Published August 30, 2016

Requirement for digestible calcium by eleven- to twenty-five–kilogram pigs as determined by growth performance, bone ash concentration, calcium and phosphorus balances, and expression of genes involved in transport of calcium in intestinal and kidney cells[1]

J. C. González-Vega,* Y. Liu,*[2] J. C. McCann,* C. L. Walk,† J. J. Loor,* and H. H. Stein‡*[3]

*Department of Animal Sciences, University of Illinois, Urbana 61801;
†AB Vista, Marlborough, SN8 4AN, UK; and ‡Division of Nutritional Sciences, University of Illinois, Urbana 61801

ABSTRACT: Two experiments were conducted to determine the requirement for standardized total tract digestible (STTD) Ca by 11- to 25-kg pigs based on growth performance, bone ash, or Ca and P retention and to determine the effect of dietary Ca on expression of genes related to Ca transport in the jejunum and kidneys. Six diets were formulated to contain 0.36% STTD P and 0.32, 0.40, 0.48, 0.56, 0.64, or 0.72% STTD Ca by including increasing quantities of calcium carbonate in the diets at the expense of cornstarch. Two additional diets contained 0.72% STTD Ca and 0.33% or 0.40% STTD P to determine if 0.36% STTD P had negative effects on the Ca requirement. The same batch of all diets was used in both experiments. In Exp. 1, 256 pigs (11.39 ± 1.21 kg initial BW) were randomly allotted to the 8 diets with 4 pigs per pen and 8 replicate pens per diet in a randomized complete block design. On the last day of the experiment, 1 pig from each pen was euthanized and the right femur and intestine and kidney samples were collected. Results indicated that ADG and G:F started to decline (linear and quadratic, $P < 0.05$) at 0.54 and 0.50% STTD Ca, respectively. In contrast, bone ash increased (quadratic, $P < 0.05$) as dietary Ca increased and reached a plateau indicating that the requirement for STTD Ca to maximize bone ash was 0.48%. Bone ash, but not ADG or G:F, increased (linear, $P < 0.01$) as STTD *P* increased in the diets. The mRNA expression of genes related to transcellular Ca transport decreased (linear, $P < 0.01$) in the jejunum and in kidneys (linear and quadratic, $P < 0.01$) as dietary Ca increased. In Exp. 2, 80 pigs (13.12 ± 1.79 kg initial BW) were placed in metabolism crates and randomly allotted to the 8 diets with 10 replicate pigs per diet in a randomized complete block design. Fecal and urine samples were collected using the marker-to-marker approach. Results indicated that the requirement for STTD Ca to maximize Ca and P retention (g/d) was 0.60 and 0.49%, respectively. In conclusion, the STTD Ca requirement by 11- to 25-kg pigs to maximize bone ash was 0.48%; however, ADG and G:F declined if more than 0.54 or 0.50% STTD Ca, respectively, was fed, and the minimum concentration of Ca needed to maximize ADG and G:F could not be determined under the conditions of this experiment. Increasing dietary Ca decreased the mRNA expression of several genes related to transcellular Ca transport in the jejunum and the kidneys.

Key words: bone ash, calcium balance, calcium requirement, calcium retention, digestible calcium, pigs

 J. Anim. Sci. 2016.94:3321–3334
doi:10.2527/jas2016-0444

INTRODUCTION

An optimum utilization of Ca and P by pigs is expected if diets are formulated to meet requirements for standardized total tract digestible (**STTD**) Ca and STTD P. Requirements for total Ca in diets fed to growing pigs may be calculated by multiplying the requirements for STTD P by 2.15 (NRC, 2012), but no STTD Ca requirements have been reported because of a lack of data for standardized total tract digestibility

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

[2]Present address: Department of Animal Science, University of California, Davis, CA 95616.

[3]Corresponding author: hstein@illinois.edu

Received March 5, 2016.
Accepted May 14, 2016.

of Ca (NRC, 2012). However, recent experiments have generated values for standardized total tract digestibility of Ca in several commonly used Ca sources (González-Vega et al., 2014, 2015a,b). Therefore, requirements for digestible Ca may now be established. The concentration of STTD Ca needed to maximize growth performance is expected to be less than the concentration needed to maximize bone ash and Ca retention in young growing pigs (NRC, 2012), but because 96 to 99% of Ca is deposited in skeletal tissue (Crenshaw, 2001), no differences between concentrations of STTD Ca needed to maximize bone ash and Ca retention are expected.

Vitamin D may influence the expression of genes involved in transcellular transport of Ca, which is mainly used if dietary Ca is low (Kutuzova and DeLuca, 2004), and in dogs, GH and IGF-1 may indirectly affect Ca absorption because of regulation of vitamin D synthesis (Tryfonidou et al., 2003). Research has been conducted in broiler chickens, rats, and mice to evaluate the effect of dietary Ca concentrations on expression of genes involved in transcellular transport of Ca in the small intestine and kidneys (Armbrecht et al., 1980, 2003; Rosenberg et al., 1986; Hurwitz et al., 1995; van de Graaf et al., 2004; Healy et al., 2005; Ko et al., 2009), but limited data have been reported for pigs. Therefore, the objectives of the present experiments were to determine the requirement for STTD Ca by 11- to 25-kg pigs to maximize growth performance, bone ash, and Ca retention and to determine the effect of dietary Ca on the expression of genes involved in transcellular transport of Ca in jejunal and kidney cells.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the University of Illinois reviewed the protocols for the 2 experiments. Pigs used in both experiments were the offspring of G-Performer boars and Fertilis 25 females (Genetiporc, Alexandria, MN).

Experiment 1: Growth Performance, Bone Ash, and Gene Expression

Animals, Diets, and Feeding. Two hundred fifty-six pigs (11.39 ± 1.21 kg initial average BW) were randomly allotted to 8 diets with 8 replicate pens per diet in a randomized complete block design. Pen was the experimental unit and 2 barrows and 2 gilts were housed in each pen. The experiment was conducted in 2 blocks; 1 block with 3 replicate pens per diet and 1 block with 5 replicate pens per diet. The Experimental Animal Allotment Program (Kim and Lindemann, 2007) was used to allot pigs to experimental diets. Pigs were housed in pens with fully slatted floors, and room temperature was controlled (27.5 ± 2.1°C maximum temperature and 24.1 ± 2.2°C minimum temperature).

Table 1. Composition of ingredients, as-fed basis, Exp. 1 and 2

	Ingredient				
Item	Corn	Soybean meal	Lactose	Calcium carbonate	Monocalcium phosphate
GE, kcal/kg	3,913	4,160	3,685	–	–
DM, %	90.70	90.45	95.13	99.93	94.62
CP, %	8.96	47.69	0.13	–	–
NDF, %	8.49	7.42	–	–	–
ADF, %	3.42	5.86	–	–	–
Ash, %	1.40	8.11	0.46	93.30	80.93
Ca, %	0.01	0.52	0.04	41.16	19.03
P, %	0.25	0.58	0.01	1.13	22.48
Phytate, %	0.60	1.38	–	–	–
Phytate-bound P,[1] %	0.17	0.39	–	–	–
Nonphytate P,[2] %	0.08	0.19	–	–	–

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

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

Diets were based on corn, soybean meal, and lactose (Table 1). A diet containing 0.36% STTD P and 0.32% STTD Ca was formulated (Tables 2 and 3). Five additional diets were formulated to contain 0.36% STTD P and 0.40, 0.48, 0.56, 0.64, or 0.72% STTD Ca, by including increasing quantities of calcium carbonate in the diets at the expense of cornstarch. The 3 diets with the least concentrations of Ca contained less total Ca than the requirement according to the NRC (2012) and the 3 diets with the greatest concentrations of Ca met or exceeded the Ca requirement, but the concentration of STTD P in all diets was 10% above the requirement (NRC, 2012; Table 3). Two additional diets were formulated to contain 0.72% STTD Ca and 0.33% (NRC [2012] requirement) or 0.40% STTD P. Microbial phytase was not included in any diets.

Growth Performance and Bone Measurements. Pigs were allowed ad libitum access to feed and water throughout the experiment, and pigs were weighed at the beginning of the experiment, on d 10, and at the conclusion of the experiment (d 22). The amount of feed offered was recorded every day, and the amount of feed left in the feeder at the conclusion of the experiment was subtracted from the quantity of feed offered. On the last day of the experiment, 1 barrow in each pen that had a BW closest to the average BW of the pen was euthanized via captive bolt stunning. The right front foot and the right hind leg were removed and stored at −20°C and later autoclaved separately at 125°C for 55 min. The third and fourth metacarpals were removed from the feet and femurs were removed from the hind legs. The marrow of the broken metacarpals and femurs

Table 2. Ingredient composition of experimental diets, as-fed basis, Exp. 1 and 2

	0.36% STTD[1] P						0.33% STTD P	0.40% STTD P
Ingredient, %	0.32% STTD Ca	0.40% STTD Ca	0.48% STTD Ca	0.56% STTD Ca	0.64% STTD Ca	0.72% STTD Ca	0.72% STTD Ca	0.72% STTD Ca
Ground corn	53.00	53.00	53.00	53.00	53.00	53.00	53.00	53.00
Soybean meal, 47% CP	29.50	29.50	29.50	29.50	29.50	29.50	29.50	29.50
Lactose	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Cornstarch	3.79	3.13	2.49	1.89	1.25	0.61	0.76	0.48
Choice white grease	0.90	1.21	1.52	1.80	2.10	2.41	2.33	2.47
Calcium carbonate	0.05	0.40	0.73	1.05	1.39	1.72	1.80	1.59
Monocalcium phosphate	1.15	1.15	1.15	1.15	1.15	1.15	1.00	1.35
L-Lys HCL	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
DL-Met	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-Thr	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-Val	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Sodium chloride	0.62	0.62	0.62	0.62	0.62	0.62	0.62	0.62
Vitamin–mineral premix[2]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

[1]STTD = standardized total tract digestible.

