracting Ca in urine and endogenous losses of Ca from dietary Ca absorbed.

Data were analyzed as a randomized complete block design with a 4 × 2 factorial arrangement using the Proc MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The UNIVARIATE procedure was used to identify outliers and outliers were identified as values that deviated from the treatment mean by more than 3 times the interquartile range (Devore and Peck, 1993). Four pigs were identified as outliers and removed from the data set (1 pig was fed 0.08% Ca without phytase, 1 pig was fed 0.08% Ca with phytase, 1 pig was fed 0.16% Ca with phytase, and 1 pig was fed 0.24% Ca with phytase). In the model, Ca level, phytase, and the interaction between Ca level and phytase were the fixed effects, and pig was the random effect. The pig was the experimental unit for all analyses. The LSMEANS procedure was used to calculate the mean values for the treatments and an α level of 0.05 was used to assess differences among means.

Linear effects of Ca intake on apparent total tract digested Ca in canola meal diets without and with phytase were determined using orthogonal CONTRAST statements. The REG procedure was used to estimate the *Y*-intercept and the slope to determine endogenous losses of Ca and the TTTD of Ca, respectively. Intercepts and slopes obtained for the diets without microbial phytase were compared with values obtained for diets that contained microbial phytase using the 95% confidence interval derived from the SE of the respective regression coefficients (Dilger and Adeola, 2006). Linear and quadratic effects of the proportion of endogenous Ca in the fecal output of pigs fed canola meal either without or with microbial phytase at different levels of Ca intake were determined using orthogonal CONTRAST statements. Linear and quadratic analyses were analyzed using the PROC REG procedure of SAS when linear and quadratic effects were significant.

Table 4. Analyzed and calculated concentrations of Ca and P in experimental diets (as-fed basis)

	Diet							
	Ca from canola meal, %				Ca from canola meal + phytase,[1] %			
Item	0.08	0.16	0.24	0.32	0.08	0.16	0.24	0.32
Ca concentration, %								
Calculated value	0.08	0.16	0.24	0.32	0.08	0.16	0.24	0.32
Analyzed value	0.15	0.21	0.29	0.36	0.15	0.21	0.29	0.38
Total P concentration, %								
Calculated value	0.45	0.54	0.63	0.73	0.45	0.54	0.63	0.73
Analyzed value	0.51	0.60	0.63	0.75	0.50	0.57	0.63	0.76
Nonphytate P concentration,[2] %								
Calculated value	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
Analyzed value	0.33	0.35	0.31	0.35	0.35	0.33	0.37	0.40

[1]Each diet contained 1,500 units of microbial phytase/kg (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK).

[2]Calculated by subtracting phytate P (i.e., 28.2% of phytate; Tran and Sauvant, 2004) from total P concentration.

RESULTS

The analyzed concentrations of Ca in the diets were between 0.04 and 0.07 percentage units greater than expected (Table 4), but the expected differences among diets were obtained. This was true for the diets without microbial phytase and for the diets with phytase. The analyzed concentrations of total P in the diets were up to 0.06 percentage units greater than expected, and the values for nonphytate P in the diets were between 0.01 percentage units less and 0.08 percentage units greater than expected. These differences were assumed not to affect the results of the experiment.

All pigs consumed their diets and remained healthy throughout the experiment. Feed intake, Ca intake, urine Ca output, and endogenous Ca increased ($P < 0.01$) by increasing Ca level in the diets and were not affected by inclusion of phytase in the diets (Table 5). The ATTD of Ca and the Ca retention expressed as percentage of Ca intake increased ($P < 0.05$) with increasing Ca level in the diets and also were greater ($P < 0.01$) when phytase was added to the diets than when no phytase was used. Therefore, Ca excretion expressed as percentage of intake decreased ($P < 0.01$) with increasing dietary Ca level and also was less ($P < 0.01$) when phytase was added to the diets than when no phytase was used. The TTTD of Ca was greater ($P < 0.01$) for diets containing phytase than for diets with no phytase, but was not affected by dietary Ca level. Calcium output in feces and excretion of Ca in grams per day increased ($P < 0.01$) with dietary Ca to a greater extent if no phytase was added to the diet than when phytase was used (interaction, $P < 0.05$). In contrast, absorbed Ca and retention of Ca in grams per day increased ($P < 0.01$) with dietary Ca to a greater extent if phytase was added to the diet than if no phytase was used (interaction, $P < 0.05$).

