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Non Ruminant Nutrition

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Increasing calcium from deficient to adequate concentration in diets for gestating sows decreases digestibility of phosphorus and reduces serum concentration of a bone resorption biomarker

Su A. Lee,[*] L. Vanessa Lagos,[†] Mike R. Bedford,[‡] and Hans H. Stein[*,†,1]

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

[1]Corresponding author: hstein@illinois.edu

ABSTRACT

The objective of this experiment was to test the hypothesis that the concentration of Ca in diets fed to late gestating sows affects the apparent total tract digestibility (**ATTD**) and retention of Ca and P, serum concentrations of Ca and P, hormones, and blood biomarkers for bone formation and resorption. Thirty-six sows (average parity = 2.8) were housed in metabolism crates from day 91 to day 104 of gestation and fed 1 of 4 experimental diets containing 25, 50, 75, or 100% of the requirement for Ca. All diets met the requirement for P. The initial 5 d of each period were the adaptation period, which was followed by 4 d of quantitative collection of feces and urine. At the end of the collection period, a blood sample was collected from all sows. Results indicated that feed intake, weights of dried fecal and urine samples, and the ATTD of DM were not affected by dietary Ca, but ATTD of Ca increased (quadratic, $P < 0.05$) as Ca in diets increased. Urine Ca output was not affected by dietary Ca, but Ca retention increased (quadratic, $P < 0.05$) as Ca intake increased. Fecal P output increased (linear, $P < 0.001$) as dietary Ca increased, which resulted in a linear decrease ($P < 0.001$) in the ATTD of P. Urine P output also decreased (linear, $P < 0.001$) as dietary Ca increased, but P retention increased (linear, $P < 0.05$). Regressing the apparent total tract digestible Ca against dietary Ca intake resulted in a regression line with a slope of 0.33, indicating that true total tract digestibility of Ca in calcium carbonate was 33%. Serum concentrations of Ca and P and estrogen, calcitonin, and parathyroid hormone were not affected by dietary Ca. Serum concentration of carboxyterminal cross-linked telopeptide of type I collagen (**CTX-I**) decreased (linear, $P < 0.05$) as dietary Ca increased, which is a result of reduced bone resorption as dietary Ca increased. Serum bone-specific alkaline phosphatase tended to decrease (linear, $P < 0.10$) as Ca in diets increased, but the concentration of osteocalcin (**OC**) in serum was not affected by dietary Ca. The ratio between OC and CTX-I tended to increase ($P < 0.10$) as dietary Ca increased, which indicated that there was more bone formation than resorption in sows as dietary Ca increased. In conclusion, P digestibility in late gestating sows decreased, but retention of P increased, as dietary Ca increased from inadequate to adequate levels and blood biomarkers for bone resorption changed as Ca and P retention increased.

Key words: biomarkers, calcium, digestibility, phosphorus, retention, sows

Abbreviations

ATTD	apparent total tract digestibility
BAP	bone-specific alkaline phosphatase
CTX-I	carboxyterminal cross-linked telopeptide of type I collagen
ME	metabolizable energy
OC	osteocalcin
PTH	parathyroid hormone
TTTD	true total tract digestibility

Introduction

Values for apparent total tract digestibility (**ATTD**) of P in sows fed Ca-free diets are greater than values from sows fed diets containing corn and calcium carbonate (Lee et al., 2019b), and increasing dietary Ca linearly reduces P digestibility in growing pigs (Stein et al., 2011). These observations indicate that there is an interaction between dietary Ca and P, which is probably a result of precipitation of Ca and P in the intestinal tract of pigs.

