Basal endogenous loss, standardized total tract digestibility of calcium in calcium carbonate, and retention of calcium in gestating sows change during gestation, but microbial phytase reduces basal endogenous loss of calcium¹

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ABSTRACT: The objective was to test the hypothesis that the standardized total tract digestibility (STTD) of Ca and the response to microbial phytase on STTD of Ca and apparent total tract digestibility (ATTD) of P in diets fed to gestating sows are constant throughout gestation. The second objective was to test the hypothesis that retention of Ca and P does not change during gestation. Thirty-six gestating sows (parity = 3.3 ± 1.5; d of gestation = 7 d) were allotted to 4 diets. Two diets containing 0 or 500 units of microbial phytase per kilogram were based on corn, potato protein concentrate, and calcium carbonate. Two Ca-free diets were also formulated without or with microbial phytase to estimate basal endogenous loss of Ca. Daily feed allowance was 1.5 times the maintenance energy requirement. Sows were housed individually in gestation stalls and fed a common gestation diet, but they were moved to metabolism crates from days 7 to 20 (early gestation), days 49 to 62 (midgestation), and again from days 91 to 104 (late gestation). When sows were in metabolism crates, they were fed experimental diets and feces and urine were quantitatively collected for 4 d after 4 d of adaptation. Results indicated that outcomes were not influenced by the interaction between period of

gestation and dietary phytase. The basal endogenous loss of Ca was greater ($P < 0.05$) by sows in early gestation than by sows in mid- or late-gestation, but supplementation of microbial phytase to the Ca-free diet decreased ($P < 0.01$) the basal endogenous loss of Ca and tended ($P = 0.099$) to increase ATTD of P. Supplementation of microbial phytase did not affect ATTD of DM, STTD of Ca, Ca retention, ATTD of P, or P retention in sows fed the calcium carbonate-containing diet. The ATTD of DM was not affected by period of gestation, but the ATTD of Ca, the ATTD of P, and the retention of Ca were least ($P < 0.05$) in midgestation, followed by early and late gestation, respectively, and the STTD of Ca in midgestation was also reduced ($P < 0.05$) compared with sows in early or late gestation. Phosphorus retention was greater ($P < 0.05$) in late gestation than in the earlier periods. In conclusion, Ca retention was less negative and ATTD of P tended to increase with supplementation of microbial phytase to the Ca-free diet regardless of gestation period. The basal endogenous loss, STTD of Ca, ATTD of P, and retention of Ca and P in gestating sows change during gestation with the greatest digestibility values observed in late gestation.

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

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INTRODUCTION

Standardized total tract digestibility (STTD) of Ca has been determined for most Ca containing ingredients fed to growing pigs ([González-Vega](#)

et al., 2015a; Stein et al., 2016; Zhang et al., 2016), but the STTD of Ca by sows in midgestation is less than by growing pigs (Lee et al., 2018). As a consequence, if diets for gestating sows are formulated using STTD values determined in growing pigs, provisions of digestible Ca will be less than calculated.

Exogenous phytase increases not only P digestibility, but also Ca digestibility, in diets and feed ingredients including Ca carbonate when fed to growing pigs (Almeida et al., 2013; González-Vega et al., 2015a). The efficacy of phytase to release Ca and P is believed to be influenced by the physiological status of the animal with phytase fed to sows in midgestation releasing less Ca and P compared with growing pigs or sows in late-gestation (Kemme et al., 1997; Sulabo, 2004). It is also possible that the digestibility of Ca and P by sows changes during gestation with sows in late gestation having greater digestibility than sows in midgestation (Kemme et al., 1997; Jongbloed et al., 2004, 2013; Nyachoti et al., 2006). However, to our knowledge, possible changes during gestation of the basal endogenous loss, the STTD of Ca, and retention of Ca and P have not been reported, and it is, therefore, not known if values for STTD of Ca or retention of Ca and P obtained in a specific time in gestation is representative of the entire gestation period.

Therefore, the objective of this experiment was to test the hypothesis that the basal endogenous loss of Ca, the STTD of Ca in Ca carbonate, and the response to microbial phytase on STTD of Ca and apparent total tract digestibility (ATTD) of P in P-adequate-corn-based diets fed to gestating sows are constant throughout gestation. The second objective was to test the hypothesis that retention of Ca and P does not change during gestation.

