Concentration of dietary calcium supplied by calcium carbonate does not affect the apparent total tract digestibility of calcium, but decreases digestibility of phosphorus by growing pigs

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J ANIM SCI 2011, 89:2139-2144.
doi: 10.2527/jas.2010-3522 originally published online February 18, 2011

Concentration of dietary calcium supplied by calcium carbonate does not affect the apparent total tract digestibility of calcium, but decreases digestibility of phosphorus by growing pigs[1]

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ABSTRACT: A regional experiment was conducted to test the hypothesis that the concentration of dietary Ca does not affect the digestibility of Ca or P in diets fed to growing pigs. Six diets based on corn, potato protein isolate, cornstarch, and soybean oil were formulated. All diets also contained monosodium phosphate, crystalline AA, salt, and a vitamin-micromineral premix. The only difference among the diets was that varying concentrations of calcium carbonate were used to create diets containing 0.33, 0.46, 0.51, 0.67, 0.92, and 1.04% Ca. All diets contained between 0.40 and 0.43% P. Six universities participated in the experiment and each university contributed 2 replicates to the experiment for a total of 12 replicates (initial BW: 23.1 ± 4.4 kg). Pigs were placed in metabolism cages that allowed total, but separate, collection of feces and urine from the pigs. Pigs within each replicate were randomly allotted to the 6 diets and fed experimental diets for 14 d with urine and feces being collected over a 5-d period. Diets, feces, and urine samples were analyzed for Ca and P, and the daily balance, the apparent total tract digestibility (ATTD), and the retention of Ca and P were calculated. Results indicated that intake, fecal excretion, and urinary excretion of Ca increased (linear, $P < 0.05$) as dietary Ca concentration increased. The daily intake of P was not affected by the dietary concentration of Ca, but fecal excretion of P increased (linear, $P < 0.05$) as dietary Ca concentrations increased. In contrast, urinary P output was decreased (linear, $P < 0.05$) as dietary Ca increased. The retention of Ca increased (linear, $P < 0.05$) from 1.73 to 4.60 g/d, whereas the retention of P decreased (linear, $P < 0.05$) from 1.98 to 1.77 g/d as dietary Ca concentrations increased. However, if calculated as a percentage of intake, both Ca and P retention were decreased (linear, $P < 0.05$) as dietary Ca concentration increased (from 55.4 to 46.1% and from 48.4 to 43.5%, respectively). The ATTD of Ca was not affected by the dietary concentration of Ca, but the ATTD of P was decreased (linear, $P < 0.05$) from 56.9 to 46.2% as dietary Ca concentration increased. It is concluded that the dietary concentration of Ca does not affect the ATTD of Ca in calcium carbonate, but increased concentrations of dietary Ca may decrease the ATTD of P in diets based on corn, potato protein isolate, and monosodium phosphate.

Key words: calcium, calcium carbonate, digestibility, phosphorus, pig

J. Anim. Sci. 2011. 89:2139–2144
doi:10.2527/jas.2010-3522

INTRODUCTION

Both Ca and P are needed for bone tissue synthesis (Crenshaw, 2001), and considerable quantities of P are also needed for synthesis of muscle. It is, therefore, recommended that total Ca and total P are added to swine diets at a ratio between 1:1 and 1.25:1, although pigs tolerate a wide ratio between the 2 minerals (Crenshaw, 2001). Sometimes, the ratio is based on digestible P rather than total P, in which case, the Ca-to-P

[1]The donation of calcium carbonate for this work by Iowa Limestone Company, Alden, IA, is greatly appreciated.

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Received September 19, 2010.
Accepted February 11, 2011.

ratio should be between 2:1 and 3:1 (Crenshaw, 2001). Because only the digestible portion of the dietary Ca can be used for bone synthesis, it is more accurate if the ratios are expressed as digestible Ca to digestible P (Eeckhout et al., 1995). Although it is well documented that the digestibility of P is less than 100% in most sources of dietary P, there is limited information about the digestibility of Ca in the sources of Ca that are usually fed to pigs. Unlike P, only a small portion of the Ca needs of a pig is provided by the vegetable feed ingredients that are most often used in diets fed to pigs. The majority of Ca in the diets is provided by inorganic sources such as calcium carbonate, limestone, and calcium phosphates, but the digestibility of Ca in pigs in these sources has not been reported.