Relatively more Ca and P are needed for fetus development in late gestation compared with earlier gestation periods (Bikker and Blok, 2017; Lee et al., 2019b), but an assessment of the exact requirements for Ca and P in sows is challenging and expensive. However, biomarkers for bone turnover including carboxyterminal cross-linked telopeptide of type I collagen (**CTX-I**), osteocalcin (**OC**), and bone-specific alkaline phosphatase (**BAP**) have been used in humans, beef breeder cows, and growing pigs as indicators of Ca and P adequacy in diets (Larsen et al., 2000; Vasikaran et al., 2011; Anderson et al., 2017; Sørensen et al., 2018). Therefore, changes in dietary Ca and P, retained Ca and P in the body, and bone turnover may be estimated from serum concentrations of biomarkers, but this relationship has not been demonstrated in sows, and it is not known if blood biomarkers can be used to estimate Ca and P status of gestating sows.

Blood Ca levels are regulated by several hormones including parathyroid hormone (**PTH**) and calcitonin and, occasionally, estrogen (Heaney, 1990; Crenshaw, 2001). It is also possible that hormone levels are affected by dietary Ca and P, but pig data to demonstrate this are lacking. Therefore, the objective of this experiment was to test the hypothesis that the concentration of Ca in diets fed to late gestating sows affects ATTD and retention of Ca and P, blood Ca and P concentrations, serum hormone levels, and concentrations of serum biomarkers.

Materials and Methods

The Institutional Animal Care and Use Committee at the University of Illinois reviewed and approved the protocol for the experiment before the animal work was initiated.

Animals, housing, diets, and sample collection

Thirty-six gestating Camborough sows (PIC, Hendersonville, TN; average parity = 2.8) were allotted to 3 blocks of 12 sows using a randomized complete block design. Four diets were fed to the 12 sows in each block with 3 sows per diet; thus, there was a total of 9 replicate sows for each treatment. Sows were housed individually in metabolism crates from approximately day 91 to day 104 of gestation. Metabolism crates were equipped with a feeder, a nipple drinker, and fully slatted tribar floors. A screen floor and a urine pan were installed below the tribar floors to allow for collection of feces and urine, respectively.

Experimental diets were based on corn, soybean meal, and sugar beet pulp (Table 1). All diets were formulated to contain P at the requirement for total P by late gestating sows in parity greater than 3 (i.e., 0.55%; NRC, 2012), but Ca was included at 25%, 50%, 75%, or 100% of the requirement for sows in late gestation that are in their third or greater parity (Table 2). Thus, the 4 experimental diets were formulated to contain 0.18%, 0.36%, 0.54%, or 0.72% Ca, and these concentrations were achieved by adding increasing concentrations of calcium carbonate to the diets at the expense of cornstarch. All vitamins and minerals except Ca were included in all diets to meet requirements (NRC, 2012).

Daily feed allotments were provided in 2 equal meals that were fed at 0800 and 1600 h throughout the experiment. The daily feed allowance was 1.5 times the maintenance energy requirement for gestating sows based on the body weight of sows when they were moved to the metabolism crates (i.e., 100 kcal metabolizable energy (**ME**)/kg body weight$^{0.75}$; NRC, 2012). Water was available at all times.

The initial 5 d of each period in the metabolism crates were considered the adaptation period to the diets, and this period was followed by 4 d of total fecal collection using the marker to marker procedure (Adeola, 2001). Fecal collection was initiated when the first marker (i.e., indigo carmine) appeared in the feces and ceased when the second marker (i.e., chromic oxide) appeared (Adeola, 2001). It took 3 to 4 d for the second marker to appear in the feces, and as a consequence, sows were housed for 13 d in the metabolism crates. Fecal samples were stored at −20 °C as soon as collected. Urine was collected in buckets placed under the urine pans with 50 mL of 3N HCl from day 6 in the morning until day 10 in the morning. Buckets were emptied daily, the weight of the collected urine was recorded, and 10% of the collected urine was stored at −20 °C until subsampling. Following fecal and urine collections, sows were fasted for 24 h (Vasikaran et al., 2011) and a blood sample was collected from the vena cava. Blood samples were immediately centrifuged and serum was collected and stored at −20 °C.