The estimated endogenous losses of Ca for canola meal without phytase and canola meal with phytase

Table 5. Calcium balance, apparent total tract digestibility (ATTD), and true total tract digestibility (TTTD) of Ca for pigs fed canola meal without or with microbial phytase at different levels of Ca

	Canola meal without phytase				Canola meal with phytase[1]					*P*-value		
Item Ca, %:	0.08	0.16	0.24	0.32	0.08	0.16	0.24	0.32	SEM	Ca level	Phytase	Ca level × phytase
Feed intake, g/d	679	723	734	786	702	711	727	765	38	<0.01	0.716	0.571
Ca intake, g/d	0.52	1.16	1.76	2.52	0.57	1.14	1.76	2.45	0.09	<0.01	0.728	0.709
Fecal Ca output, g/d	0.35	0.76	1.07	1.45	0.30	0.46	0.60	0.89	0.09	<0.01	<0.01	0.012
Urine Ca output, mg/d	127	123	160	205	123	135	137	182	24	<0.01	0.521	0.817
Absorbed Ca, g/d	0.18	0.40	0.69	1.07	0.26	0.67	1.16	1.56	0.07	<0.01	<0.01	0.012
Ca retention, g/d	0.06	0.27	0.53	0.86	0.14	0.54	1.03	1.37	0.07	<0.01	<0.01	<0.01
Ca retention, %	9.75	24.15	30.52	34.77	24.89	45.56	58.35	56.90	5.47	<0.01	<0.01	0.733
Ca excretion, g/d	0.48	0.88	1.23	1.65	0.42	0.59	0.73	1.08	0.10	<0.01	<0.01	0.017
Ca excretion, %	90.25	75.85	69.48	65.23	75.11	54.44	41.65	43.10	5.47	<0.01	<0.01	0.733
ATTD of Ca, %	33.71	34.65	39.60	42.96	45.89	57.30	65.91	64.19	4.94	0.030	<0.01	0.560
Endogenous Ca,[2] g/d	0.11	0.12	0.12	0.13	0.11	0.11	0.12	0.12	0.01	<0.01	0.758	0.704
TTTD of Ca,[3] %	53.95	44.65	46.28	47.93	65.96	67.34	72.59	69.18	4.94	0.862	<0.01	0.548

[1]Each diet contained 1,500 units of microbial phytase/kg (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK).

[2]Endogenous Ca was calculated by multiplying 0.160 and 0.189 g by daily DMI (kg) for pigs fed canola meal without phytase and canola meal with phytase, respectively.

[3]TTTD of Ca was calculated by correcting the ATTD values for the average (0.175 g/kg DMI) of the endogenous losses of Ca estimated in the linear regression.

Table 6. Regression of apparent total tract digested Ca (g/kg DMI) on dietary Ca intake (g/kg DM)[1]

Item	Regression equation	Slope		Intercept		R^2	Estimated TTTD[2] of Ca, %	Estimated ECaL,[2] g/kg DMI
		SE	*P*-value	SE	*P*-value			
Canola meal	$y = 0.4661x - 0.1598$	0.0428	<0.01	0.1045	0.138	0.85	46.6[b]	0.160
Canola meal + phytase	$y = 0.7026x - 0.1892$	0.0438	<0.01	0.1047	0.085	0.92	70.3[a]	0.189

[a,b]Means within a column with no common superscript are different ($P < 0.05$).

[1]Regression analyses of apparent total tract digested Ca on dietary Ca intake was linear ($P < 0.01$).

[2]TTTD = true total tract digestibility; ECaL = endogenous losses of Ca.

were 0.160 and 0.189 g/kg DMI, respectively, and these values were not different (Table 6; Fig. 1). The estimated average TTTD of Ca in canola meal without phytase (46.6%) was less ($P < 0.05$) than in canola meal with microbial phytase (70.3%).