It is possible that the digestibility of Ca is affected by the concentration of Ca in the diet because Ca absorption may be related to the concentration of 1,25-dehydroxycholecalciferol, and the concentration of this hormone is regulated by plasma Ca concentrations (Crenshaw, 2001). It is also possible that the dietary concentration of Ca may influence the digestibility of P in the diet because Ca can form complexes with P in the intestinal tract of pigs, which may render the P undigestible. However, to our knowledge, there are no data on the effects of dietary Ca concentration on the digestibility of Ca and P. It was, therefore, the objective of this experiment to test the hypothesis that dietary Ca concentration does not affect the digestibility of Ca and P by growing pigs.

MATERIALS AND METHODS

This experiment was conducted at 6 universities as part of the research efforts in the North Central Coordinating Committee on Swine Nutrition (NCCC-42). The 6 participating universities were North Carolina State University, Purdue University, The Ohio State University, University of Illinois, University of Kentucky, and University of Nebraska. The protocol for the experiment was reviewed and approved by the Institutional Animal Care and Use Committee at each participating university.

Diets, Ingredients, and Experimental Design

Six diets were formulated (Tables 1 and 2). Diets were based on corn, potato protein isolate, corn starch, and soybean oil, and the only difference among the 6 diets was that the concentration of calcium carbonate varied to create diets that contained graded amounts of dietary Ca (0.33, 0.46, 0.51, 0.67, 0.92, and 1.04%). The concentration of calcium carbonate in the diets was increased at the expense of cornstarch. Corn contains very little Ca, and potato protein isolate was used as a protein source because it is a rich source of indispensable AA but almost devoid of Ca. Using these ingredients, it was, therefore, possible to formulate a basal diet that contained very little organic Ca. All diets contained 0.80% monosodium phosphate and were calculated to contain 0.46% total P. The concentration of all other nutrients was constant among diets and calculated to meet or exceed current requirement estimates for growing pigs (NRC, 1998). The calcium carbonate used in the experiment (Iowa Limestone Company, Alden, IA) contained 97.08% calcium carbonate and 38.83% Ca by analysis. The average particle size of the calcium carbonate was 550 μm. The monosodium phosphate and the potato protein isolate were obtained from a commercial company (ADM, Decatur, IL). The corn was a commercial hybrid that was sourced from the University of Illinois Feed Mill (Champaign, IL). The same batch of each ingredient was used in all diets, and each diet was mixed in 1 batch at the University of Illinois, analyzed for Ca and P, and shipped to participating universities. All diets were fed in a mash form.

The experiment was conducted as a randomized complete block design with 6 dietary treatments and 6 pigs per replicate. There were 2 replicates at each of the participating universities for a total of 12 replicates and 72 pigs. White crossbred pigs were used at all universities, and the genetic background of the pigs reflected the genetic material that was available at each university at the time of the experiment.

Animals, Feeding, and Sample Collection

For each replicate, 6 growing barrows (initial BW: 23.1 ± 4.4 kg) were placed in metabolism cages and randomly allotted to the 6 treatment diets. Pigs were fed a corn-soybean meal, pretest diet containing 0.60% Ca and 0.50% P during the 14 d preceding the start of the experiment. The pretest diet was manufactured at each participating university. Inorganic sources of Ca and P in this diet were calcium carbonate, ground calcitic limestone, dicalcium phosphate, or monocalcium phosphate depending on availability at each participating university. No microbial phytase was used in the pretest diet.

Pigs were fed experimental diets with approximately 3 times the maintenance requirement for energy (i.e., 106 kcal of ME/kg of $BW^{0.75}$; NRC, 1998). Pigs were fed the experimental diets for 14 d with the initial 7 d being an adaptation period to the diet. In the morning meals of d 8 and 13, 0.25% of indigo carmine was mixed into the diet. Urine collection started immediately after feeding the morning meal on d 8 and ceased immediately after feeding the morning meal on d 13. Urine was collected over a preservative of 20 mL of 6 *N* HCl. Fecal collection commenced at the first sign of the marker in the feces after d 8 and ceased when the marker appeared in the feces for the first time after d 13, as described previously (Stein et al., 2008). Urine and fecal samples were collected quantitatively during the collection period. The total quantity of urine that was collected daily was weighed, and a subsample of 20% was stored at −20°C. Fecal samples were also stored at −20°C as they were collected. All samples were mixed within pig at the conclusion of the experiment.