At the conclusion of the experiment, urine samples were thawed and mixed within animal and collection period and

Table 1. Analyzed nutrient composition of feed ingredients (as-is basis)

Item	Corn	Soybean meal	Sugar beet pulp	Calcium carbonate	Monosodium phosphate	Vitamin–mineral premix	Sodium chloride
DM, %	86.3	92.0	89.5	99.9	99.7	96.4	99.7
GE, kcal/kg	3,859	4,240	3,630	—	—	—	—
CP, %	7.1	49.8	7.3	—	—	—	—
Acid hydrolyzed ether extract, %	4.3	2.3	3.0	—	—	—	—
Ash, %	1.3	6.6	6.6	93.3	91.4	55.9	99.8
Ca, %	—	0.30	0.82	37.8	0.05	2.04	0.25
P, %	0.29	0.80	0.09	—	26.7	0.12	—

Table 2. Composition of experimental diets (as-is basis)

Item	Ca level, % of the requirement[1]			
	25	50	75	100
Ingredient, %				
Corn	76.45	76.45	76.45	76.45
Soybean meal	11.00	11.00	11.00	11.00
Sugar beet pulp	8.00	8.00	8.00	8.00
Calcium carbonate	0.15	0.62	1.09	1.55
Monosodium phosphate	1.10	1.10	1.10	1.10
Cornstarch	1.40	0.93	0.46	—
Soybean oil	1.20	1.20	1.20	1.20
L-Lys·HCl, 78.8% Lys	0.10	0.10	0.10	0.10
L-Thr, 99% Thr	0.05	0.05	0.05	0.05
Sodium chloride	0.40	0.40	0.40	0.40
Vitamin–mineral premix[2]	0.15	0.15	0.15	0.15
Analyzed composition, %				
ME, kcal/kg[3]	3,347	3,328	3,310	3,291
DM	88.1	88.1	87.6	87.9
CP	12.1	11.3	12.0	11.6
Ash	3.2	4.1	4.5	4.8
Total dietary fiber[4]	14.3	13.6	16.3	14.5
Soluble dietary fiber	2.0	1.4	2.6	2.0
Insoluble dietary fiber	12.3	12.2	13.7	12.5
Ca	0.18	0.36	0.59	0.71
P	0.61	0.61	0.61	0.57
Ca:P ratio	0.30:1	0.59:1	0.97:1	1.25:1

[1]The requirement estimate is based on the requirement for Ca by gestating sows that are in their third parity and in late gestation (NRC, 2012).
[2]The vitamin–micromineral premix provided the following quantities of vitamins and microminerals per kg of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D_3 as cholecalciferol, 2,210 IU; vitamin E as selenium yeast, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.42 mg; thiamin as thiamine mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.6 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride. Wheat bran, but not limestone, was used as a carrier in the premix to avoid Ca being added from the premix.
[3]Values for ME were calculated rather than analyzed (NRC, 2012).
[4]Total dietary fiber = soluble dietary fiber + insoluble dietary fiber.

subsamples were collected. Urine subsamples were filtered through a 4- to 8-µm P4 filter (Fisher Scientific International, Inc., Hampton, NH). Fecal samples were dried at 65 °C in a forced-air oven and finely ground through a 1-mm screen before analysis using a Wiley mill (Model 4; Thomas Scientific, Swedesboro, NJ).

Chemical analyses

Calcium and P in ingredients, diets, feces, urine, and serum were analyzed by inductively coupled plasma spectroscopy (AOAC Int., 2007; method 985.01 A, B, and C) after wet ash sample preparation [AOAC Int., 2007; method 975.03 B(b)]. Ingredient, diet, and fecal samples were analyzed for DM (AOAC Int., 2007; method 930.15), and ash was analyzed in all ingredient and diet samples (AOAC Int., 2007; method 942.05). Crude protein in corn, soybean meal, sugar beet pulp, and all diets was calculated as N × 6.25 and N was analyzed by combustion (AOAC Int., 2007; method 990.03) using a LECO FP628 apparatus (LECO Corp., Saint Joseph, MI). Insoluble dietary fiber and soluble dietary fiber in diets were analyzed according to method 991.43 (AOAC Int., 2007) using the Ankom^{TDF} Dietary Fiber Analyzer (Ankom Technology, Macedon, NY). Acid hydrolyzed ether extract in corn, soybean meal, and sugar beet pulp was analyzed by acid hydrolysis using 3N HCl (AnkomHCl, Ankom Technology) followed by fat extraction using petroleum ether (AnkomXT15, Ankom Technology). The GE in corn, soybean meal, and sugar beet pulp was measured using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL).