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. Camborough sows (PIC, Hendersonville, TN) were used in the experiment.

Animals, Housing, and Sample Collection

Thirty-six gestating sows (initial BW: 219.1 ± 33.4 kg; average parity: 3.3 ± 1.5) that were 1 wk post-breeding 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;

thus, there was a total of 9 replicate sows for each treatment.

Experimental diets included a corn-based diet in which Ca carbonate was the sole source of Ca and a Ca-free diet (Tables 1 and 2). Each diet was prepared with no microbial phytase and with addition of 500 units of phytase (Quantum Blue; AB Vista, Marlborough, UK). All vitamins and minerals except Ca in the Ca-free diet were included in all diets to meet estimated nutrient requirements (NRC, 2012). Daily feed allotments were provided in 2 equal meals that were fed at 0800 and 1600 h. Daily feed allowance was 1.5 times the maintenance energy requirement for gestating sows (i.e., 100 kcal ME/kg BW^{0.75}; NRC, 2012). Water was available at all times.

Sows were housed individually in gestation stalls throughout gestation. However, from days 7 to 20 (early gestation), days 49 to 62 (midgestation), and again from days 91 to 104 (late gestation), sows were moved to metabolism crates, where they were fed 1 of the 4 experimental diets. Sows were fed the same experimental diet every time they were placed in the metabolism crates, but when sows were housed in the gestation stalls, they were fed a common conventional gestation diet that was formulated to meet the requirement estimates for all nutrients (NRC, 2012). Metabolism crates were equipped with a feeder, a nipple drinker, and a fully slatted T-bar floor. A screen floor and a urine pan were installed below the T-bar floor to allow for collection of feces and urine, respectively. The initial 4 d of each period in the metabolism crates were considered the adaptation period to the diets. A 4-d adaptation period was used to reduce the length

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

Item	Corn	Potato protein concentrate	Calcium carbonate
DM, %	89.77	93.57	99.95
CP, %	6.95	79.87	—
Ash, %	1.42	0.65	93.38
Ca, %	<0.01	0.02	39.63
Total P, %	0.25	0.13	0.01
Phytate ¹ , %	0.73	0.28	—
Phytate-bound P, %	0.21	0.08	—
Phytate-bound P, % of total P	82.00	62.99	—
Nonphytate P ² , %	0.05	0.05	—

¹Phytate was calculated by dividing the analyzed phytate-bound P by 0.282 (Tran and Sauvant, 2004).

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

Table 2. Ingredient composition of diets (as-is basis)

Ingredient, %	Calcium carbonate ¹	Ca-free ¹	Conventional diet ²
Corn	86.19	88.34	74.58
Soybean meal	—	—	14.00
Sugar beet pulp	—	—	7.00
Potato protein concentrate	8.50	8.50	—
Calcium carbonate	2.10	—	1.30
Soybean oil	1.00	1.00	1.20
L-Lys-HCl	—	—	0.12
L-Thr	—	—	0.05
Monocalcium phosphate	—	—	1.20
Monosodium phosphate	1.15	1.10	—
Potassium carbonate	0.40	0.40	—
Magnesium oxide	0.10	0.10	—
Sodium chloride	0.40	0.40	0.40
Vitamin-mineral premix ³	0.15	0.15	0.15
Phytase premix ⁴	0.01	0.01	—

¹Diets were formulated without or with 500 units of microbial phytase (Quantum Blue, AB Vista, Marlborough, UK).

²Conventional diet was fed to gestating sows before and between collection periods, and the conventional diet did not contain any exogenous phytase.

³The vitamin-micromineral premix provided the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 11,150 IU; vitamin D₃ as cholecalciferol, 2,210 IU; vitamin E as DL- α tocopheryl acetate, 66 IU; vitamin K as menadiol dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamin mononitrate, 1.10 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 1.00 mg; vitamin B₁₂, 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 sulfate; Fe, 125 mg as iron sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganous sulfate; Se, 0.30 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc sulfate.