The proportion of endogenous Ca in the fecal output of pigs fed canola meal without phytase and pigs fed canola meal with phytase decreased quadratically ($P < 0.01$) and linearly ($P < 0.01$), respectively, as dietary Ca levels increased (Fig. 2). Dietary Ca excreted in feces increased ($P < 0.01$) with dietary Ca intake to a greater extent if no phytase was added to the diets than when phytase was added (interaction, $P < 0.05$; Table 7). Therefore, dietary Ca absorbed and dietary Ca retained increased ($P < 0.01$) with dietary Ca intake to a greater extent if phytase was added to the diets than when phytase was not added (interaction, $P < 0.05$).

Phosphorus intake, P excretion in grams per day and as percentage of intake, and P retention in grams per day increased ($P < 0.01$) by increasing Ca level in the diets, but P retention as percentage of intake decreased ($P < 0.01$) by increasing Ca level in the diets (Table 8). However, P intake, P excretion, and P retention either in grams per day or as percentage of intake were not affected by addition of microbial phytase to the diets. Phosphorus output in feces increased ($P < 0.01$) with increasing levels of dietary Ca to a greater extent if no phytase was added to the diet than when phytase was used (interaction, $P < 0.01$). Phosphorus excreted in the urine was not affected by increasing dietary Ca, but P excreted in urine and absorbed P increased more with increasing dietary Ca intake if phytase was added to the diets than when no phytase was used (interaction, $P < 0.05$). The ATTD of P decreased ($P < 0.01$) as dietary Ca increased to a lesser extent if phytase was added to the diets than when phytase was not added (interaction, $P < 0.01$); therefore, microbial phytase increased ($P < 0.01$) the ATTD of P.

DISCUSSION

The concentration of Ca, total P, and phytate-bound P in canola meal used in this experiment is within the range of reported values (Sauvant et al., 2004; de Blass et al., 2010; Rostagno et al., 2011; NRC, 2012). The concentration of nonphytate P was kept constant in the diets by decreasing the amount of monosodium phosphate as the inclusion of canola meal increased, assuming the ATTD of P in monosodium phosphate is 92% (Petersen and Stein, 2006) and the ATTD of P in canola meal is 32% (Sauvant et al., 2004). The low ATTD of P in canola meal is mainly due to the high concentration of phytate, which binds most of the P and reduces P digestibility (Akinmusire and Adeola, 2009). Therefore, increasing the concentration of canola meal in the diet was expected to reduce the ATTD of P, which was also observed. It is possible that the ATTD of P was negatively affected as the level of Ca in the diets increased

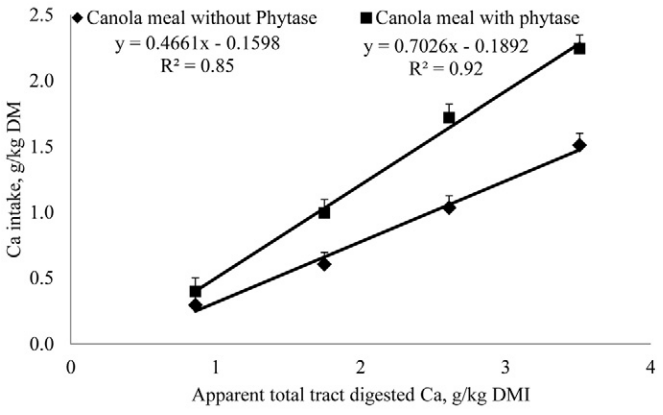

Figure 1. Regression of apparent total tract digestible Ca (g/kg DMI) on dietary Ca intake (g/kg DM) for canola meal diets without and with phytase.

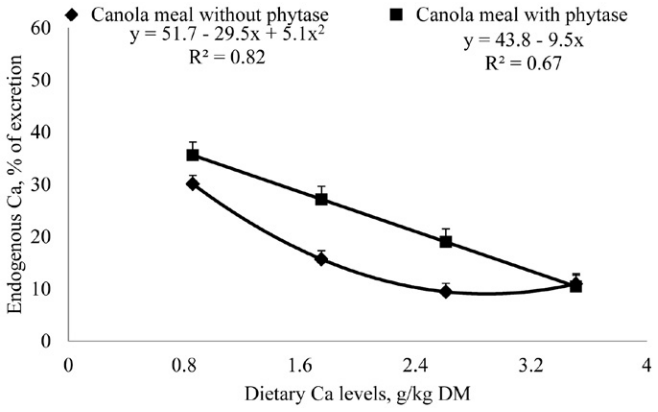

Figure 2. Endogenous Ca in fecal output of pigs fed canola meal without (quadratic, $P < 0.01$) or with microbial phytase (linear, $P < 0.01$) at different levels of Ca intake.