[2]The vitamin–micromineral premix provided the following quantities of vitamins and microminerals per kilogram of complete diet: 11,136 IU vitamin A as retinyl acetate, 2,208 IU vitamin D_3 as cholecalciferol, 66 IU vitamin E as DL-alpha tocopheryl acetate, 1.42 mg vitamin K as menadione dimethylprimidinol bisulfite, 0.24 mg thiamin as thiamine mononitrate, 6.59 mg riboflavin, 0.24 mg pyridoxine as pyridoxine hydrochloride, 0.03 mg vitamin B_{12}, 23.5 mg D-pantothenic acid as D-calcium pantothenate, 44.1 mg niacin, 1.59 mg folic acid, 0.44 mg biotin, 20 mg Cu as copper sulfate, 126 mg Fe as iron sulfate, 1.26 mg I as ethylenediamine dihydriodide, 60.2 mg Mn as manganous sulfate, 0.25 mg Se as sodium selenite (0.15 mg) and selenium yeast (0.10 mg), and 124.9 mg Zn as zinc sulfate.

was removed and the bones were dried and soaked in petroleum ether under a chemical hood for 72 h to remove the remaining marrow and fat. Bones were dried overnight at 130°C and ashed at 600°C for 16 h to calculate the concentration of bone ash.

Sample Analysis. Corn, soybean meal, lactose, calcium carbonate, monocalcium phosphate, and diets were analyzed for DM by oven drying at 135°C for 2 h (method 930.15; AOAC Int., 2007), for ash (method 942.05; AOAC Int., 2007), and for Ca and P by inductively coupled plasma–optical emission spectrometry (**ICP–OES**; method 985.01 A, B, and C; AOAC Int., 2007) after wet ash sample preparation (method 975.03 B(b); AOAC Int., 2007). The ash from the femurs was also analyzed for Ca and P. Corn, soybean meal, and lactose were analyzed for GE using an isoperibol bomb calorimeter (model 6300; Parr Instruments, Moline, IL). Benzoic acid was used as the standard for calibration. Corn, soybean meal, lactose, and diets were analyzed for N using the combustion procedure (method 990.03; AOAC Int., 2007) on an Elementar Rapid N-cube protein/nitrogen apparatus (Elementar Americas Inc., Mt. Laurel, NJ) and CP was calculated as N × 6.25. Corn, soybean meal, and diets were also analyzed for ADF (method 973.18; AOAC Int., 2007) and NDF (Holst, 1973). Corn and soybean meal were calorimetrically analyzed for P in phytic acid with an assay kit (K-PHYT; Megazyme International, Wicklow, Ireland). Diets were analyzed for phytate P using a Foss near-infrared spectrometer (AB Vista, Memphis, TN) with the phytate P levels predicted using AUNIR (Northamptonshire, UK) calibration standards based on the K-PHYT assay kit (Megazyme International, Co. Wicklow, Ireland).

Sample Collection for Gene Expression. From 48 euthanized pigs that were fed diets containing 0.36% STTD P and 0.40, 0.48, 0.56, 0.64, or 0.72% STTD Ca, 5-cm jejunum tissue samples were collected from the middle region of the small intestine, cut longitudinally, washed with PBS, and scraped with microscope slides to recover the mucosal layer (Mao et al., 2015). Kidney samples were collected from these pigs between the renal cortex and the renal medulla. Scraped mucosal layer and kidney samples were snap-frozen in liquid N immediately after collection and stored at −80°C.

Ribonucleic Acid Extraction and Quantitative Reverse-Transcription PCR. The RNA was extracted from 100 mg of tissue using the PureLink RNA Mini Kit (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. The RNA quantity and quality were assessed using the ND-1000 NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE) and the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA), respectively (average RNA integrity number = 9.63 ± 0.36). The RNA was subjected to reverse transcription by using the Superscript III First-Strand Synthesis SuperMix (Invitrogen) to synthesize the double-stranded cDNA. Double-stranded cDNA was diluted and used for quantitative reverse-transcription

Table 3. Analyzed composition of experimental diets, as-fed basis, Exp. 1 and 2

	0.36% STTD[1] P						0.33% STTD P	0.40% STTD P
Item	0.32% STTD Ca	0.40% STTD Ca	0.48% STTD Ca	0.56% STTD Ca	0.64% STTD Ca	0.72% STTD Ca	0.72% STTD Ca	0.72% STTD Ca
DM, %	90.07	90.08	90.13	90.12	90.10	90.08	90.51	90.23
Ash, %	4.13	4.62	4.93	4.74	5.79	5.36	5.87	5.59
CP, %	18.64	18.98	18.61	18.77	19.72	18.33	18.89	19.60
NDF, %	7.76	8.31	8.01	7.98	7.91	7.55	9.07	8.71
ADF, %	4.79	4.95	4.36	4.47	4.78	4.02	3.96	4.62
Ca, %	0.38	0.50	0.72	0.77	0.86	1.03	1.02	1.06
P, %	0.56	0.58	0.58	0.56	0.58	0.56	0.54	0.63
Phytate,[2] %	0.50	0.60	0.60	0.60	0.60	0.67	0.67	0.71
Phytate-bound P, %	0.14	0.17	0.17	0.17	0.17	0.19	0.19	0.20
Nonphytate P,[3] %	0.42	0.41	0.41	0.39	0.41	0.37	0.35	0.43

[1]STTD = standardized total tract digestible.

[2]Phytate was calculated by dividing the phytate-bound P by 0.282 (Tran and Sauvant, 2004).

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

PCR. Each 10-μL reaction consisted of 5 μL SYBR Green (Applied Biosystems, Foster City, CA), 4 μL diluted cDNA sample, 0.4 μL of 10 μ*M* forward and reverse primer, and 0.2 μL deoxyribonuclease/ribonuclease–free water. The reactions were performed in an ABI Prism 7900 HT (Applied Biosystems) using the following conditions: 2 min at 50°C, 10 min at 95°C, 40 cycles of 15 s at 95°C, and 1 min at 60°C. An additional dissociation stage was added to verify the presence of a single PCR product. All reactions were run in triplicate. Data were analyzed using the 7900 HT Sequence Detection Systems Software (version 2.2.1; Applied Biosystems).

Two internal control genes, *glyceraldehyde 3-phosphate dehydrogenase* (*GAPDH*) and *hydroxymethylbilane synthase* (*HMBS*), were used to normalize the expression of tested genes (Vigors et al., 2014). Tested genes included *S100 calcium binding protein G* (*S100G*), *transient receptor potential cation channel, subfamily V, member 6* (*TRPV6*), *ATPase, Ca^{2+} transporting, plasma membrane 1* (*ATP2B1 or PMCA1*), and *vitamin D* (*1, 25-dihydroxyvitamin $D_{3)}$ receptor* (*VDR*) in jejunal samples and *S100G, calbindin 1, 28 kDa* (*CALB1*), *TRPV6, transient receptor potential cation channel, subfamily V, member 5* (*TRPV5*), *ATP2B1*, and *VDR* in kidney samples. All of these genes are important for transcellular transport of Ca. Primers are listed in Table 4 and primers for *TRPV5* and *CALB1* were designed using the Primer3 program (Ye et al., 2012). All primers were commercially synthesized by Applied Biosystems. Primers for *TRPV5* and *CALB1* were verified by gel electrophoresis and sequencing.

Calculations and Statistical Analysis. The percentage of phytate-bound P in corn and soybean meal was calculated as 28.2% of phytate (Tran and Sauvant, 2004). The percentage of phytate in diets was calculated by dividing the analyzed phytate-bound P by 0.282, and to calculate the percentage of nonphytate P, the amount of phytate-bound P was subtracted from the amount of total P. The dietary cation–anion difference (**DCAD**) was calculated using the following equation:

$$\text{DCAD} = [(\text{Na} \times 10{,}000)/23] + [(\text{K} \times 10{,}000)/39] - [(\text{Cl} \times 10{,}000)/35.5], \quad [1]$$

in which DCAD is expressed in milliequivalents per kilogram and Na, K, and Cl are expressed in percentage in the diet. Values for Na, K, and Cl were calculated rather than analyzed (NRC, 2012).

The ADG, ADFI, and G:F were calculated for each pen and treatment group. Bone ash percentage was calculated by dividing the quantity of bone ash by the quantity of fat-free dried bone and multiplied by 100. To obtain the relative gene expression, the average of the quantity of triplicate samples were calculated and divided by the geometric mean of the 2 internal control genes. The gene expression data were expressed as an *n*-fold change relative to the average of the diet containing the least concentration of Ca (0.32% STTD Ca).

Normality of residuals and identification of outliers were determined by the UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC). Gene expression data that were expressed as n-fold change were $\log_{10}$ transformed to align measures to a normal distribution. Body weight, ADG, ADFI, G:F, bone variables, and logarithmic-scale gene expression data were analyzed using PROC MIXED of SAS. The model included diet as the fixed effect and block as the random effect. The LSMEANS procedure was used to calculate mean values for treatments. Gene expression data presented

Table 4. Gene-specific primer sets, Exp. 1

Gene[1]	Forward	Reverse	Source
TRPV5	5′- AGGGTCGGTTTCTCTCGCTA-3′	5′- GGCATAGGTGATGGTGATGACA-3′	This study
TRPV6	5′- TCCAGACAGAGGACCCTAACAAG-3′	5′- GTGAGAAACAGCTCAAAGGTGCTA-3′	Vigors et al., 2014
S100G	5′- CGCAACAGTCCCATTTAAGGA-3′	5′- TCAGCAGAGACATGGGTGGTT-3′	Vigors et al., 2014
CALB1	5′- ACGCTGACGGAAGTGGTTAC-3′	5′- ATCCAGCCTTCTTTCGTGCC-3′	This study
ATP2B1	5′- GGGCGGGCAGGTCATT-3′	5′- CCGCCGGGAGAAGATCA-3′	Vigors et al., 2014
VDR	5′- AGGCTTCTTCAGACGGAGCATGAA-3′	5′- ACTCCTTCATGCCGATGTCCA-3′	Gupta et al., 2012
Internal control gene			
GAPDH	5′- CAGCAATGCCTCCTGTACCA-3′	5′- ACGATGCCGAAGTTGTCATG-3′	Vigors et al., 2014
HMBS	5′- CTGAACAAAGGTGCCAAGAACA-3′	5′- GCCCCGCAGACCAGTTAGT-3′	Vigors et al., 2014

[1]*TRPV5 = transient receptor potential cation channel, subfamily V, member 5*; *TRPV6 = transient receptor potential cation channel, subfamily V, member 6*; *S100G = S100 calcium binding protein G*; *CALB1 = calbindin 1, 28kDa*; *ATP2B1 = ATPase, Ca++ transporting, plasma membrane 1*; *VDR = vitamin D (1, 25-dihydroxyvitamin D_3) receptor*; *GAPDH = glyceraldehyde 3-phosphate dehydrogenase*; *HMBS = hydroxymethylbilane synthase*.

in figures were back-transformed using the antilogarithm. Linear and quadratic effects of increasing levels of STTD Ca were determined using CONTRAST statements. The NLIN procedure of SAS was used for broken-line analysis if the linear effect was significant and for quadratic analyses if the quadratic effect was significant. If both linear and quadratic effects were significant, the intersection of the broken line and the quadratic line was determined (Baker et al., 2002). Pen was the experimental unit, and results were considered significant at $P \leq 0.05$ and considered a trend at $P \leq 0.15$.