⁴The phytase premix contained 5,000 units of phytase/g; corn starch was used at the expense of phytase premix in diets without microbial phytase.

of feeding the Ca-free diet as much as possible to prevent bone damage to the sows. The adaptation period was followed by 4 d of 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., ferric oxide) appeared (Adeola, 2001). Passage of the marker was expected to take up to 4 d, which is the reason sows were kept in the metabolism crates for 13 d. Urine was collected in buckets placed under the urine pans and 50 mL of 3N HCl was added to each bucket every 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. At the end of each collection period, sows were moved back to the gestation stalls. All sows were checked for pregnancy using an ultrasound scanner (VSS700 EZ Preg Checker;

Veterinary Sales and Service Inc., Elmhurst, IL) on day 28 after breeding. Nonpregnant sows were removed from the experiment.

At the conclusion of the experiment, urine samples were thawed and mixed within animal and collection period and subsamples were collected. Fecal samples were stored at -20 °C as soon as collected, and at the conclusion of the experiment, samples were dried at 65 °C in a forced air oven, finely ground through a 1-mm screen using a Wiley Mill (Model 4; Thomas Scientific, Swedesboro, NJ), and mixed within sow and collection period. A subsample of the ground feces was then collected.

Chemical Analysis

Calcium and P in corn, potato protein concentrate, calcium carbonate, diets, feces, and urine samples 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)]. Diets were analyzed for phytase activity (ESC, Ystrad Mynach, UK) by the ELISA method using Quantiplate Kits for Quantum Blue and feed ingredients were also analyzed for phytate-bound P (Megazyme method; ESC, Ystrad Mynach, UK). All ingredient and diet samples were analyzed for DM (AOAC Int., 2007; method 930.15) and ash (AOAC Int., 2007; method 942.05). Crude protein in corn, potato protein concentrate, and diets was calculated as N $\times$ 6.25 and N was analyzed by combustion (AOAC Int., 2007; method 990.03) using a LECO FP628 Nitrogen Analyzer (LECO Corp., Saint Joseph, MI). Acid hydrolyzed ether extract in diet samples was analyzed by acid hydrolysis using 3N HCl (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY) followed by fat extraction (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY). Diets were also analyzed for acid detergent fiber (ADF) and neutral detergent fiber (NDF) using Ankom Technology methods 12 and 13, respectively (Ankom 2000 Fiber Analyzer, Ankom Technology, Macedon, NY).

Calculations

The ATTD of DM, Ca, and P in experimental diets was calculated as previously outlined using Eq. [1] (Almeida and Stein, 2010):

$$\text{ATTD} = \frac{\text{int ake} - \text{output}}{\text{int ake}} \times 100, \quad [1]$$

where nutrient intake and output in feces are expressed as gram per day.

Basal endogenous loss of Ca, which was estimated as the fecal flow of Ca from sows fed the Ca-free diet, was expressed as gram per kilogram of DMI. The daily basal endogenous loss of Ca from sows fed the 2 diets containing Ca carbonate without or with microbial phytase was calculated by multiplying values for basal endogenous loss of Ca by the daily DMI of sows.

Values for STTD of Ca (%) were calculated from Eq. [2] (Almeida and Stein, 2010):

$$\text{STTD} = \frac{\text{intake} - (\text{output} - \text{daily basal endogenous loss})}{\text{intake}} \times 100, \quad [2]$$

where intake, output, and daily basal endogenous loss are in g/d.

Retention of Ca and P (%) in experimental diets was calculated using Eq. [3] (Fernández, 1995):

$$\text{Retention} = \frac{\text{intake} - (\text{fecal output} + \text{urinary output})}{\text{intake}} \times 100, \quad [3]$$

where intake and fecal and urinary outputs are expressed as gram per day.