Table 7. Partitioning of dietary Ca from pigs fed canola meal without or with microbial phytase at different levels of Ca intake

	Canola meal without phytase				Canola meal with phytase[1]					*P*-value		
Item	0.08	0.16	0.24	0.32	0.08	0.16	0.24	0.32	SEM	Ca level	Phytase	Ca level × phytase
Ca intake, g/d	0.52	1.16	1.76	2.52	0.57	1.14	1.75	2.45	0.09	<0.01	0.728	0.709
Dietary Ca in feces,[2] g/d	0.24	0.64	0.95	1.32	0.19	0.35	0.48	0.77	0.08	<0.01	<0.01	0.012
Dietary Ca absorbed,[3] g/d	0.29	0.51	0.81	1.20	0.37	0.79	1.27	1.68	0.07	<0.01	<0.01	0.016
Dietary Ca retained,[4] g/d	0.06	0.27	0.53	0.87	0.14	0.54	1.03	1.37	0.07	<0.01	<0.01	<0.01

[1]Each diet contained 1,500 units of microbial phytase/kg (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK).

[2]Dietary Ca in feces was calculated by subtracting endogenous losses of Ca from total fecal output of Ca.

[3]Dietary Ca absorbed was calculated by subtracting dietary Ca in feces from Ca intake.

[4]Dietary Ca retained was calculated by subtracting Ca in urine and endogenous losses of Ca from dietary Ca absorbed.

because Ca–P complexes may be formed in the small intestine, thereby reducing P digestibility (Clark, 1969; Stein et al., 2011). There may also be an indirect effect of dietary Ca on P digestibility because vitamin D is activated at low Ca concentration, which may enhance both Ca and P absorption (Berner, 1997), and therefore ,increase P digestibility at low Ca concentrations.

The values of 0.11 to 0.13 g of endogenous losses of Ca per day that were obtained in the present experiment are less than the value of 1.49 g/d reported by Besançon and Guéguen (1969) and the value of 0.55 g/d reported by Fernández (1995). However, the latter values were obtained using the isotope dilution technique whereas our values were calculated using the regression procedure. Endogenous losses of P may be different if different methods are used to estimate the losses (Dilger and Adeola, 2006; Almeida and Stein, 2010) and it is possible that this is also true for Ca. It is also possible that this difference may be a result of the greater dietary Ca levels in the diets used with the isotope dilution technique studies because greater dietary Ca levels may increase the endogenous losses of Ca (Fernández, 1995). Nevertheless, results indicate that there is a measurable intestinal endogenous loss of Ca from pigs. Possible sources of Ca of endogenous origin may be saliva (Tryon and Bibby, 1966), epithelial intestinal cells (Bronner, 1997; Frandson et al., 2009), gastric juice (Trautmann and Kirchhof, 1937; Moore and Tyler, 1955), pancreatic juice (Gamble and McIver, 1928; Partridge et al., 1982; Bronner, 1997), bile (Sullivan et al., 1981; Bronner, 1997), and intestinal secretions in the form of intestinal enzymes and mucin (Moore and Tyler, 1955; Bronner, 1997). The observation that the proportion of Ca in the feces of endogenous origin decreases as Ca intake increase indicates that principles for endogenous Ca excretion are similar to principles for excretion of endogenous P, which also decreases as P intake increases (Fan et al., 2001).

The increased total daily endogenous loss of Ca that was associated with increased daily Ca intake has been demonstrated previously in pigs (Fernández, 1995) and also in ruminants (Vitti et al., 2010). It is, however, possible that some of this increase may be due to an increased intake of antinutritional factors and fiber as dietary Ca increased because Ca was increased by increasing the concentration of canola meal in the diet, and both antinutritional factors and fiber may influence endogenous losses of Ca (Cowieson et al., 2004) and P (Fang et al., 2007). It is, therefore, not possible to determine if the increase in endogenous loss of Ca that was observed as dietary Ca increased is a direct effect of increased Ca in the diet or a result of the increased concentration of canola meal. As a consequence, it is possible that the endogenous loss of Ca will be different if other ingredients with less fiber are included in the diets, and further