Experiment 2. Ca and P Balances

Animals, Diets, and Feeding. Eighty pigs (13.12 ± 1.79 kg initial average BW) were randomly allotted to 8 diets with 10 replicate pigs per diet in a randomized complete block design. The 8 diets used in Exp. 1 were also used in Exp. 2, and the amount of each diet that was needed for both experiments was mixed in 1 batch. This experiment was conducted in 2 blocks with 40 pigs and 5 replicate pigs per diet in each block. The Experimental Animal Allotment Program (Kim and Lindemann, 2007) was used to allot pigs to experimental diets. Pigs were individually housed in metal metabolism crates that were equipped with a slatted floor, a feeder, and a nipple drinker. A screen floor and a urine pan were placed under each crate, and a bucket was placed under each urine pan, which allowed for total collection of feces and urine. Pigs had free access to water throughout the experiment. The room temperature was controlled (27.5 ± 1.6°C maximum temperature and 23.8 ± 1.1°C minimum temperature). Pigs were fed 3 times the daily maintenance energy requirement (i.e., 197 kcal of ME/kg $BW^{0.60}$; NRC, 2012). The daily allotments of feed were divided into 2 equal meals and provided at 0700 and 1700 h. Pigs were fed each diet for 13 d, and the initial 5 d were an adaptation period to the diets, and fecal samples were quantitatively collected from the feed provided from d 6 to 11 using the marker-to-marker approach (Adeola, 2001). The beginning of fecal collections was marked by adding 0.5% indigo carmine to the morning meal on d 6, and the conclusion of fecal collection was marked by adding 0.5% ferric oxide to the morning meal on d 11. Fecal samples were collected every morning and afternoon during the collection period. Urine samples were collected every morning from d 6 to 11, and 50 mL of 6 *N* HCl was added to each bucket after they were emptied. 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 feed intake was calculated by subtracting the orts from the feed provided.

Sample Analysis and Statistical Analysis. Before analysis, urine samples were thawed at room temperature and thoroughly mixed, and a subsample of 10 mL was collected. Urine samples were analyzed for Ca and P as explained for Exp. 1. Fecal samples were dried in a forced-air oven at 65°C and then ground in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ) using a 1-mm screen. Fecal samples were analyzed for DM as explained for Exp. 1 and for Ca and P by ICP–OES (method 965.17; 985.01; AOAC Int., 2007). Values for absorption and retention of Ca and P were calculated as explained by González-Vega et al. (2013). Data were analyzed using PROC MIXED of SAS as explained for Exp. 1. Pig was the experimental unit, and results were considered significant at $P \leq 0.05$ and considered a trend at $P \leq 0.15$.

RESULTS

Experiment 1: Growth Performance, Bone Ash, and Gene Expression

All pigs remained healthy and consumed their diets without apparent problems, but 1 gilt died due to meningitis and data for ADG, ADFI, and G:F of the remaining

Table 5. Body weight and ADFI of pigs fed diets containing 0.32, 0.40, 0.48, 0.56, 0.64, or 0.72% standardized total tract digestible (STTD) Ca and 0.36% STTD P, Exp. 1

	Diets							*P*-value	
STTD Ca, %	0.32	0.40	0.48	0.56	0.64	0.72	SEM	Linear	Quadratic
BW, kg									
d 1	11.39	11.35	11.44	11.37	11.45	11.33	0.42	0.982	0.909
d 10	16.44	16.90	16.81	16.47	16.28	15.65	0.65	0.195	0.256
d 22	25.21	25.29	25.47	25.76	24.23	23.74	0.83	0.117	0.172
ADFI, g/d									
d 1–10	790	830	778	778	776	762	56	0.233	0.738
d 10–22	1,097	1,070	1,117	1,189	1,048	1,075	53	0.785	0.223
d 1–22	957	961	963	1,003	927	934	52	0.506	0.316

pigs in the pen for this pig were adjusted (Lindemann and Kim, 2007). There were no effects of increasing concentration of STTD Ca on final BW or ADFI (Table 5). However, overall ADG decreased (quadratic, $P < 0.05$) as the concentration of STTD Ca increased (Fig. 1). The concentration of STTD Ca at which ADG started to decline was 0.54% as determined by the intersection between the quadratic and the broken-line analyses. Likewise, overall G:F decreased (quadratic, $P < 0.01$) as the concentration of dietary STTD Ca increased (Fig. 2). The G:F intersection between the quadratic and the broken-line analyses was at 0.50% STTD Ca. In contrast, total bone ash and total bone Ca in grams in femurs increased (linear, $P < 0.05$, and quadratic, $P < 0.05$) as the concentration of STTD Ca increased (Fig. 3 and 4, respectively). Likewise, total bone P in grams in femurs and total bone ash in grams in metacarpals increased (quadratic, $P < 0.05$) as the concentration of STTD Ca increased (Fig. 5 and 6, respectively). The concentrations of dietary STTD Ca to maximize total bone ash, total bone Ca, and total bone P in femurs and bone ash in metacarpals were 0.48, 0.50, 0.56, and 0.54%, respectively.

There was no effect of increasing concentration of STTD P on BW, ADG from d 1 to 10 or from d 1 to 22, ADFI, G:F from d 1 to 10, or bone P percentage in femurs (Table 6). However, ADG from d 10 to 22, G:F from d 10 to 22 and from d 1 to 22, bone ash, bone Ca, and bone P in grams in femurs and bone ash in grams in metacarpals linearly increased ($P < 0.05$) as the concentration of STTD P increased. Bone Ca percentage in femurs tended to increase ($P = 0.144$) as concentration of STTD P increased. Bone ash in percent of dried bone weight in metacarpals increased (linear, $P < 0.01$, and quadratic, $P < 0.01$) as the concentration of STTD P increased.

Gene Expression. The effect of increasing the concentration of STTD Ca was evaluated for the internal control genes in the jejunum and kidneys, and as expected, the mRNA expression of the internal control genes was not affected by the concentration of STTD Ca in diets. In the jejunum, the mRNA expression of *TRPV6* linearly decreased ($P = 0.01$) as the concentration of STTD Ca increased (Fig. 7). There was a tendency ($P < 0.15$) for a linear and quadratic decrease in the mRNA expression of *S100G* as the concentration of STTD Ca increased. However, the mRNA expression of *ATP2B1* increased (quadratic, $P < 0.001$) as dietary STTD Ca increased, reaching the maximum expression for the diet containing 0.64% STTD Ca and 0.36% STTD P (STTD Ca:STTD P ratio: 1.78). Likewise, the mRNA expression of VDR increased (quadratic, $P < 0.01$) as the concentration of STTD Ca increased, reaching the maximum expression for the diet containing 0.64% STTD Ca and 0.36% STTD P (STTD Ca:STTD P ratio: 1.78). However in the kidneys, the mRNA expression of TRPV6 (linear, $P < 0.001$), *TRPV5* (linear, $P < 0.001$), *S100G* (linear, $P < 0.001$, and quadratic $P = 0.056$), and *CALB1* (linear and quadratic, $P < 0.01$) decreased as the concentration of STTD Ca increased (Fig. 8). Increasing concentration of STTD Ca increased (quadratic, $P < 0.01$) the mRNA expression of VDR in the kidneys, reaching the maximum expression for the diet containing 0.56% STTD Ca and 0.36% STTD P (STTD Ca:STTD P ratio: 1.56). However, increasing concentration of STTD Ca had no effect on mRNA expression of ATP2B1 in the kidneys.

Experiment 2. Ca and P Balances

Balance of Ca. Pigs consumed the diets and remained healthy throughout the experiment. There was no effect of increasing concentration of STTD Ca on feed intake (Table 7). However, Ca intake, Ca absorption, and Ca excretion as percentage of intake increased (linear, $P < 0.001$) as dietary STTD Ca increased. Likewise, fecal and urine Ca output and Ca excreted in grams per day increased (linear, $P < 0.001$ and quadratic, $P < 0.05$) as the concentration of dietary STTD Ca increased. However, Ca retained as percentage of intake decreased (linear, $P < 0.001$) as dietary STTD Ca increased. The quantity of Ca retained in grams per day increased (linear, $P < 0.001$, and quadratic, $P < 0.05$) as dietary concentration of STTD Ca increased. The concentration of STTD Ca needed to maximize Ca retention in grams per day

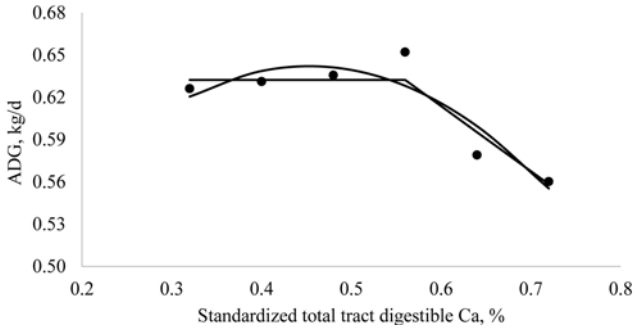

Figure 1. Fitted broken-line of ADG (kg/d; d 1 to 22) as a function of standardized total tract digestible (STTD) Ca (Exp. 1). The mean of each diet (●) represents the mean of 8 replicate pens per diet. The break point was 0.56 ± 0.10% STTD Ca if the broken-line analysis was used (plateau = 0.63 kg/d ADG). The maximum ADG determined from the quadratic analysis was observed at 0.45 ± 0.04% STTD Ca. The intersections between the broken-line analysis and the quadratic analysis were at 0.36 and 0.54% STTD Ca.