Statistical Analysis

Normality of data was verified using the UNIVARIATE procedure (SAS Inst. Inc., Cary, NC). Outliers were identified as values that deviated

from first quartile and third quartile by more than 3 times the interquartile range within treatment. The sow was the experimental unit for all analyses. Data were analyzed as repeated measures using MIXED procedures of SAS. The statistical model included phytase, period of gestation, and the interaction between phytase and period of gestation as fixed effects and block and replicate within block as random effects. However, only a few interactions were observed, and the final model, therefore, included only the main effects of phytase and period of gestation. In the few instances where an interaction between phytase and period of gestation was observed, the SLICE option of SAS was used to analyze data. Least square means were separated using the PDIF option with Tukey's adjustment. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

RESULTS

The analyzed concentrations of Ca, total P, phytate, and phytase were in agreement with formulated values (Table 3). Sows remained healthy during the experimental period and very little feed refusals were observed with the exception that one sow refused to consume the assigned diet and had to be removed. One sow was removed from the experiment because of abortion. Four of the 36 sows that were allotted to the experiment were not pregnant and had to be removed. Therefore, there were 30

Table 3. Analyzed nutrient concentrations in experimental diets

Item, %	Calcium carbonate		Ca free		Conventional diet ¹
	0	500	0	500	
ME ²	3,318	3,318	3,391	3,391	3,299
DM	87.73	88.05	87.53	87.34	87.39
CP	12.7	12.6	13.0	13.1	12.5
Ash	4.58	5.27	3.34	3.13	5.42
NDF	6.39	5.65	6.42	7.13	9.96
ADF	1.33	1.25	1.41	2.16	4.44
Acid hydrolyzed ether extract	2.35	2.36	2.68	2.45	2.06
Ca	0.87	0.89	0.02	0.01	0.86
Total P	0.55	0.54	0.52	0.53	0.49
Phytase activity, phytase units/kg	<50	687	<50	587	<50
Phytate ³ , %	0.65	0.65	0.67	0.67	0.78
Phytate-bound P ⁴ , %	0.18	0.18	0.19	0.19	0.22
Phytate-bound P, % of total P	34.55	35.19	36.54	35.85	42.65
Nonphytate P ⁵ , %	0.36	0.35	0.33	0.34	0.28

¹The conventional gestation diet was fed to gestating sows before and between collection periods and this diet did not contain any exogenous phytase.

²Values for ME were calculated rather than analyzed (NRC, 2012).

³Phytate was calculated by dividing the analyzed phytate-bound P by 0.282 (Tran and Sauvant, 2004).

⁴Phytate values were calculated from analyzed phytate in the ingredients.

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

sows that completed all 3 periods of the experiment. The number of sows per treatment that completed the experiment was 8 for all dietary treatments except for the diet containing calcium carbonate without supplemental phytase ($n = 6$). A sow in early gestation fed the Ca-free diet with phytase was identified as an outlier in most response criteria, and this sow was, therefore, not included in the final analysis.

Basal Endogenous Loss of Ca and Balance of P by Sows Fed Ca-Free Diets

Interactions between period of gestation and phytase were not observed for sows fed the Ca-free diets (data not shown). Supplementation of microbial phytase reduced ($P < 0.01$) fecal Ca output, which resulted in a reduction ($P < 0.01$) in the basal endogenous loss of Ca (Table 4). Calcium retention was less negative ($P < 0.05$) if microbial phytase was used than if no phytase was included in the diet. The ATTD of DM and P retention were not affected by microbial phytase, but the ATTD of P tended to be greater ($P = 0.099$) if sows were fed the diet with supplemental phytase.

Feed intake increased ($P < 0.05$) from early- to mid- to late-gestation and the ATTD of DM was greater ($P < 0.05$) in sows in late gestation than in early gestation. Fecal Ca output was reduced ($P < 0.05$) from early- and midgestation to late-gestation. The

basal endogenous loss of Ca (mg/kg DMI) was greatest ($P < 0.05$) by sows in early gestation, followed by sows in mid- and late-gestation periods, respectively. There was no gestation period effect for total urinary excretion, but urine Ca output was greater ($P < 0.05$) in early- or midgestation periods than in late-gestation period, resulting in less Ca retention ($P < 0.05$) in early- or midgestation periods compared with the late-gestation period. The increased feed intake from early- to mid- to late-gestation periods resulted in the greatest ($P < 0.05$) P intake by sows in the late-gestation period, followed by sows in mid- and early-gestation periods, respectively, and fecal P output from sows in the late-gestation period was less ($P < 0.05$) than from sows in the midgestation period. Absorbed P and the ATTD of P were greater ($P < 0.05$) in the late-gestation period than in early- or midgestation periods. Phosphorus retention (g/d) increased ($P < 0.05$) from the early-gestation period to the late-gestation period, and P retention (% of intake) was greater ($P < 0.05$) in late- and midgestation periods compared with the early-gestation period.