Table 8. Phosphorus balance and apparent total tract digestibility (ATTD) of P for pigs fed canola meal without or with microbial phytase at different levels of Ca

	Canola meal without phytase				Canola meal with phytase[1]					*P*-value		
Item	0.08	0.16	0.24	0.32	0.08	0.16	0.24	0.32	SEM	Ca level	Phytase	Ca level × phytase
P intake, g/d	3.44	4.34	4.63	5.89	3.52	4.05	4.63	5.81	0.24	<0.01	0.302	0.443
Fecal P output, g/d	0.78	1.64	2.26	3.03	0.50	0.80	1.36	1.71	0.15	<0.01	<0.01	<0.01
Urine P output, g/d	1.24	0.96	0.91	0.88	1.62	1.61	1.63	1.90	0.18	0.071	<0.01	<0.01
Absorbed P, g/d	2.68	2.70	2.36	2.87	3.02	3.24	3.25	4.11	0.18	<0.01	<0.01	0.010
P retention, g/d	1.45	1.73	1.45	1.98	1.39	1.62	1.64	2.21	0.11	<0.01	0.452	0.325
P retention, %	41.11	40.31	31.89	34.49	40.27	40.07	35.57	38.09	2.50	<0.01	0.304	0.579
P excretion, g/d	2.02	2.60	3.17	3.91	2.11	2.42	2.97	3.61	0.24	<0.01	0.138	0.524
P excretion, %	58.89	59.69	68.11	65.51	59.73	59.93	64.43	61.91	2.50	<0.01	0.304	0.579
ATTD of P, %	77.06	62.39	51.25	49.07	85.56	79.71	70.53	70.67	1.92	<0.01	<0.01	<0.01

[1]Each diet contained 1,500 units of microbial phytase per kg (Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK).

research is, therefore, needed to elucidate the effect of fiber on the endogenous loss of Ca.

To our knowledge, the effect of phytase on the endogenous loss of Ca in pigs has not been reported. We speculated that phytate may elicit increased secretions of endogenous Ca into the intestinal tract, and that this effect might be eliminated if microbial phytase was added to the diet. However, the endogenous flow of Ca was not reduced by microbial phytase, which indicates that there is either no increased intestinal loss induced by phytate, or that phytase added at 1,500 phytase units/kg of feed was ineffective in reducing the loss induced by phytate. However, the fact that there was no influence of microbial phytase on the endogenous loss of Ca is in agreement with recent observations in broiler chickens (Cowieson et al., 2004; Pirgozliev et al., 2009).

Correction of ATTD values of a nutrient for basal endogenous losses results in calculation of values for the standardized total tract digestibility, whereas correction of ATTD values for total endogenous losses results in calculation of values for TTTD (NRC, 2012). In the present experiment, we used the regression procedure to estimate endogenous losses, which results in estimation of total endogenous losses (Fan et al., 2001). As a consequence, when we corrected ATTD values for the total endogenous losses estimated in this experiment, we calculated values for TTTD of Ca.

To our knowledge, TTTD values for Ca in canola meal have not been previously reported. It was demonstrated in the present experiment that TTTD values for Ca are different from ATTD values because of the loss of endogenous Ca from the gastrointestinal tract. The increase in the ATTD of Ca, which was observed as dietary Ca increased, is a result of the reduced contribution of endogenous Ca to total Ca output with an increase in Ca intake. However, because endogenous Ca is subtracted from Ca output, as values for TTTD of Ca are calculated, TTTD values are unaffected by the level of dietary Ca, which indicates that the only reason for the increase in ATTD of Ca that was observed as Ca intake increased is the reduced contribution of endogenous Ca to total Ca output. This observation indicates that digestibility of Ca in pigs and excretion of endogenous Ca follow the principles observed for AA (Mosenthin et al., 2000) and P (Fan et al., 2001). We are not aware of any other data illustrating this principle for Ca in pigs. Because TTTD values for Ca were not influenced by the level of dietary Ca, these values are expected to be additive in mixed diets.

The observation that phytase supplementation increases ATTD of Ca is in agreement with results from previous experiments (Guggenbuhl et al., 2007; Almeida and Stein, 2010; Poulsen et al., 2010), but to our knowledge, the effect of phytase on TTTD of Ca has not been reported. The positive effect of phytase on the ATTD and TTTD of Ca in canola meal is believed to be primarily a result of the hydrolysis of phytate esters, which reduces the ability of phytate to chelate Ca (Selle et al., 2009).