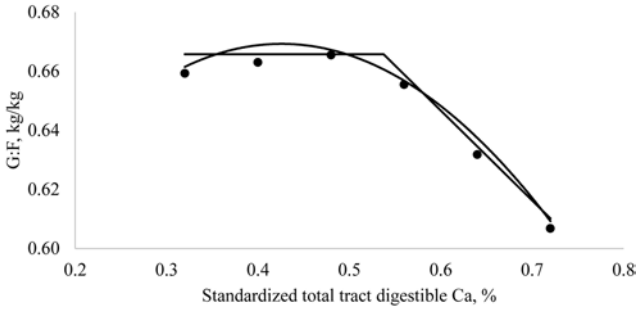

Figure 2. Fitted broken line of G:F (kg/kg; d 1 to 22) as a function of standardized total tract digestible (STTD) Ca (Exp. 1). The mean of each diet (●) represents the mean of 8 replicate pens per diet. The break point for G:F was at 0.54 ± 0.04% STTD Ca (plateau = 0.67 kg/kg G:F). The maximum G:F determined from the quadratic analysis was observed at 0.43 ± 0.04% STTD Ca. The intersections between the broken-line analysis and the quadratic analysis were observed at 0.35 and 0.50% STTD Ca.

was 0.60% (Fig. 9). Increasing dietary STTD P did not affect feed intake, Ca intake, or absorbed Ca. However, increasing concentration of STTD P decreased fecal Ca output (linear and quadratic, $P < 0.05$), Ca excreted in grams per day (linear and quadratic, $P < 0.05$), urinary Ca output (linear, $P < 0.001$), and Ca excretion as percent of intake (linear, $P < 0.001$). In contrast, Ca retention as percent of intake increased (linear, $P < 0.001$) as the concentration of STTD P increased.

Balance of P. Intake, absorption, and excretion of P in grams per day were not affected by increasing the concentration of STTD Ca in the diet (Table 8). However, urine P output and P excretion as percent of intake decreased (linear, $P < 0.05$, and quadratic, $P < 0.01$) as the concentration of STTD Ca increased. There was a tendency ($P < 0.15$) for an increase in the quantity of P retained in grams per day as the concentration of STTD Ca increased. The concentration of STTD Ca needed to maximize P retention in grams per day was 0.49% (Fig. 10). Fecal P output increased (linear, $P < 0.01$) and P retention (% of intake) increased (linear, $P < 0.05$, and quadratic, $P < 0.01$) as the concentration of STTD Ca increased. There were no effects of increasing concentration of STTD P in the diet on fecal or urinary output of P or P excretion in grams per day. However, the quantity of absorbed and retained P in grams per day increased (linear, $P < 0.05$) as dietary STTD P increased. There was a tendency ($P < 0.15$) for an increase in P intake and P retention in percent of intake as concentration of STTD P increased; however, P excretion in percent of intake tended to decrease ($P = 0.07$) as concentration of STTD P increased.

DISCUSSION

The concentration of Ca and P in corn and monocalcium phosphate were in agreement with reported values (de Blas et al., 2010; Rostagno et al., 2011; NRC, 2012), but the concentration of Ca and P in soybean meal and calcium carbonate were greater than reported values (Sauvant et al., 2004; de Blas et al., 2010; Rostagno et al., 2011; NRC, 2012). However, all ingredients were analyzed before diet mixing so these differences did not influence concentrations of Ca and P in the diets, and the analyzed Ca and P in all diets were close to calculated values. All diets had DCAD values (182.4 mEq/kg) that were below values that may reduce growth performance of pigs (from 269 to 388 mEq/kg; Guzmán-Pino et al., 2015).

The ratio of Ca and P in the diets is important to consider because an excess or deficiency of one mineral may affect the utilization of the other (Crenshaw, 2001). In the present experiment, 0.36% STTD P was used, which is 10% above the requirement (NRC, 2012) to make sure pigs were not deficient in P. Results indicated that there were no differences in growth performance between pigs fed the 0.36% STTD P and those fed the 0.33% STTD P. The reduced quantity of bone ash in pigs fed the diet containing 0.33% STTD P was expected because P requirements are established to maximize growth performance but not bone ash (NRC, 2012). The observation that pigs fed 0.40% STTD P had greater G:F and Ca retention than pigs fed 0.36% STTD P indicates that pigs fed 0.36% STTD P may have been marginally deficient in STTD P for these variables despite receiving 10% more P than the requirement.

Results illustrate that the quantity of STTD Ca that is needed to maximize retention of Ca and maximize bone ash is different from the quantity needed to maximize growth performance, which is in agreement with previous reports (Maxon and Mahan, 1983; Combs et al., 1991a,b). Increasing concentrations of Ca may increase formation of Ca–P complexes in the gastrointestinal tract, which reduces digestibility of P (Clark, 1969; Brink et al., 1992; Stein et al., 2011; González-Vega et al., 2014). This negative effect on digestibility

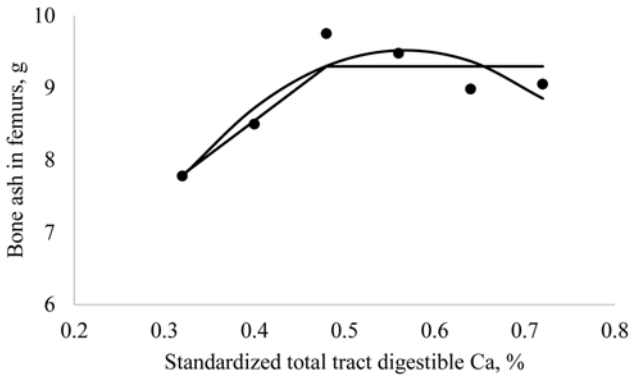

Figure 3. Fitted broken line of bone ash in femurs (g) as a function of standardized total tract digestible (STTD) Ca (Exp. 1). The mean of each diet (●) represents the mean of 8 replicate pens per diet. The break point for grams bone ash was at 0.48 ± 0.11% STTD Ca if the broken-line analysis was used (plateau = 9.30 g bone ash). The maximum concentration of bone ash determined from the quadratic analysis was observed at 0.57 ± 0.03% STTD Ca. The intersections between the broken-line analysis and the quadratic analysis were observed at 0.48 and 0.66% STTD Ca.

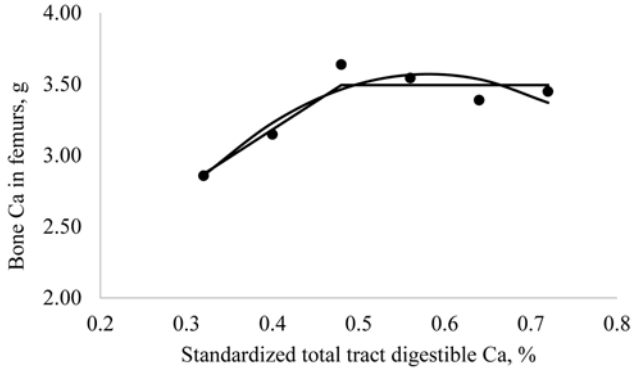

Figure 4. Fitted broken line of bone Ca in femurs (g) as a function of standardized total tract digestible (STTD) Ca (Exp. 1). The mean of each diet (●) represents the mean of 8 replicate pens per diet. The break point for bone Ca was observed at 0.48 ± 0.11% STTD Ca if the broken-line analysis was used (plateau = 3.49 g bone Ca). The maximum quantity of bone Ca determined from the quadratic analysis was at 0.58 ± 0.04% STTD Ca. The intersections between the broken-line analysis and the quadratic analysis were observed at 0.50 and 0.67% STTD Ca.

of P may be one of the reasons for the negative effect of increasing concentrations of dietary STTD Ca on ADG and G:F. In 25-kg pigs that were fed increasing concentrations of true total tract digestible Ca and a constant concentration of true total tract digestible P, G:F was also reduced as dietary Ca increased (Fan and Archbold, 2012). The observation that ADG and G:F were not negatively affected by the lowest concentration of STTD Ca indicates that pigs that were fed diets with the lowest concentrations of Ca may have been able to mobilize Ca from the bones to compensate for the deficiency of dietary Ca. It is possible that 22 d was too short a period to deplete mobile bone Ca stores and therefore observe negative effects on growth performance. Nevertheless, the fact that a negative response to ADG and G:F was not observed at the lowest level of dietary Ca prevented us from determining the minimum concentration of STTD Ca that is needed to maximize growth performance. However, the strong negative responses on ADG and G:F of including STTD Ca in diets above the identified break points are of great practical importance in feed formulation and indicate that STTD Ca in diets fed to 11- to 25-kg pigs should not exceed approximately 0.50%.

The interactions between Ca and P are illustrated by the observation that as dietary STTD Ca increased, fecal excretion of P increased but P retention also increased. The reason for this response is that as dietary STTD Ca increased, increased amounts of Ca was available for bone synthesis and, as a result, increased amounts of absorbed P could be retained and less P was excreted in the urine. The reduction in urinary P excretion was greater than the increase in fecal P excretion as dietary STTD Ca increased and as a result, P retention increased.