Digestibility of Ca and Ca Balance by Sows Fed Ca carbonate-Containing Diets

Interactions between gestation period and phytase were not observed for sows fed the diet

Table 4. Basal endogenous loss (BEL) of Ca and balance of P by sows fed Ca-free diets without or with microbial phytase fed to sows in early-, mid-, and late-gestation periods

Item, %	Phytase units/kg		SEM	P	Period of gestation			SEM	P
	0	500			Early	Mid	Late		
Number of observations, <i>n</i>	24	23	—	—	15	16	16	—	—
Feed intake ¹ , kg/d	2.65	2.64	0.20	0.926	2.43 ^c	2.63 ^b	2.86 ^a	0.19	<0.001
Dry fecal excretion ¹ , kg/d	0.27	0.26	0.02	0.472	0.26	0.26	0.26	0.02	0.997
Urinary excretion ¹ , kg/d	7.32	6.97	1.31	0.856	7.54	6.19	7.70	1.28	0.552
ATTD of DM ² , %	89.13	89.34	0.33	0.641	88.49 ^b	89.21 ^{ab}	90.01 ^a	0.42	0.047
Calcium									
Fecal Ca output ¹ , g/d	2.64	1.95	0.35	0.003	2.66 ^a	2.39 ^a	1.83 ^b	0.35	<0.001
BEL of Ca ² , mg/kg DMI	1,141	858	85	0.002	1,225 ^a	1,036 ^b	737 ^c	87	<0.001
Urine Ca output ¹ , g/d	0.13	0.22	0.05	0.210	0.23 ^a	0.20 ^a	0.09 ^b	0.04	0.001
Ca retention ¹ , g/d	-2.52	-1.94	0.37	0.012	-2.68 ^b	-2.35 ^b	-1.66 ^a	0.37	<0.001
Phosphorus									
P intake ¹ , g/d	13.76	13.71	1.03	0.922	12.66 ^c	13.66 ^b	14.89 ^a	1.01	<0.001
Fecal P output ¹ , g/d	6.50	6.16	0.68	0.088	6.38 ^{ab}	6.81 ^a	5.80 ^b	0.71	0.040
Absorbed P ¹ , g/d	7.29	7.63	0.38	0.395	6.36 ^b	6.89 ^b	9.13 ^a	0.40	<0.001
ATTD of P, %	52.37	54.59	1.62	0.099	50.03 ^b	49.87 ^b	60.54 ^a	2.05	<0.001
Urine P output ¹ , g/d	6.32	6.56	0.76	0.589	6.85	5.72	6.75	0.79	0.086
P retention ¹ , g/d	0.91	0.97	0.46	0.852	-0.58 ^b	1.10 ^{ab}	2.31 ^a	0.60	0.001
P retention, % of intake	6.46	6.93	3.71	0.829	-3.86 ^b	7.95 ^a	15.99 ^a	4.49	0.001

¹All values for intake, output, or retention are the average values for the 4-d collection period.

²ATTD = apparent total tract digestibility; BEL = basal endogenous loss.

containing Ca carbonate (data not shown). Supplementation of microbial phytase did not affect the ATTD of DM, the ATTD of Ca, the STTD of Ca, or Ca retention (Table 5). However, supplementation of microbial phytase reduced ($P < 0.001$) daily basal endogenous loss of Ca (mg/d).

Feed intake was greater ($P < 0.05$) in the late-gestation period, followed by mid- and early-gestation periods, respectively, and fecal excretion from sows in the late-gestation period was greater ($P < 0.05$) compared with sows in the early-gestation period. Nevertheless, the ATTD of DM was not affected by gestation period. Calcium intake was greatest ($P < 0.05$) in the late-gestation period, followed by mid- and early-gestation periods, respectively, but fecal Ca output was greater ($P < 0.05$) in the mid-gestation period than in early- or late-gestation periods. Absorbed Ca was greater ($P < 0.05$) in sows during the late-gestation period compared with earlier gestation periods. The ATTD of Ca was least ($P < 0.05$) in the mid-gestation period, followed by early- and late-gestation periods, respectively, and the STTD of Ca for sows in the mid-gestation period was also lower ($P < 0.05$) than for sows in early- or late-gestation periods. Urine Ca output was greater ($P < 0.05$) in the early-gestation period compared with the later-gestation periods, and Ca retention

(g/d and % of intake) was greater ($P < 0.05$) in the late-gestation period than in early-gestation periods.