Table 1. Ingredient composition of diets (as-fed basis)

	Ca from calcium carbonate, %					
Ingredient, %	0.30	0.45	0.60	0.75	0.90	1.05
Ground corn	84.00	84.00	84.00	84.00	84.00	84.00
Potato protein isolate	8.00	8.00	8.00	8.00	8.00	8.00
Cornstarch	2.50	2.11	1.72	1.34	0.95	0.57
Soybean oil	3.00	3.00	3.00	3.00	3.00	3.00
Calcium carbonate	0.77	1.16	1.55	1.93	2.32	2.70
Monosodium phosphate	0.80	0.80	0.80	0.80	0.80	0.80
L-Lysine·HCl	0.30	0.30	0.30	0.30	0.30	0.30
L-Tryptophan	0.03	0.03	0.03	0.03	0.03	0.03
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin-mineral premix[1]	0.30	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00	100.00

[1]Provided the following quantities of vitamins and minerals per kilogram of complete diet: vitamin A, 11,128 IU as vitamin A acetate; vitamin D_3, 2,204 IU of D-activated animal sterol; vitamin E, 66 IU as α-tocopherol acetate; vitamin K, 1.42 mg as menadione dimethylpyrimidinol bisulfite; thiamine, 0.24 mg as thiamine mononitrate; riboflavin, 6.58 mg; pyridoxine, 0.24 mg as pyridoxine hydrochloride; vitamin B_{12}, 0.03 mg; D-pantothenic acid, 23.5 mg as calcium pantothenate; niacin, 44.00 mg; folic acid, 1.58 mg; biotin, 0.44 mg; Cu, 10 mg as copper sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as potassium iodate; Mn, 60 mg as manganese sulfate; Se, 0.3 mg as sodium selenite; and Zn, 100 mg as zinc oxide.

Chemical Analyses

Fecal samples were oven-dried at 65°C, weighed, and ground through a 1-mm screen. Ground fecal samples and frozen urine samples were shipped overnight to the University of Illinois where they were stored until analyzed. Subsamples of urine and feces were then transported to the University of Missouri (Columbia) and analyzed for Ca and P using inductively coupled plasma spectroscopy (method 985.01; AOAC International, 2007) after wet ash sample preparation (method 975.03; AOAC International, 2007).

Calculations and Statistical Analysis

Total balances for Ca and P were calculated for each diet. The apparent total tract digestibility (**ATTD**) and retention rate for Ca and P in each experimental diet were also calculated using the direct procedure (Adeola, 2001). Analyzed concentrations of Ca and P in the diets were used in these calculations. Data were analyzed using the MIXED Procedure (SAS Inst. Inc., Cary, NC). The model statement included dietary treatment as a fixed effect, and the random statement included station and the station × treatment interaction. Polynomial contrasts were used to test linear and quadratic effects of dietary Ca concentration on intake, output, digestibility, and retention of Ca and P. Appropriate coefficients for unequally spaced Ca concentration in the diets were obtained using the interactive matrix language procedure of SAS (PROC IML). The pig was the experimental unit for all analyses, and an α value of 0.05 was used to assess significance among means.

RESULTS

The analyzed concentrations of Ca in 5 of the 6 experimental diets were between 0.02 and 0.12% less than expected (Table 2), but the differences among Ca concentrations in the diets were close to calculated values. The analyzed values for P were also slightly less than expected, but the values in the 6 diets were similar and assumed not to affect the results of the experiment.

Table 2. Analyzed and calculated concentrations of Ca and P in experimental diets (as-fed basis)[1]

	Ca from calcium carbonate, %					
Item	0.30	0.45	0.60	0.75	0.90	1.05
Ca concentration, %						
Calculated value	0.33	0.48	0.63	0.78	0.93	1.08
Analyzed value	0.33	0.46	0.51	0.67	0.92	1.04
P concentration, %						
Calculated value	0.46	0.46	0.46	0.46	0.46	0.46
Analyzed value	0.42	0.43	0.40	0.42	0.42	0.43

[1]The calculated concentration of ME in the 6 diets was 3,535, 3,519, 3,504, 3,489, 3,473, and 3,458 kcal/kg (NRC, 1998) and the calculated concentration of digestible P in all diets was 0.23%. All diets also were calculated (NRC, 1998) to contain 13.1% CP, 0.65% Arg, 0.44% His, 0.58% Ile, 0.90% Leu, 0.95% Lys, 0.30% Met, 0.56% Met + Cys, 0.74% Phe, 0.63% Thr, 0.17% Trp, and 1.29% Val.