The STTD Ca:STTD P ratios in the diets ranged from 0.88:1 to 2.00:1. The values at which dietary STTD Ca negatively affected G:F and ADG (0.50 or 0.54%, respectively) correspond to STTD Ca:STTD P ratios between 1.39:1 and 1.50:1. To maximize bone ash, bone Ca,

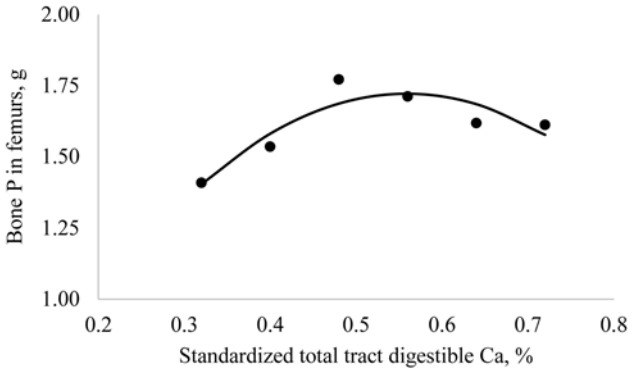

Figure 5. Fitted quadratic line of bone P in femurs (g) as a function of standardized total tract digestible (STTD) Ca (Exp. 1). The mean of each diet (●) represents the mean of 8 replicate pens per diet. The maximum quantity of bone P was observed at 0.56 ± 0.03% STTD Ca.

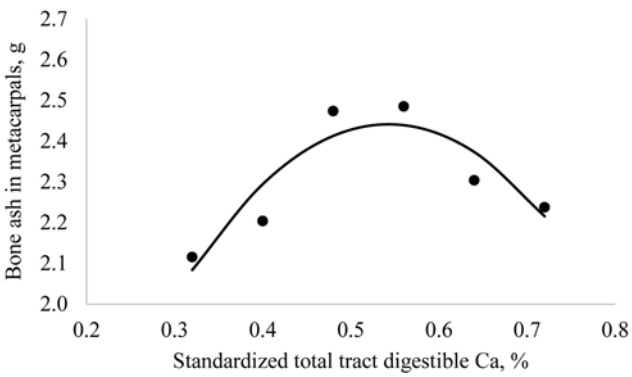

Figure 6. Fitted quadratic line of bone ash in metacarpals (g) as a function of standardized total tract digestible (STTD) Ca (Exp. 1). The mean of each diet (●) represents the mean of 8 replicate pens per diet. The maximum concentration of bone ash in metacarpals was observed at 0.54 ± 0.02% STTD Ca.

Table 6. Growth performance and bone mineralization of pigs fed diets containing 0.72% standardized total tract digestible (STTD) Ca and 0.33, 0.36, or 0.40% STTD P, Exp. 1

	Diets				*P*-value	
STTD P, %	0.33	0.36	0.40	SEM	Linear	Quadratic
BW, kg						
d 1	11.42	11.33	11.42	0.43	0.987	0.868
d 10	16.07	15.65	15.96	0.85	0.937	0.659
d 22	24.32	23.74	25.05	1.00	0.517	0.412
ADG, g/d						
d 1–10	466	435	453	54	0.833	0.535
d 10–22	689	674	759	22	0.027	0.113
d 1–22	587	565	622	31	0.230	0.183
ADFI, g/d						
d 1–10	779	762	748	80	0.618	0.939
d 10–22	1,127	1,075	1,129	57	0.909	0.363
d 1–22	970	934	957	64	0.867	0.536
G:F						
d 1–10	0.60	0.56	0.60	0.02	0.928	0.281
d 10–22	0.61	0.63	0.68	0.03	0.008	0.751
d 1–22	0.61	0.61	0.65	0.01	0.003	0.127
Femurs						
Bone ash, %	55.46	57.21	57.59	0.60	<0.001	0.076
Bone ash, g	8.11	9.05	10.23	0.42	0.002	0.941
Bone Ca, %	37.70	38.07	37.29	0.37	0.307	0.144
Bone Ca, g	3.05	3.45	3.82	0.17	0.006	0.747
Bone P, %	17.93	17.84	17.93	0.28	1.000	0.535
Bone P, g	1.45	1.62	1.84	0.09	0.003	0.989
Metacarpals						
Bone ash, %	51.29	53.43	53.08	0.36	0.004	0.006
Bone ash, g	2.19	2.25	2.55	0.11	0.022	0.492

and bone P, dietary STTD Ca above 0.48% were needed, and to maximize retention of Ca and P, a minimum of 0.60 and 0.49% STTD Ca, respectively, were needed, which correspond to STTD Ca:STTD P ratios between 1.33:1 and 1.67:1. These values are within the range of values for the Ca:P ratio in the whole body (Rymarz et al., 1982; Hendriks and Moughan, 1993; Mahan and Shields, 1998; Wiseman et al., 2009; Pettey et al., 2015).

In studies aimed at determining Ca or P requirements in pigs, the dietary concentration of one mineral may be constant while responses to graded levels of the other mineral is observed (Fan and Archbold, 2012). Alternatively, a constant Ca:P ratio may be used (Zhai and Adeola, 2013). In this experiment, a constant concentration of dietary STTD P was used in diets with varying dietary Ca. In studies that used a constant concentration of dietary Ca, increasing concentrations of STTD P increased growth performance (Ekpe et al., 2002; Saraiva et al., 2009; Viana et al., 2013; Zhai and Adeola, 2013). However, in this experiment, increasing concentrations of STTD Ca decreased growth performance, which is in agreement with results observed by

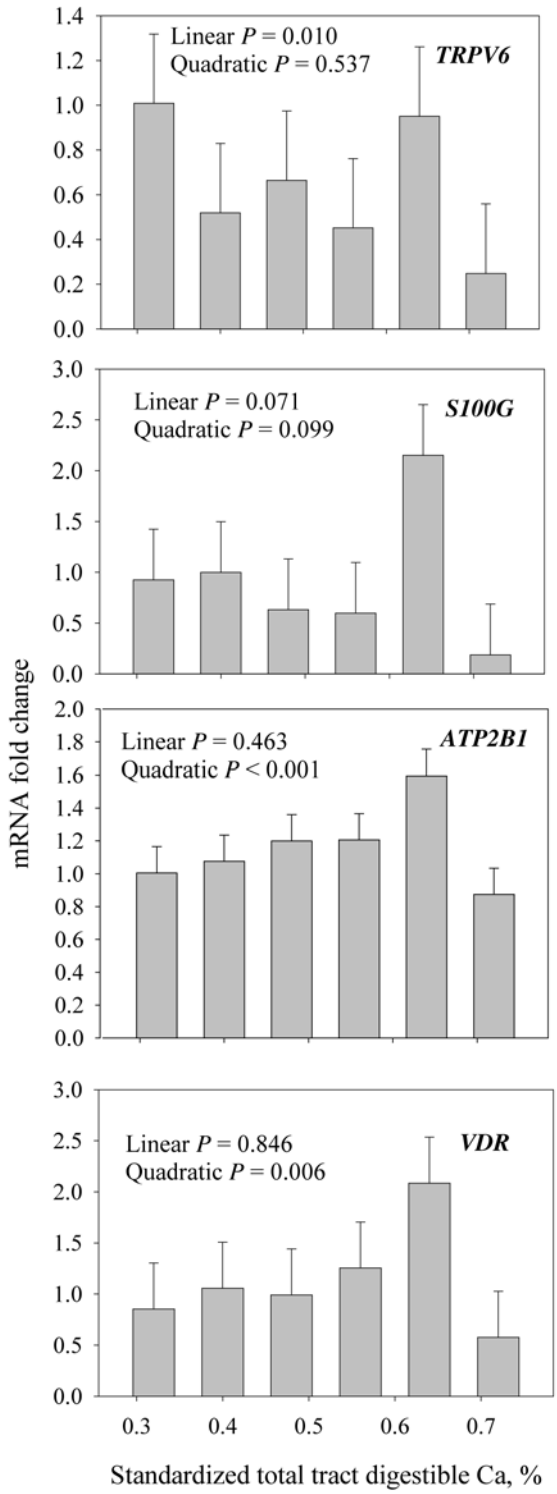

Figure 7. Expression of genes related to transcellular transportation of Ca in the jejunum of pigs fed diets containing 0.32, 0.40, 0.48, 0.56, 0.64, or 0.72% standardized total tract digestible (STTD) Ca and 0.36% STTD P. The *P*-values for linear and quadratic effect of increasing concentration of STTD Ca and means for each diet and SE (vertical bars) are indicated. *TRPV6 = transient receptor potential cation channel, subfamily V, member 6; S100G = S100 calcium binding protein G; ATP2B1 = ATPase, Ca^{2+} transporting, plasma membrane 1; VDR = vitamin D (1, 25-dihydroxyvitamin D_3) receptor.*