Serum samples were analyzed for CTX-I using a Pig Cross-Linked C-Telopeptide of Type I Collagen ELISA Kit (Abbexa Ltd., Cambridge, UK) that had an intra-assay and an interassay CV of 10%. Concentrations of OC in serum were analyzed using an N-MID Osteocalcin ELISA Kit (Immunodiagnostic Systems Ltd., The Boldons, UK), and BAP was analyzed using an Ostase BAP Enzyme Immunoassay Kit (Immunodiagnostic Systems Ltd.). The intra-assay CV was 1.8% and 4.2% for OC and BAP, respectively, and the interassay CV was 4.0% and 5.5%, respectively. Serum samples were also analyzed for calcitonin (Porcine Calcitonin ELISA Kit; MyBioSource, San Diego, CA; intra-assay CV = 8.0%, interassay CV = 12.0%), PTH (Porcine PTH ELISA Kit; MyBioSource; intra-assay CV = 4.8%, interassay CV = 4.9%), and estrogen as estradiol (Porcine Estrogen ELISA Kit; MyBioSource; intra-assay CV = 8.0%, interassay CV = 12.0%).

Calculations

The ATTD of DM, Ca, and P in experimental diets was calculated as outlined by Almeida and Stein (2010), and retention of Ca and P (%) were calculated according to Petersen and Stein (2006). Apparent total tract digested Ca (g/d) was regressed against dietary Ca intake (g/d) using the following equation, which was adopted from Fan et al. (2001):

$$\text{Apparent total tract digested Ca} = -B + (A \times \text{dietary Ca intake}),$$

where A is the slope of the regression and represents the coefficient for true total tract digestibility (**TTTD**), and B is the intercept of the regression and represents the endogenous loss of Ca (g/d).

Statistical analysis

Data were analyzed using the PROC MIXED (SAS Inst. Inc., Cary, NC), and homogeneity of the variance among treatments and normality was confirmed using the PROC UNIVARIATE of SAS. Outliers were identified and eliminated if values deviated from the first or third quartiles by more than 3 times the interquartile range (Tukey, 1977). Sow was the experimental unit for all analyses. The statistical model included diet as fixed effect and parity, block, and replicate within block as random effects and LSmeans of each treatment were calculated. Polynomial contrasts were used to test for linear and quadratic effects of increasing dietary Ca. The PROC REG of SAS was used to estimate the y-intercept and the slope to determine the endogenous losses of Ca and the TTTD of Ca, respectively. Significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

Calcium and P balance

Feed intake, fecal excretion, urine excretion, and the ATTD of DM by sows were not affected by the level of Ca in the diets (Table 3), but Ca intake, fecal Ca output, and absorbed Ca increased (linear,

Table 3. Calcium and P balances for sows in late gestation fed diets containing different levels of Ca[1]