Phosphorus Balance

Interactions between gestation period and phytase were not observed for P balance by sows fed the diets containing calcium carbonate with the exception that urine P output was reduced from sows in late gestation compared with early- and mid-gestation periods if no phytase was used, but that was not the case if phytase was included in the diet (interaction, $P = 0.005$; Table 6). Supplementation of microbial phytase did not affect the ATTD of P or P retention by gestating sows.

Phosphorus intake was greatest ($P < 0.05$) in the late-gestation period, followed by mid- and early-gestation periods, but fecal P output was greater ($P < 0.05$) in the mid-gestation period than in early- or late-gestation periods. Absorbed P was greater ($P < 0.05$) in the late-gestation period compared with earlier periods and the ATTD of P was least ($P < 0.05$) in the mid-gestation period, followed by early- and late-gestation periods, respectively. Phosphorus retention was also greater ($P < 0.05$) in the late-gestation period than in the earlier gestation periods.

Table 5. Apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) of Ca and retention of Ca in diets containing calcium carbonate fed to sows in early-, mid-, and late-gestation periods

Item, %	Phytase units/kg				Period of gestation				
	0	500	SEM	<i>P</i>	Early	Mid	Late	SEM	<i>P</i>
Number of observations, <i>n</i>	18	24	—	—	14	14	14	—	—
Feed intake ¹ , kg/d	2.73	2.65	0.19	0.242	2.46 ^c	2.69 ^b	2.92 ^a	0.19	<0.001
Dry fecal excretion ¹ , kg/d	0.27	0.25	0.02	0.312	0.24 ^b	0.27 ^{ab}	0.28 ^a	0.02	0.039
Urinary excretion ¹ , kg/d	8.73	5.74	1.50	0.127	9.40	6.33	5.98	1.47	0.058
ATTD of DM, %	88.94	89.38	0.53	0.571	89.18	88.98	89.33	0.48	0.794
Ca intake ¹ , g/d	24.12	23.37	1.65	0.241	21.74 ^c	23.70 ^b	25.79 ^a	1.63	<0.001
Fecal Ca output ¹ , g/d	16.91	16.20	1.88	0.480	15.19 ^b	19.62 ^a	14.86 ^b	1.94	0.001
Absorbed Ca ¹ , g/d	7.16	7.08	0.65	0.933	6.47 ^b	4.01 ^b	10.87 ^a	0.78	<0.001
ATTD of Ca, %	28.70	29.84	3.65	0.795	29.44 ^b	17.35 ^c	41.02 ^a	3.80	<0.001
BEL of Ca ² , mg/d	2,723	1,990	173	<0.001	2,719 ^a	2,463 ^b	1,888 ^c	171	<0.001
STTD of Ca ³ , %	40.08	38.57	3.65	0.733	41.83 ^a	27.72 ^b	48.43 ^a	3.80	<0.001
Urine Ca output ¹ , g/d	0.38	0.33	0.09	0.444	0.55 ^a	0.29 ^b	0.22 ^b	0.09	<0.001
Ca retention ¹ , g/d	6.74	6.71	0.66	0.982	5.89 ^b	3.68 ^b	10.60 ^a	0.78	<0.001
Ca retention, % of intake	27.42	28.72	3.81	0.755	27.33 ^b	16.43 ^c	40.46 ^a	4.00	<0.001

^{a-c}Within a row, means without a common superscript differ ($P < 0.05$).

¹All values for intake, output, or retention are the average values for the 4-d collection period.

²BEL = basal endogenous loss; the daily BEL of Ca (mg/d) was calculated by multiplying the BEL of Ca (mg/kg DMI) by the daily DMI (kg/d) of each experimental diet.