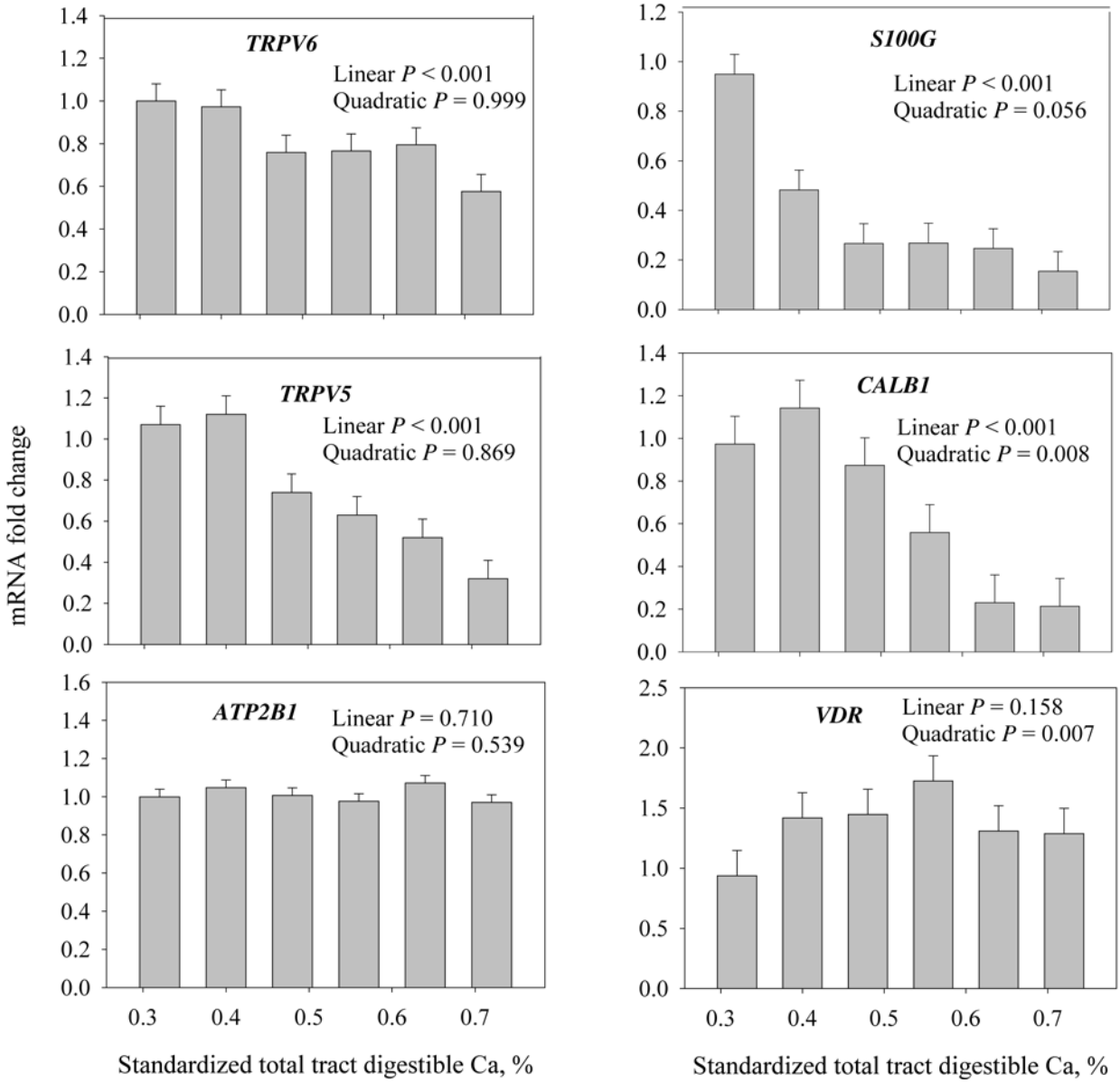

Figure 8. Expression of genes related to transcellular transportation of Ca in the kidneys of pigs fed diets containing 0.32, 0.40, 0.48, 0.56, 0.64, or 0.72% standardized total tract digestible (STTD) Ca and 0.36% STTD P. The *P*-values for linear and quadratic effect of increasing concentration of STTD Ca and means for each diet and SE (vertical bars) are indicated. *TRPV6 = transient receptor potential cation channel, subfamily V, member 6; TRPV5 = transient receptor potential cation channel, subfamily V, member 5; ATP2B1 = ATPase, Ca^{2+} transporting, plasma membrane 1; S100G = S100 calcium binding protein G; CALB1 = calbindin 1, 28 kDa; VDR = vitamin D (1, 25-dihydroxyvitamin D_3) receptor.*

Fan and Archbold (2012). This observation indicates that to maximize growth performance in pigs, the ratio of STTD Ca to STTD P likely is more important than the absolute concentration of both minerals.

Calcium may be absorbed by either paracellular or transcellular routes. If luminal Ca concentration is high, most Ca is absorbed by passive diffusion using the paracellular route (Hurwitz, 1996; Fleet and Schoch, 2010). In contrast, if luminal Ca concentration is low, most Ca is absorbed by active transport using the transcellular route, which requires Ca channels, Ca transporters, and energy (Hurwitz, 1996; Bouillon et al., 2003; Fleet and Schoch, 2010). Calcium crosses the brush border membrane using Ca channels (*TRPV6* and/or *TRPV5*), and in the cytoplasm, Ca is bound to Ca-binding proteins (calbindin-D9k and/or calbindin-D28k), which transport Ca to the basolateral membrane (Schröder et al., 1996; Bouillon et al., 2003; Schwaller, 2010), where it is released from the cell via *PMCA1* or by a Na^+/Ca^{2+} exchanger (Bouillon et al., 2003; Proszkowiec-Weglarz and Angel, 2013). Therefore, dietary Ca concentration may affect the expression of genes related to Ca transport. As indicated by the results from the current experiment, the mRNA expression of Ca-binding proteins and Ca-channel proteins were regulated by dietary Ca concentrations in both the jejunum and the kidneys. These

Table 7. Calcium balance for pigs fed diets containing between 0.32 and 0.72% standardized total tract digestible (STTD) Ca and 0.36% STTD, P, and diets containing 0.72% STTD Ca and 0.33 or 0.40% STTD P, Exp. 2[1]

	Diets								Diets with 0.36% STTD P			Diets with 0.72% STTD Ca		
	STTD Ca, %									*P*-value			*P*-value	
	0.32	0.40	0.48	0.56	0.64	0.72	0.72	0.72						
	STTD P, %													
Item	0.36	0.36	0.36	0.36	0.36	0.36	0.33	0.40	SEM	Linear	Quadratic	SEM	Linear	Quadratic
Feed intake, g/d	771	759	781	746	782	761	748	717	29	0.964	0.953	30	0.412	0.465
Ca intake, g/d	3.10	4.14	5.33	6.07	7.45	8.30	8.19	7.70	0.26	<0.001	0.985	0.26	0.158	0.326
Fecal Ca output, g/d	0.82	1.19	1.54	1.93	2.34	3.03	2.89	2.56	0.10	<0.001	0.039	0.11	0.021	0.035
Urine Ca output, mg/d	122	204	383	731	1,124	1,534	1,677	1,096	65	<0.001	<0.001	73	<0.001	0.243
Ca excretion, g/d	0.94	1.39	1.92	2.66	3.46	4.55	4.69	3.47	0.14	<0.001	<0.001	0.14	<0.001	0.031
Ca excretion, % of intake	30.26	33.66	35.97	43.83	46.89	52.17	55.37	45.49	1.62	<0.001	0.385	1.65	<0.001	0.611
Absorbed Ca, g/d	2.28	2.96	3.79	4.14	4.95	5.25	5.09	5.03	0.19	<0.001	0.283	0.20	0.774	0.450
Ca retention, % of intake	69.74	66.34	64.03	56.17	53.11	47.83	44.63	54.51	1.62	<0.001	0.385	1.65	<0.001	0.611

[1]Each least squares mean represents 10 observations.

results indicate reduced transport of Ca via the transcellular route as dietary Ca concentration increases, and these observations are in close agreement with data obtained in mice, rats, and broiler chickens (Armbrecht et al., 1980, 2003; Rosenberg et al., 1986; Hurwitz et al., 1995; van de Graaf et al., 2004; Ko et al., 2009). However, the observation that the absorption of Ca did not reach a plateau and continued to increase as dietary Ca increased indicates that as the transcellular uptake of Ca is reduced at greater dietary Ca concentrations, more Ca is absorbed via the paracellular route.

Calcium homeostasis is mainly regulated by 2 hormones, parathyroid hormone and calcitonin (Crenshaw, 2001). Secretion of parathyroid hormone increases at low dietary Ca concentrations due to low plasma Ca concentrations (Eklou-Kalonji et al., 1999). Parathyroid hormone increases the production of 1-hydroxylase in the kidneys, which forms 1,25-dihydroxycholecalciferol (**1,25-$(OH)_2D_3$**), the active form of vitamin D (Hurwitz, 1996; Crenshaw et al., 2011). If 1,25-$(OH)_2D_3$ is bound to *VDR*, upregulation of several genes involved in transcellular Ca transport may occur (Healy et al., 2005), which may increase Ca absorption in the duodenum and jejunum and Ca reabsorption in the kidneys (Bouillon et al., 2003). In broiler chickens, rats, and mice, the expression of *TRPV6, TRPV5, S100G*, and/or *PMCA1* was increased in the small intestine and/or in the kidneys at low dietary Ca concentrations (Armbrecht et al., 1980, 2003; Rosenberg et al., 1986; Hurwitz et al., 1995; van de Graaf et al., 2004; Ko et al., 2009). Results from this experiment support these observations because the mRNA expression of *TRPV6* and *S100G* in the jejunum and the mRNA expression of *TRPV5, TRPV6, S100G*, and *CALB1* in the kidneys were downregulated as dietary Ca increased. In mice, high concentrations of dietary Ca increased mRNA expression of *VDR* in the kidneys but not in the duodenum (Healy et al., 2005). However, the results of the present experiment indicate that in pigs, mRNA expression of *VDR* is increased only as concentrations of Ca are below or at the requirement, whereas provision of Ca above the requirement results in a reduction of the mRNA expression of *VDR*. Therefore, results of this experiment

Table 8. Phosphorus balance for pigs fed diets containing between 0.32 STTD P, and in diets containing 0.72% STTD Ca and 0.33 or 0.40% STTD P, Exp. 2[1]

	Diets								Diets with 0.36% STTD P			Diets with 0.72% STTD Ca		
	STTD Ca, %									*P*-value			*P*-value	
	0.32	0.40	0.48	0.56	0.64	0.72	0.72	0.72						
	STTD P, %													
Item	0.36	0.36	0.36	0.36	0.36	0.36	0.33	0.40	SEM	Linear	Quadratic	SEM	Linear	Quadratic
P intake, g/d	4.34	4.31	4.46	4.29	4.53	4.35	4.11	4.56	0.17	0.699	0.842	0.17	0.066	0.809
Fecal P output, g/d	1.52	1.60	1.69	1.71	1.76	1.78	1.72	1.75	0.07	0.006	0.489	0.07	0.801	0.668
Urine P output, mg/d	548	287	131	82	84	88	93	84	15	<0.001	<0.001	12	0.567	0.951
P excretion, g/d	1.95	1.89	1.82	1.80	1.86	1.87	1.82	1.84	0.08	0.415	0.217	0.08	0.867	0.687
P excretion, % of intake	46.91	43.63	40.88	41.84	41.45	42.82	44.24	40.79	1.33	0.021	0.009	1.33	0.070	0.972
Absorbed P, g/d	2.82	2.70	2.77	2.58	2.77	2.58	2.39	2.80	0.13	0.246	0.892	0.13	0.023	0.951
P retention, g/d	2.30	2.42	2.64	2.50	2.67	2.49	2.29	2.72	0.12	0.144	0.148	0.12	0.017	0.941
P retention, % of intake	53.09	56.37	59.12	58.16	58.55	57.18	55.77	59.21	1.33	0.021	0.009	1.33	0.070	0.972

[1]Each least squares mean represents 10 observations.