	Ca level, % of the requirement[2]					P-value[3]	
Item	25	50	75	100	SEM	Lin.	Quad.
Feed intake, kg/d	3.05	3.09	3.05	3.08	0.13	0.834	1.000
Fecal excretion, kg DM/d	0.29	0.31	0.30	0.32	0.02	0.116	0.884
Urine excretion, kg/d	3.99	4.76	7.87	4.80	1.39	0.380	0.177
ATTD of DM, %	89.11	88.80	88.82	88.47	0.54	0.156	0.955
Ca balance							
Ca intake, g/d	5.7	10.8	16.0	21.0	0.5	<0.001	0.935
Fecal Ca output, g/d	6.2	9.3	12.8	16.5	1.0	<0.001	0.571
Absorbed Ca, g/d	–0.4	1.6	3.3	4.7	0.8	<0.001	0.601
ATTD of Ca, %	–7.49	14.34	20.59	22.04	5.95	<0.001	0.039
Urine Ca output, g/d	0.1	0.1	0.1	0.1	0.04	0.300	0.581
Ca retention, g/d	–0.5	1.6	3.2	4.6	0.7	<0.001	0.592
Ca retention, % of intake	–9.33	13.53	19.83	21.45	5.87	<0.001	0.034
Ca retention, % of absorbed[4]	—	99.88	99.80	96.08	5.29	0.491	0.705
P balance							
P intake, g/d	18.6	18.8	18.6	18.8	0.8	0.822	0.967
Fecal P output, g/d	11.5	13.1	13.2	14.4	0.9	<0.001	0.673
Absorbed P, g/d	7.0	5.7	5.3	4.3	0.6	<0.001	0.680
ATTD of P, %	39.02	31.26	29.72	23.97	3.01	<0.001	0.677
Urine P output, g/d	4.9	3.8	2.2	1.3	0.3	<0.001	0.770
P retention, g/d	2.3	2.0	3.2	3.3	0.5	0.031	0.704
P retention, % of intake	12.33	10.90	17.87	17.86	3.19	0.026	0.755
P retention, % of absorbed	28.39	29.49	50.83	69.03	7.93	<0.001	0.093

[1]Each least squares mean represents 9 observations.
[2]The requirement estimate is based on the requirement for Ca by gestating sows that are in their third parity and in late gestation (NRC, 2012).
[3]Lin. = linear effect of Ca level; Quad. = quadratic effect of Ca level.
[4]Regardless of calculated value, Ca retention was assumed to be close to zero. Therefore, the first diet was excluded to test the linear and quadratic effects of dietary Ca.

$P < 0.001$) with increasing Ca in diets. Values for the ATTD of Ca increased (quadratic, $P < 0.05$) as Ca in diets increased. Urine Ca output and Ca retention, expressed as percent of absorbed, were not affected by dietary Ca, but Ca retention expressed as g/d increased (linear, $P < 0.001$), and Ca retention, expressed as percent of intake, increased quadratically ($P < 0.05$).

Phosphorus intake was not affected by dietary Ca because all diets had the same concentration of P. Fecal P output increased (linear, $P < 0.001$) as dietary Ca increased, which resulted in linear decreases ($P < 0.001$) in ATTD of P and absorbed P (g/d). Urine P output also decreased (linear, $P < 0.001$) as dietary Ca increased, whereas P retention expressed as g/d and as percent of P intake and as percent of absorbed P increased as dietary Ca increased (linear, $P < 0.05$). The slope of the regression line that was developed by regressing apparent total tract digestible Ca against dietary Ca intake was 0.33, which indicates that the TTTD of Ca in calcium carbonate was 33% (Table 4). The intercept of the regression line and the vertical axis was at 0.79, indicating that total endogenous loss of Ca was 0.79 g per kg DM intake.

Calcium and P levels, hormones, and biomarkers in blood samples

Serum concentrations of Ca and P and estrogen, calcitonin, and PTH in serum samples were not affected by Ca concentration in diets (Table 5). However, the concentration of CTX-I decreased (linear, $P < 0.05$) as Ca concentration increased in the diets, indicating that reduced quantities of Ca were mobilized from the bones as dietary Ca increased. In contrast, serum BAP tended to decrease (linear, $P < 0.10$) as Ca in diets increased. The concentration of OC in serum was not affected by dietary Ca, but the ratio of OC to CTX-I tended to increase ($P < 0.10$) as dietary Ca increased, which indicates that bone formation increased more than bone resorption increased.

Table 4. Regression of apparent total tract digested Ca (g/d) against dietary Ca intake (g/d) of sows fed diets containing 4 levels of calcium carbonate[1]

Item	Calcium carbonate
Regression equation	Y = –2.1230 + 0.3318X
SE of slope	0.0186 (P = 0.003)
SE of intercept	0.2705 (P = 0.016)
Coefficient of determination (r^2)	0.994
Endogenous loss of Ca, g/d	2.12
Endogenous loss of Ca[2], g/kg DM intake	0.79
True total tract Ca digestibility[2], %	33.18

[1]The number of observations (n) = 36.
[2]The SE of the endogenous loss was 0.10; the SE for the true total tract digestibility = 1.86 (the SE of the slope × 100).