Table 3. Daily balance and apparent total tract digestibility (ATTD) of Ca in pigs fed experimental diets (as-fed basis)[1]

	Analyzed Ca concentration, %							*P*-value	
Item	0.33	0.46	0.51	0.67	0.92	1.04	SEM	Linear	Quadratic
Initial BW, kg	22.2	22.6	23.5	23.9	22.7	23.1	1.8	0.53	0.10
Diet intake, kg/d	0.96	0.96	0.96	0.94	0.94	0.93	0.08	0.23	0.79
Ca intake, g/d	3.09	4.42	4.90	6.31	8.66	9.65	0.55	0.01	0.61
Feces output, g/d	90	85	89	88	97	94	9	0.05	0.43
Ca in feces, %	1.37	1.49	1.89	2.68	3.04	3.79	0.26	0.01	0.94
Fecal Ca output, g/d	1.22	1.23	1.60	2.27	2.88	3.47	0.22	0.01	0.35
Urine output, kg/d	2.61	3.71	3.30	4.07	3.60	3.55	1.32	0.59	0.43
Ca in urine, mg/kg	115	232	398	517	722	771	154	0.01	0.37
Urinary Ca output, g/d	0.17	0.37	0.82	1.23	1.34	1.58	0.14	0.01	0.03
Absorbed Ca, g/d	1.90	3.19	3.30	3.99	5.78	6.18	0.62	0.01	0.45
Retained Ca, g/d	1.73	2.82	2.49	2.75	4.44	4.60	0.65	0.01	0.75
ATTD of Ca, %	60.9	70.9	65.6	62.4	65.2	62.9	4.7	0.62	0.25
Ca retention, % of intake	55.4	62.8	48.4	42.2	48.8	46.1	6.2	0.01	0.09
Ca retention, % of absorbed	90.2	88.5	71.8	65.0	72.6	72.7	5.1	0.01	0.01

[1]Each least squares means represents 12 observations.

Pigs easily consumed the diets, and no problems with feed intake were observed. The daily intake of Ca increased (linear, $P < 0.01$) as the concentration of Ca in the diets increased (Table 3). Total fecal output also increased (linear, $P < 0.05$) as Ca concentration in the diet increased, but dietary Ca concentration did not affect urine Ca output. The concentration (%) and quantity (g/d) of Ca in the feces increased (linear, $P < 0.01$) as the Ca concentration in the diet increased. Likewise, urinary Ca concentration (mg/kg) and quantity (g/d) increased (linear, $P < 0.01$) with increasing dietary Ca.

The quantity (g/d) of absorbed and retained Ca increased (linear, $P < 0.01$) as dietary Ca concentration increased, but the ATTD of Ca was not affected by dietary Ca concentration. The retention of Ca calculated as a percentage of Ca intake or as a percentage of absorbed Ca decreased as dietary Ca increased (linear, $P < 0.01$, and linear and quadratic, $P < 0.01$, respectively).

Daily intake of P was not affected by the concentration of Ca in the diets (Table 4), but concentration (%) and quantity (g/d) of P in feces increased (linear, $P < 0.01$), whereas P concentration and quantity in urine decreased (linear, $P < 0.01$) as Ca in the diet increased. The quantity (g/d) of P that was absorbed and retained and the ATTD of P were decreased (linear, $P < 0.01$) as dietary Ca increased. The retention of P calculated as percentage of P intake also decreased (linear $P < 0.01$) as dietary Ca increased, but if calculated as a percentage of P absorbed, P retention increased (linear and quadratic, $P < 0.05$) as Ca intake increased.