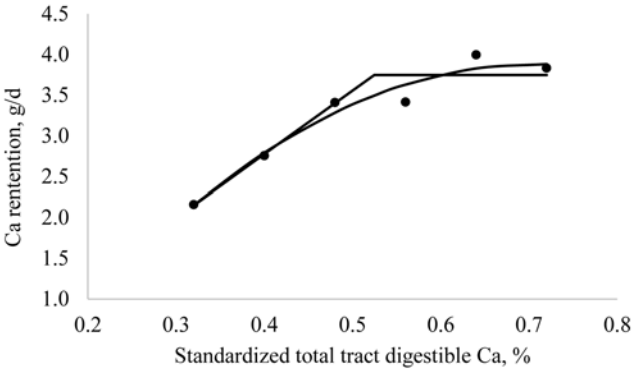

Figure 9. Fitted broken line of Ca retention (g/d) as a function of standardized total tract digestible (STTD) Ca (Exp. 2). The mean of each diet (●) represents the mean of 10 replicated pigs per diet. The break point for Ca retention was at 0.52 ± 0.03% STTD Ca (plateau = 3.75 g/d of Ca retention). The maximum Ca retention determined from the quadratic analysis was at 0.71 ± 0.08% STTD Ca. The intersection between the broken-line analysis and the quadratic analysis was at 0.60% STTD Ca.

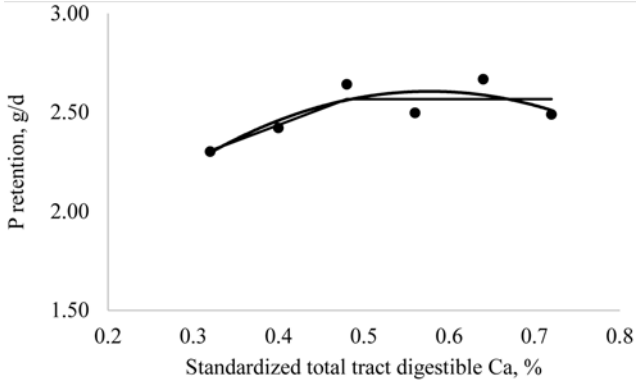

Figure 10. Fitted broken line of P retention (g/d) as a function of standardized total tract digestible (STTD) Ca (Exp. 2). The mean of each diet (●) represents the mean of 10 replicated pigs per diet. The break point for P retention was at 0.48 ± 0.16% STTD Ca (plateau = 2.57 g/d of P retention). The maximum P retention determined from the quadratic analysis was at 0.58 ± 0.05% STTD Ca. The intersection between the broken-line analysis and the quadratic analysis was at 0.49% STTD Ca.

indicate that although, at high dietary concentrations of Ca, mRNA expression of most Ca transporters was downregulated in the jejunum, most Ca may have been absorbed using the paracellular route. As a consequence, Ca that was not needed for bone tissue synthesis was subsequently excreted in the urine, indicating that Ca balance was regulated mainly at the renal level. In contrast, at low concentrations of dietary Ca, most Ca is likely absorbed using the transcellular route. As a consequence, vitamin D–dependent transport may play a role in increasing Ca absorption from the small intestine and in Ca reabsorption in the kidneys at low concentrations of dietary Ca. In dogs, it has been demonstrated that Ca homeostasis is influenced by plasma concentrations of GH and IGF-1 via regulation of vitamin D synthesis (Tryfonidou et al., 2003). However, we are not aware of any reports on effects of GH and IGF-I on Ca balance in pigs.

Conclusions

Growth performance of pigs was reduced if dietary Ca exceeded approximately 0.50% STTD Ca in diets containing 0.36% STTD P. However, the minimum concentration of dietary STTD Ca needed to maximize growth performance of pigs could not be determined under the conditions of this experiment, which is possibly because diets were not fed long enough to deplete the mobile bone stores of Ca. Bone ash, bone Ca, and bone P were maximized by dietary Ca at or above 0.48% STTD Ca, and retention of Ca and P was maximized if dietary Ca was at or above 0.60 and 0.49% STTD Ca, respectively. Based on these results, it is likely that the requirement for STTD Ca for 11- to 25-kg pigs is approximately 1.35 times the required STTD P, but further experiments need to be conducted to verify this value. Increasing concentrations of dietary Ca decreased expression of genes related to transcellular transport of Ca in the jejunum and kidneys of pigs, but the effect of this decrease on absorption of Ca was limited, which is likely due to increased paracellular absorption of Ca as dietary Ca increases. These observations indicate that the main site for regulation of Ca balance appears to be in the kidneys.

LITERATURE CITED

Adeola, O. 2001. Digestion and balance techniques in pigs. In: A. J. Lewis and L. L. Southern, editors, Swine nutrition. 2nd ed. CRC Press, Washington, DC. p. 903–916.

AOAC International. 2007. Official Methods of Analysis of AOAC Int. 18th ed. Rev. 2. W. Hortwitz and G. W. Latimer, Jr., editors. AOAC Int., Gaithersburg, MD.

Armbrecht, H. J., M. A. Boltz, and M. E. H. Bruns. 2003. Effect of age and dietary calcium on intestinal calbindin D-9k expression in the rat. Arch. Biochem. Biophys. 420:194–200. doi:10.1016/j.abb.2003.09.025

Armbrecht, H. J., T. V. Zenser, C. J. Gross, and B. B. Davis. 1980. Adaptation to dietary calcium and phosphorus restriction changes with age in the rat. Am. J. Physiol. 239:E322–E327.

Baker, D. H., A. B. Batal, T. M. Parr, N. R. Augspurger, and C. M. Parsons. 2002. Ideal ratio (relative to lysine) of tryptophan, threonine, isoleucine, and valine for chicks during the second and third weeks posthatch. Poult. Sci. 81:485–494. doi:10.1093/ps/81.4.485

Bouillon, R., S. Van Cromphaut, and G. Carmeliet. 2003. Intestinal calcium absorption: Molecular vitamin D mediated mechanisms. J. Cell. Biochem. 88:332–339. doi:10.1002/jcb.10360

Brink, E. J., A. C. Beynen, P. R. Dekker, E. C. H. van Beresteijn, and R. van der Meer. 1992. Interaction of calcium and phosphate decreases ileal magnesium solubility and apparent magnesium absorption in rats. J. Nutr. 122:580–586.

Clark, I. 1969. Importance of dietary Ca:PO_4 ratios on skeletal, Ca, Mg, and PO4 metabolism. Am. J. Physiol. 217:865–870.

Combs, N. R., E. T. Kornegay, M. D. Lindemann, and D. R. Notter. 1991a. Calcium and phosphorus requirements of swine from weaning to market: I. Development of response curves for performance. J. Anim. Sci. 69:673–681.

Combs, N. R., E. T. Kornegay, M. D. Lindemann, D. R. Notter, J. W. Wilson, and J. P. Mason. 1991b. Calcium and phosphorus requirements of swine from weaning to market: II. Development of response curves for bone criteria and comparison of bending and shear bone testing. J. Anim. Sci. 69:682–693.

Crenshaw, T. D. 2001. Calcium, phosphorus, vitamin D, and vitamin K in swine nutrition. In: A. J. Lewis and L. L. Southern, editors, Swine nutrition. 2nd ed. CRC Press, Boca Raton, FL. p. 187–212.

Crenshaw, T. D., L. A. Rortvedt, and Z. Hassen. 2011. Triennial growth symposium: A novel pathway for vitamin D-mediated phosphate homeostasis: Implications for skeleton growth and mineralization. J. Anim. Sci. 89:1957–1964. doi:10.2527/jas.2010-3411

de Blas, C., G. G. Mateos, and P. García-Rebollar, editors. 2010. Tablas FEDNA de composición y valor nutritivo de alimentos para la fabricación de piensos compuestos. 3rd ed. (In Spanish.) Fund. Esp. Desarrollo Nutr. Anim., Madrid, Spain.

Eklou-Kalonji, E., E. Zerath, C. Colin, C. Lacroix, X. Holy, I. Denis, and A. Pointillart. 1999. Calcium-regulating hormones, bone mineral content, breaking load and trabecular remodeling are altered in growing pigs fed calcium-deficient diets. J. Nutr. 129:188-193.