Discussion

Calcium and P balance

The requirement for Ca in late gestation sows is 0.72% (NRC, 2012). Concentrations of analyzed Ca in the 4 diets were 0.18%, 0.36%, 0.59%, and 0.71%, which is equivalent to 25%, 50%, 82%, and 99% of the requirement, respectively. Thus, analyzed Ca in

Table 5. Calcium and P concentrations, bone resorption and formation biomarkers, and hormone concentrations in serum samples of late gestation sows fed diets containing different levels of Ca[1]

	Ca level, % of the requirement[2]					P-value[3]	
Item	25	50	75	100	SEM	Lin.	Quad.
Ca, mg/L	93	92	91	92	2.1	0.713	0.539
P, mg/L	81	79	79	83	2.8	0.560	0.162
Hormones							
Estrogen, µg/L	2.1	2.0	2.0	2.0	0.16	0.546	0.706
Calcitonin, µg/L	2.7	2.7	2.5	2.5	0.14	0.230	0.935
Parathyroid hormone, µg/L	1.7	1.8	1.6	1.6	0.10	0.348	0.758
Bone resorption biomarker							
CTX-I, µg/L	1.5	1.0	1.4	0.2	0.39	0.033	0.296
Bone formation biomarkers							
Bone alkaline phosphatase, µg/L	12.1	10.7	10.5	10.2	1.15	0.091	0.506
Osteocalcin, µg/L	16.6	18.7	18.8	19.0	1.37	0.176	0.446
Osteocalcin/CTX-I	25	42	43	82	21.5	0.055	0.570

[1]Each least squares mean represents 9 observations.
[2]The requirement estimate is based on the requirement for Ca by gestating sows that are in their third parity and in late gestation (NRC, 2012).
[3]Lin. = linear effect of Ca level; Quad. = quadratic effect of Ca level.

the 4 diets was in agreement with formulated concentrations. The observations that urine Ca output was not affected by dietary Ca intake, whereas Ca retention increased as dietary Ca increased, indicates that sows retained absorbed Ca with the same efficiency regardless of dietary Ca intake. This indicates that even at the greatest level of Ca intake, sows retained almost all absorbed Ca. A similar observation was made in growing pigs that were fed diets with increasing dietary concentrations of Ca (González-Vega et al., 2016a).

The requirement for P is 0.55% (NRC, 2012), and all diets were formulated to meet the requirement. The analyzed P in all diets was 0.02 to 0.06 percentage units greater than formulated, indicating that all diets met the requirement for P as was intended. The reason for the slightly greater analyzed than formulated concentrations was that corn and soybean meal contained slightly more P than expected.

Values for the ATTD of Ca and P in the diets were in agreement with previous values for gestating sows (Nyachoti et al., 2006; Jang et al., 2014; Lee et al., 2019b). The quadratic increase in the ATTD of Ca that was observed as dietary Ca increased is a result of a greater proportion of endogenous Ca in the feces of sows fed diets with a low concentration of Ca compared with sows fed diets with greater Ca (González-Vega et al., 2013). The negative values for ATTD of Ca and retention of Ca as percent of intake for sows fed the diet with the least concentration of Ca demonstrate that these sows had endogenous losses of Ca that were greater than the daily Ca intake.

The TTTD of Ca in calcium carbonate was close to the standardized total tract digestibility of Ca in calcium carbonate fed to late gestating sows (Lee et al., 2019b). The standardized total tract digestibility and TTTD of Ca in calcium carbonate by growing pigs were between 69% and 76% (González-Vega et al., 2015; Merriman and Stein, 2016; Zhang and Adeola, 2017; Lee et al., 2019a). The observation that the TTTD of Ca in calcium carbonate was 33% indicates that sows have much less digestibility of Ca compared with growing pigs, which concurs with previous data (Lee et al., 2018a,b).