DISCUSSION

Ca Digestibility and Balance

The requirement for Ca in 20- to 50-kg pigs is 0.60% (NRC, 1998). The diets used in the present experiment contained between 0.33 and 1.04% Ca, which is between 55 and 173% of the requirement (NRC, 1998). The Ca in the diets originated from corn, potato protein isolate, and calcium carbonate. Corn and potato protein isolate, however, contributed only a total of about 0.04% Ca to the diet, and the majority of Ca in

Table 4. Daily balance and apparent total tract digestibility (ATTD) of P in pigs fed experimental diets (as-fed basis)[1]

	Analyzed Ca concentration, %							*P*-value	
Item	0.33	0.46	0.51	0.67	0.92	1.04	SEM	Linear	Quadratic
P intake, g/d	4.01	4.11	3.84	3.95	3.95	3.98	0.33	0.68	0.49
P in feces, %	1.89	1.91	1.93	2.26	2.16	2.28	0.10	0.01	0.38
Fecal P output, g/d	1.70	1.59	1.69	1.92	2.05	2.10	0.13	0.01	0.73
P in urine, mg/kg	167	42	39	32	28	30	20	0.01	0.01
Urinary P output, g/d	0.34	0.11	0.12	0.09	0.09	0.10	0.04	0.01	0.01
Absorbed P, g/d	2.32	2.52	2.15	2.04	1.91	1.88	0.31	0.01	0.70
Retained P, g/d	1.98	2.41	2.03	1.94	1.82	1.77	0.33	0.01	0.23
ATTD of P, %	56.9	60.6	55.1	50.1	47.4	46.2	4.0	0.01	0.88
P retention, % of intake	48.4	57.7	51.6	47.2	44.9	43.5	4.6	0.01	0.21
P retention, % of absorbed	83.1	95.1	93.6	94.8	94.5	94.0	3.0	0.03	0.01

[1]Each least squares means represents 12 observations.

all diets was, therefore, provided by calcium carbonate. To our knowledge, the ATTD of Ca in calcium carbonate fed to pigs has not been measured. However, the ATTD of Ca in a wheat-barley-corn-based diet fed to pigs, in which calcium carbonate contributes 86% of the Ca, is between 63 and 74% (Malde et al., 2010) and the ATTD of Ca in calcitic limestone fed to Japanese quail is 72% (Kim et al., 1985). The ATTD values for Ca measured in the present experiment were between 60.9 and 70.9%, which agrees with previous research and indicates that the ATTD of Ca in calcium carbonate is similar to that of calcitic limestone. The bioavailability of Ca in calcitic limestone is between 96 and 102% relative to that of calcium carbonate (Ross et al., 1984), which further indicates that there is no difference in the digestibility of Ca between calcitic limestone and calcium carbonate.

It has been suggested that intestinal absorption of Ca is downregulated if the concentration of Ca in the diet exceeds the requirement for Ca (Hurwitz and Bar, 1969; Pointillart et al., 1989; Soares, 1995). The biological basis for this downregulation is that Ca absorption is regulated by calcitonin and parathyroid hormone and at increased serum concentrations of Ca, secretion of calcitonin from the C-cells in the parathyroid glands will increase, which may lead to a decrease in the active absorption of Ca from the small intestine. However, Ca is absorbed by an active energy-dependent mechanism, as well as by a passive paracellular mechanism (Allen, 1982; Bronner, 1987; Crenshaw, 2001). It is possible that as the energy-dependent uptake of Ca is downregulated by calcitonin (Berdanier, 1998), the paracellular uptake may compensate for this and allow more Ca to be absorbed, which results in a constant Ca digestibility (Pointillart et al., 1989).

In the present experiment, no attempts to distinguish Ca absorption between the 2 absorption mechanisms were made, but the values for ATTD of Ca that were measured indicate that the ATTD of Ca in calcium carbonate is not affected by the concentration of Ca in the diet if dietary Ca is between 55 and 173% of the requirement. This observation is in agreement with data showing that the ATTD of Ca in a diet containing 1.51% Ca is not different from that of a diet containing 0.91% Ca (Pointillart et al., 1989). The ATTD of Ca in 90-kg pigs is also slightly greater than in 60-kg pigs (Kemme et al., 1997), although the requirement for Ca is decreased as pigs become heavier, which further indicates that supplying Ca in the diet in excess of the requirement does not decrease Ca digestibility. It appears, therefore, that the ATTD of Ca is not affected by the dietary content of Ca.