Ekpe, E. D., R. T. Zijlstra, and J. F. Patience. 2002. Digestible phosphorus requirement of grower pigs. Can. J. Anim. Sci. 82:541–549. doi:10.4141/A02-006

Fan, M. Z., and T. Archbold. 2012. Effects of dietary true digestible calcium to phosphorus ratio on growth performance and efficiency of calcium and phosphorus use in growing pigs fed corn and soybean meal-based diets. J. Anim. Sci. 90(Suppl. 4):254–256. doi:10.2527/jas.54005

Fleet, J. C., and R. D. Schoch. 2010. Molecular mechanism for regulation of intestinal calcium absorption by vitamin D and other factors. Crit. Rev. Clin. Lab. Sci. 47:181–195. doi:10.3109/10408363.2010.536429

González-Vega, J. C., C. L. Walk, Y. Liu, and H. H. Stein. 2013. Determination of endogenous intestinal losses of Ca and true total tract digestibility of calcium in canola meal fed to growing pigs. J. Anim. Sci. 91:4807–4816. doi:10.2527/jas.2013-6410

González-Vega, J. C., C. L. Walk, Y. Liu, and H. H. Stein. 2014. The site of net absorption of Ca from the intestinal tract of growing pigs and effect of phytic acid, Ca level and Ca source on Ca digestibility. Arch. Anim. Nutr. 68:126–142. doi:10.1080/1745039X.2014.892249

González-Vega, J. C., C. L. Walk, and H. H. Stein. 2015a. Effects of microbial phytase on apparent and standardized total tract digestibility of calcium in calcium supplements fed to growing pigs. J. Anim. Sci. 93:2255–2264. doi:10.2527/jas.2014-8215

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

Gupta, G. K., T. Agrawal, M. G. Del Core, W. J. Hunter III, and D. K. Agrawal. 2012. Decreased expression of vitamin D receptors in neointimal lesion following coronary artery angioplasty in atherosclerotic swine. PLoS One 7:e42789. doi:10.1371/journal.pone.0042789

Guzmán-Pino, S. A., D. Solà-Oriol, R. Davin, E. G. Manzanilla, and J. F. Pérez. 2015. Influence of dietary electrolyte balance on feed preference and growth performance of postweaned piglets. J. Anim. Sci. 93:2840–2848. doi:10.2527/jas.2014-8380

Healy, K. D., M. A. Frahm, and H. F. DeLuca. 2005. 1,25-Dihydroxyvitamin D3 up-regulates the renal vitamin D receptor through indirect gene activation and receptor stabilization. Arch. Biochem. Biophys. 433:466–473. doi:10.1016/j.abb.2004.10.001

Hendriks, W. H., and P. J. Moughan. 1993. Whole-body mineral composition of entire male and female pigs depositing protein at maximal rates. Livest. Prod. Sci. 33:161–170. doi:10.1016/0301-6226(93)90247-F

Holst, D. O. 1973. Holst filtration apparatus for Van Soest detergent fiber analysis. J. Assoc. Off. Anal. Chem. 56:1352–1356.

Hurwitz, S. 1996. Homeostatic control of plasma calcium concentration. Crit. Rev. Biochem. Mol. Biol. 31:41–100. doi:10.3109/10409239609110575

Hurwitz, S., I. Plavnik, A. Shapiro, E. Wax, H. Talpaz, and A. Bar. 1995. Calcium metabolism and requirements of chickens are affected by growth. J. Nutr. 125:2679–2686.

Kim, B. G., and M. D. Lindemann. 2007. A new spreadsheet method for the experimental animal allotment. J. Anim. Sci. 85(Suppl. 2):218. (Abstr.)

Ko, S.-H., G.-S. Lee, T. T. B. Vo, E.-M. Jung, K.-C. Choi, K.-W. Cheung, J. W. Kim, J.-G. Park, G. T. Oh, and E.-B. Jeung. 2009. Dietary Ca and 1,25-dihydroxyvitamin D3 regulate transcription of calcium transporter genes in calbindin-D9k knockout mice. J. Reprod. Dev. 55:137–142. doi:10.1262/jrd.20139

Kutuzova, G. D., and H. F. DeLuca. 2004. Gene expression profiles in rat intestine identify pathways for 1,25-dihydroxyvitamin D3 stimulated calcium absorption and clarify its immunomodulatory properties. Arch. Biochem. Biophys. 432:152–166. doi:10.1016/j.abb.2004.09.004

Lindemann, M. D., and B. G. Kim. 2007. Technical note: A model to estimate individual feed intake of swine in group feeding. J. Anim. Sci. 85:972–975. doi:10.2527/jas.2006-412

Mahan, D. C., and R. G. Shields Jr. 1998. Macro- and micromineral composition of pigs from birth to 145 kilograms of body weight. J. Anim. Sci. 76:506–512.

Mao, X., M. Liu, J. Tang, H. Chen, D. Chen, B. Yu, J. He, J. Yu, and P. Zheng. 2015. Dietary leucine supplementation improves the mucin production in the jejunal mucosa of the weaned pigs challenged by porcine rotavirus. PLoS One 10:e0137380. doi:10.1371/journal.pone.0137380

Maxon, P. F., and D. C. Mahan. 1983. Dietary calcium and phosphorus levels for growing swine from 18 to 57 kilograms body weight. J. Anim. Sci. 56:1124–1134.

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

Pettey, L. A., G. L. Cromwell, Y. D. Jang, and M. D. Lindemann. 2015. Estimation of calcium and phosphorus content in growing and finishing pigs: Whole empty body components and relative accretion rates. J. Anim. Sci. 93:158–167. doi:10.2527/jas.2014-7602

Proszkowiec-Weglarz, M., and R. Angel. 2013. Calcium and phosphorus metabolism in broilers: Effect of homeostatic mechanism on calcium and phosphorus digestibility. J. Appl. Poult. Res. 22:609–627. doi:10.3382/japr.2012-00743

Rosenberg, J., S. Hurwitz, and A. Bar. 1986. Regulation of kidney calcium-binding protein in the bird (*Gallus domesticus*). Comp. Biochem. Physiol. 83:277–281. doi:10.1016/0300-9629(86)90574-8

Rostagno, H. S., L. F. T. Albino, J. L. Donzele, P. C. Gomes, R. F. de Oliveira, D. C. Lopes, A. S. Ferreira, S. L. T. Barreto, and R. F. Euclides. 2011. Brazilian tables for poultry and swine. H. S. Rostagno, editor, 3rd ed. Universidade Federal de Viçosa, Viçosa, Brazil.

Rymarz, A., H. Fandrejewski, and J. Kielanowski. 1982. Content and retention of calcium, phosphorus, potassium and sodium in the bodies of growing gilts. Livest. Prod. Sci. 9:399–407. doi:10.1016/0301-6226(82)90045-8

Saraiva, A., J. L. Donzele, R. F. M. de Oliveira, M. L. T. de Abreu, F. C. O. Silva, and F. A. Santos. 2009. Available phosphorus levels in diets for swine from 15 to 30 kg genetically selected for meat deposition. Rev. Bras. Zootec. 38:307–313. doi:10.1590/S1516-35982009000200013

Sauvant, D., J.-M. Perez, and G. Tran. 2004. Tables of composition and nutritional value of feed materials: Pig, poultry, sheep, goats, rabbits, horses, fish. 2nd ed. Wageningen Academic Publishers, Wageningen, the Netherlands. doi:10.3920/978-90-8686-668-7

Schröder, B., C. Schlumbohm, R. Kaune, and G. Breves. 1996. Role of calbindin-D9k in buffering cytosolic free Ca^{2+} ions in pig duodenal enterocytes. J. Physiol. 492:715–722. doi:10.1113/jphysiol.1996.sp021340

Schwaller, B. 2010. Cytosolic Ca2+ buffers. Cold Spring Harb. Perspect. Biol. 2:a004051. doi:10.1101/cshperspect.a004051

Stein, H. H., O. Adeola, G. L. Cromwell, S. W. Kim, D. C. Mahan, and P. S. Miller. 2011. Concentration of dietary calcium supplied by calcium carbonate does not affect the apparent total tract digestibility of calcium, but reduces digestibility of phosphorus by growing pigs. J. Anim. Sci. 89:2139–2144. doi:10.2527/jas.2010-3522

Tran, G., and D. Sauvant. 2004. Chemical data and nutritional value. In: D. Sauvant, J.-M. Perez, and G. Tran, editors, Tables of composition and nutritional value of feed materials: Pig, poultry, sheep, goats, rabbits, horses, fish. 2nd ed. Wageningen Academic Publishers, Wageningen, the Netherlands. p. 17–24.

Tryfonidou, M. A., M. S. Holl, M. Vastenburg, M. A. Oosterlaken-Dijksterhuis, D. H. Birkenhäger-Frenkel, W. E. van den Brom, and H. A. W. Hazewinkel. 2003. Hormonal regulation of calcium homeostasis in two breeds of dogs during growth at different rates. J. Anim. Sci. 81:1568–1580.

van de Graaf, S. F. J., I. Boullart, J. G. J. Hoenderop, and R. J. M. Bindels. 2004. Regulation of the epithelial Ca2+ channels TRPV5 and TRPV6 by 1α,25-dihydroxy vitamin D_3 and dietary Ca^{2+}. J. Steroid Biochem. 89–90:303–308. doi:10.1016/j.jsbmb.2004.03.029

Viana, J. M., P. C. Brustolin, F. C. O. Silva, A. Saraiva, J. L. Donzele, R. F. M. Oliveira, A. S. Ferreira, F. A. Santos, and E. Paula. 2013. Níveis de fósforo disponível em dietas para suínos dos 15 a os 30kg. (In Portuguese.) Arq. Bras. Med. Vet. Zootec. 65:203–212. doi:10.1590/S0102-09352013000100030

Vigors, S., T. Sweeney, C. J. O'Shea, J. A. Browne, and J. V. O'Doherty. 2014. Improvements in growth performance, bone mineral status and nutrient digestibility in pigs following the dietary inclusion of phytase are accompanied by modifications in intestinal nutrient transporter gene expression. Br. J. Nutr. 112:688–697. doi:10.1017/S0007114514001494

Wiseman, T. G., D. C. Mahan, and N. R. St-Pierre. 2009. Mineral composition of two genetic lines of barrows and gilts from twenty to one hundred twenty-five kilograms of body weight. J. Anim. Sci. 87:2306–2314. doi:10.2527/jas.2006-545

Ye, J., G. Coulouris, I. Zaretskaya, I. Cutcutache, S. Rozen, and T. Madden. 2012. Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction. BMC Bioinf. 13:134. doi:10.1186/1471-2105-13-134

Zhai, H., and O. Adeola. 2013. True digestible phosphorus requirement of 10 to 20 kg pigs. J. Anim. Sci. 91:3716–3723. doi:10.2527/jas.2012-5875