The ATTD of P in diets containing corn, soybean meal, and monosodium phosphate is expected to be approximately 60% if the diets are fed to growing pigs (NRC, 2012). However, the observation that the ATTD of P in the 4 diets fed to sows was less than 40% further confirms that sows have much lower digestibility of Ca and P than growing pigs. It is not clear why the digestibility of Ca and P in gestating sows is so much lower than in growing pigs. In the diet with the least amount of Ca, sows were fed only 25% of the requirement, which theoretically should have upregulated the transcellular absorption of Ca, resulting in a greater digestibility of Ca. However, the fact that this did not happen indicates that sows are not able to regulate the intestinal absorption of Ca, even if Ca is fed well below the requirement. This observation is in agreement with data from growing pigs (Stein et al., 2011) and intestinal absorption of P also appears not to be upregulated if the P is provided at levels below the requirement (Stein et al., 2008).

The observation that the ATTD of P was reduced by increasing dietary Ca clearly demonstrates that P absorption is reduced by increasing Ca from calcium carbonate in the diets. This is likely due to chelation of phytate from corn, soybean meal, and sugar beet pulp with Ca^{+2} ions, which results in undigestible Ca–P complexes (Stein et al., 2011). It is also possible that dietary P binds directly to Ca ions in the intestinal tract of pigs, which results in precipitation and, therefore, reduction in digestibility (Walk et al., 2012). However, the observations that urine excretion of P decreased and retention of P increased as dietary Ca increased indicates that P was in excess in the low Ca diets because there was not enough Ca to support maximum bone tissue synthesis. However, as dietary Ca increased, more bone was synthesized, which required more P and less P was, therefore, excreted in the urine. These observations indicate that Ca was the limiting nutrient for synthesizing bone because Ca and P are needed at the same time in the body to synthesize bone tissue. This observation is in agreement with data demonstrating that bone mineralization in growing pigs increased as dietary Ca increased with a constant concentration of P (González-Vega et al., 2016a; Merriman et al., 2017; Lagos et al., 2019). However, regardless of dietary treatment, values for Ca and P retention that were calculated in this experiment were less than those observed in a previous experiment for sows in late gestation (Lee et al., 2019b). Thus, it is possible that even though the diet with the greatest concentration of Ca was formulated to meet the requirement for Ca, sows fed this diet were fed below the concentration of Ca that is required to maximize Ca retention.

As demonstrated in this experiment, the DM digestibility was in agreement with what is observed in growing pigs and AA

digestibility in gestating sows is also close to the values observed in growing pigs (Stein et al., 2001). It therefore appears that the low digestibility of Ca and P is specific to these nutrients and not something that is general for all nutrients fed to gestating sows. The digestibility of Ca and P in lactating sows is closer to values obtained in growing pigs compared with gestating sows (Kemme et al., 1997; Jongbloed et al., 2004; Nyachoti et al., 2006). It therefore seems that the very low digestibility of Ca and P that was observed in this experiment is specific to gestating sows. More research is needed to elucidate the reasons for these low digestibility values in gestating sows.

Endogenous loss of Ca

The *y*-intercept of the regression line indicated that the endogenous loss of Ca was 2.12 g/d and 0.79 g/kg DM intake if corrected for the average DM intake of sows. The total endogenous loss of Ca was in agreement with the value for the basal endogenous loss of Ca by sows in late gestation that was measured in a previous experiment (Lee et al., 2019b). However, the values obtained from sows were much greater compared with the endogenous loss of Ca in growing pigs (González-Vega et al., 2013; Zhang and Adeola, 2017; Lee et al., 2019a). The reason for this observation may be that gestating sows are fed only 1.5 times the energy requirement for maintenance, whereas growing pigs are usually fed 3.0 to 3.4 times the energy requirement for maintenance. The endogenous losses of AA measured as g per kg DM intake increases as feed intake is reduced (Stein et al., 1999; Moter and Stein, 2004), but it is unlikely that is the case for Ca because the level of feed intake does not affect ATTD of Ca and P in gestating sows (Lee et al., 2018a).