The lack of an effect of dietary Ca on the ATTD of Ca indicates that Ca homeostasis is mainly regulated at the renal level rather than at the intestinal level. This observation is in agreement with data indicating that pigs fed a P-free diet have almost the same Ca absorption as pigs fed diets with normal Ca content, but because of the lack of P in these pigs, most of the absorbed Ca is excreted in the urine (Stein et al., 2006). The rapid increase in urinary Ca that was observed in the present experiment as dietary Ca increased also supports the hypothesis that Ca homeostasis is mainly regulated at the renal level.

P Digestibility and Balance

The P in the diets used in this experiment was supplied by corn (approximately 0.21%), potato protein isolate (approximately 0.01%), and monosodium phosphate (approximately 0.21%). If it is assumed that the ATTD of P in corn and monosodium phosphate is 28 and 92%, respectively (Bohlke et al., 2005; Petersen and Stein, 2006), it can be calculated that the ATTD of P in the diets used in this experiment should be approximately 60%. The ATTD of P in the 2 diets with the least concentrations of Ca was close to the expected value (56.9 and 60.6%, respectively), but as dietary Ca concentration increased, the ATTD of P was linearly decreased and the ATTD of P in the diet with the greatest Ca concentration was only 46.2%.

The ATTD of P in phytase-supplemented diets is decreased if dietary Ca is increased (Lei et al., 1994; Liu et al., 1998; Brady et al., 2002; Selle et al., 2009), but to our knowledge, such a mechanism has not been documented for the ATTD of P in diets that are not supplemented with phytase. It has, however, been shown that serum P concentrations, bone ash concentration, and bone bending moment are decreased if the Ca-to-P ratio is increased from 1.3:1 to 2.0:1, 3.0:1, or 4.0:1 (Reinhart and Mahan, 1986), which indicates that the absorption and digestibility of P may also have been decreased as the dietary Ca-to-P ratio was increased. Likewise, tibia ash in pigs fed diets containing 1.41% Ca is less than in pigs fed diets containing 0.91% Ca if the P concentration in the diet is constant (Pointillart et al., 1989), which indicates that the ATTD of P was decreased in the diets with the greatest Ca concentration. In growing White Pekin ducklings fed low P diets, BW gain, feed intake, and tibia ash concentration are also decreased if dietary Ca concentration is increased (Xie et al., 2009), which is most likely caused by a decrease in the absorption of P. Thus, negative effects of increasing dietary Ca concentrations on measures of P status of animals have previously been reported. In the present experiment, it was, however, demonstrated that these negative effects are a result of a decreased ATTD of P as dietary Ca concentrations are increased.

The reason for the decrease in the ATTD of P as dietary Ca increases has not been elucidated. It is possible that increased dietary Ca may result in binding of P by Ca in the intestinal tract of the animals, which may form a Ca-P complex that precludes P from being absorbed. However, such mechanisms have not been demonstrated, and research in this area is, therefore, warranted. It is also possible that the decreased ATTD of P was caused by increased endogenous intestinal loss of P, but there is no information about the effects of di-

etary Ca on endogenous intestinal loss of P, so research in this area is also needed.

Pigs used in the present experiment were limit fed to 3 times the estimated energy requirement for maintenance. To our knowledge, there are no data on the possible effects of limit feeding on the digestibility of P. It has, however, been demonstrated that there is no difference in the apparent ileal digestibility of AA between pigs fed 3 times the estimated energy requirement for maintenance and pigs given ad libitum access to feed (Chastanet et al., 2007). We speculate, therefore, that data for the apparent digestibility of P obtained with limit-fed pigs are also applicable to pigs given free access to feed.

Conclusions

The observation that the digestibility of Ca in calcium carbonate is much less than 100% indicates that diets may be more accurately formulated if values for digestible Ca in feed ingredients rather than for total Ca are used. The important influence of Ca on the ATTD of P also points in this direction. There are, however, limited data on the digestibility of Ca in different feed ingredients, and more work in this area is needed before diets can be formulated on the basis of digestible Ca.

Results from the present experiment imply that dietary Ca concentration is not critical for Ca digestibility of feed ingredients if measured in diets containing between 55 and 173% of the requirement for Ca. However, results from this experiment clearly document that the dietary Ca concentration affects the ATTD of P. The dietary Ca concentration is, therefore, very important when the ATTD of P in feed ingredients is measured and it may be concluded that the greatest values for ATTD of P is obtained if the dietary Ca to P ratio is around or slightly less than 1.1:1. There is, however, a need for more research in this area.

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