Calcium may be absorbed from the intestinal tract by diffusion via the paracellular pathway or via active transport through the enterocytes (Bronner, 1987; Veum, 2010). Although active transport is downregulated as dietary Ca intake increases, Ca absorption may not be affected because more Ca is absorbed via the paracellular pathway if the concentration of Ca in the diet is increased (Bronner, 1987; Stein et al., 2011). This is likely the reason TTTD of Ca did not change as Ca intake increased. This observation indicates that at the levels of dietary Ca used in this experiment, intestinal regulation of Ca absorption plays only a minor role in Ca homeostasis.

For absorbed Ca to be retained in the body, both Ca and P have to be available (Crenshaw, 2001), but the fact that Ca retention increased as dietary Ca increased indicates that sufficient P was available to increase bone synthesis as dietary Ca increased. Likewise, more Ca was retained when phytase was used because phytase increased the amount of Ca absorbed. This observation is also in agreement with results of previous research (Poulsen et al., 2010). The reason for the relatively low values for Ca retention when calculated as a percentage of Ca intake is most likely that the intake was relatively low compared with the endogenous losses of Ca, which results in a low value for Ca retention. This was particularly true when the dietary concentration of Ca was low, but as Ca intake increased, a greater percentage of dietary Ca was retained, and if phytase was included in the diet, Ca retention increased further. The data for Ca retention, therefore, clearly illustrate the effects of endogenous Ca losses on calculated values for Ca retention.

The positive effect of phytase on P digestibility is in agreement with results from experiments in which pigs were fed diets based primarily on canola meal (Akinmusire and Adeola, 2009) or barley and canola meal (Sauer et al., 2003), and the increase in P digestibility is due to the release of P from phytate. Microbial phytase may also increase P retention (Sauer et al., 2003), but that was not observed in this experiment, which is likely a result of a lack of Ca for bone synthesis. For P to be retained in bone tissue, both Ca and P need to be available (Crenshaw, 2001; Stein et al., 2006) and because of the lack of Ca, the increased P absorption associated with use of phytase resulted in increased P excretion in the urine. Therefore, as is the case for Ca, the major regulation of P homeostasis appears to be at the renal level whereas regulation at the intestinal level seems to be less important. This observation is in agreement with previous data demonstrating that the concentration of P in a diet does not influence the digestibility of P (Stein et al., 2008).

In conclusion, there is a measurable loss of endogenous Ca from the gastrointestinal tract of pigs, but inclusion of phytase to the diets does not influence the endogenous loss of Ca. Because of the endogenous loss of Ca, values for ATTD are influenced by dietary Ca level although the influence of endogenous Ca on ATTD is less with a greater dietary concentration of Ca. In contrast, TTTD values are not influenced by dietary Ca level, and as a consequence, TTTD values for Ca are expected to be additive in mixed diets. Phosphorus digestibility is negatively affected by the levels of dietary Ca. The ATTD of both Ca and P and Ca retention is increased if microbial phytase is added to diets that are deficient in Ca. The present results indicate that Ca homeostasis in pigs fed diets containing low levels of Ca is mainly regulated at the renal level whereas regulation at the intestinal level is less important.

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Errata

doi:10.2527/jas2013-6410

In the article "Determination of endogenous intestinal losses of calcium and true total tract digestibility of calcium in canola meal fed to growing pigs" (J. Anim. Sci. 2013.91:4807−4816), Equation [4] was published incorrectly in the original version of the article. The correct equation can be found below.

$$Ca_R = \{[Ca_{intake} - (Ca_{fecal} + Ca_{urine})]/Ca_{intake}\} \times 100 \quad [4]$$

doi: 10.2527/jas.2012-6142

In the article "Nutritional intervention in early life to manipulate rumen microbial colonization and methane output by kid goats post-weaning" (J. Anim. Sci. 2013. 91:4832-4840), the daily dosage of bromochloromethane (BCM) in the section entitled 'Animals, Diets, and Experimental Design' is published incorrectly in the original version of the article. The correct dosage can be found below.

3 mg of BCM/kg BW