Calcium and P levels, hormones, and biomarkers in blood samples

Concentrations of Ca and P in serum were in agreement with expected values (Lauridsen et al., 2010; Weber et al., 2014). These results indicate that dietary Ca levels do not affect serum Ca or P in sows, which is in agreement with previous data (Larsen et al., 2000; González-Vega et al., 2016b). Blood concentrations of Ca are regulated by PTH and calcitonin (Crenshaw, 2001). If blood Ca is low, PTH is released from the parathyroid glands, which results in increased Ca absorption, efflux of Ca from bones, and reabsorption of Ca in the kidneys to increase blood Ca concentration (Crenshaw, 2001; Molina, 2013; Blaine et al., 2015). However, calcitonin is released when blood Ca is high, which results in a decrease in blood Ca concentration because of storage of more Ca in bone and reduced reabsorption of Ca from the kidney (Crenshaw, 2001; Molina, 2013). Estrogen concentration in serum is related to Ca metabolism in the body (Heaney, 1990; Ross et al., 2011; Harmon et al., 2016), and estrogen in serum increases during late gestation and during the postpartum period to support the development of mammary glands (Kensinger et al., 1982). Therefore, it was expected that estrogen, PTH, and calcitonin concentration in serum would be affected by dietary Ca, but that was not the case. It is possible that this is a result of sows being fasted for 24 h before bleeding. Sows were fasted because some bone biomarkers may be affected by food intake (Vasikaran et al., 2011).

Several biomarkers to predict bone turnover have been used in clinical practice for humans (Seibel, 2005; Vasikaran et al., 2011; Smith and Samadfam, 2017) and in some pig experiments (Weber et al., 2014; Sørensen et al., 2018). Most markers are derivatives or byproducts of bone turnover (Weber et al., 2014; Sørensen et al., 2018). The CTX-I is a collagen peptide derived from the bone matrix, which is released in greater quantities as bone breakdown increases; OC is synthesized by osteoblasts when new bone tissues are formed and BAP is an enzyme that is involved in calcification of bone tissues by using blood P as a building block for bone tissue synthesis (Vasikaran et al., 2011). When dietary Ca is low, bone tissue breakdown is increased, which results in an increase in serum concentrations of collagen fragments including CTX-I that were parts of the bone matrix as was demonstrated in this experiment. In contrast, when there is sufficient Ca, it is more likely that osteoblasts are activated to increase bone tissue formation, which results in increases in OC and BAP levels. However, the bone formation markers did not increase as dietary Ca increased and the tendency for a decrease in the serum BAP as Ca increased was unexpected. However, a reduction in serum BAP in growing pigs as dietary Ca increased was reported (Sørensen et al., 2018). It is possible this is a result of the fact that changes in bone formation take up to 3 mo whereas only 10 d are needed for bone resorption (Seibel, 2005). In this experiment, sows were fed experimental diets for 13 d and this may explain why only CTX-I concentration differed among dietary treatments. For other markers to show a change as a result of dietary changes in Ca concentration, it is possible that a longer period of feeding is required. Nevertheless, the observation that CTX-I increased and the OC to CTX-I ratio tended to increase as dietary Ca increased indicates that these biomarkers possibly can be used to estimate Ca status of gestating sows, but more research is needed to verify this hypothesis.

Conclusion

Data from this experiment indicate that P digestibility by late gestating sows decreases, but retention of P increases, as dietary Ca increases from below to at the requirement. Blood Ca, P, and hormones are not affected by dietary Ca if Ca and P levels do not exceed the requirement for Ca. Some blood biomarkers may be useful in predicting bone resorption by late gestation sows, but the present data indicate that bone formation biomarkers may be less accurate in predicting changes in bone status of animals than bone resorption biomarkers. More research is needed to verify the present results for bone biomarkers, and quantification of bone mass relative to biomarker concentrations is needed for biomarkers to be used in requirement studies for gestating sows.

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Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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