³The STTD of Ca in each diet within each period of gestation was calculated using the basal endogenous Ca loss that was specific for each period; basal endogenous losses of Ca from sows fed the Ca-free diet without microbial phytase = 1,348, 1,196, and 877 mg/kg DMI for early-, mid-, and late-gestation periods, respectively; basal endogenous losses of Ca from sows fed the Ca-free diet with microbial phytase = 1,130, 876, and 596 mg/kg DMI for early-, mid-, and late-gestation periods, respectively.

Table 6. Apparent total tract digestibility (ATTD) of P and retention of P in diets containing Ca carbonate fed to sows in early-, mid-, and late-gestation periods

Item, %	Phytase units/kg			<i>P</i>	Period of gestation			SEM	<i>P</i>
	0	500	SEM		Early	Mid	Late		
Number of observations, <i>n</i>	18	24	—	—	14	14	14	—	—
P intake ¹ , g/d	14.87	14.41	1.02	0.240	13.40 ^c	14.61 ^b	15.90 ^a	1.01	<0.001
Fecal P output ¹ , g/d	11.26	10.21	1.27	0.138	9.88 ^b	12.52 ^a	9.81 ^b	1.30	0.001
Absorbed P ¹ , g/d	3.54	4.06	0.49	0.463	3.42 ^b	1.99 ^b	5.99 ^a	0.51	<0.001
ATTD of P, %	22.65	27.36	3.41	0.349	25.06 ^b	13.66 ^c	36.29 ^a	3.35	<0.001
Urine P output without phytase ^{1,2} , g/d	—	—	—	—	1.43 ^a	1.12 ^a	0.39 ^b	0.19	<0.001
Urine P output with phytase ^{1,2} , g/d	—	—	—	—	1.28 ^a	1.03 ^a	0.91 ^{ab}	0.17	0.037
P retention ¹ , g/d	2.54	2.99	0.51	0.543	2.08 ^b	0.92 ^b	5.30 ^a	0.53	<0.001
P retention, % of intake	15.94	20.08	3.47	0.415	15.41 ^b	6.54 ^b	32.08 ^a	3.40	<0.001

^{a-c}Within a row, means without a common superscript differ ($P < 0.05$).

¹All values for intake, output, or retention are the average values for the 4-d collection period.

²There was an interaction between supplemental phytase and gestation period ($P = 0.005$). Therefore, values for urine P output were partitioned using the SLICE option of SAS (SAS Inst. Inc., Cary, NC). Each least squares mean represents 6 observations for sows fed the diet without phytase; each least squares mean represents 8 observations for sows fed the diet with phytase.

DISCUSSION

Concentrations of Ca and P in the corn, potato protein concentrate, Ca carbonate, and monosodium phosphate that were used in this experiment were in agreement with reported values (NRC, 2012; González-Vega et al., 2015a; Merriman and Stein, 2016). Feed intake and Ca and P intake by sows were greater in late-gestation than in the earlier periods because BW of sows increased from early- to mid- to late-gestation and feed intake was calculated based on the initial BW of sows at each period. This approach was used to maintain a constant feed intake relative to the metabolic BW of sows and thus avoid possible confounding effects of supplying feed at different quantities relative to the maintenance requirement of sows.

Effects of Phytase on Basal Endogenous Loss of Ca and ATTD of P

The basal endogenous loss of Ca in sows during midgestation if no phytase was used (1,196 mg/kg DMI) was close to the value (1,580 mg/kg DMI) reported by Lee et al. (2018). However, the basal endogenous loss of Ca from growing pigs fed a corn-based Ca-free diet was between 329 (Merriman and Stein, 2016) and 550 mg/kg DMI (González-Vega et al., 2015b; Merriman, 2016; Blavi et al., 2017), and the present result along with the data by Lee et al. (2018) confirms that gestating sows have much greater basal endogenous loss of Ca than growing pigs if measured as mg per kg DMI.

The efficacy of phytase depends on the feed ingredients used in diets and the P adequacy of the

diets because Ca and P digestibility in some ingredients, including dicalcium phosphate and monocalcium phosphate, are not affected by phytase (González-Vega et al., 2015a). The observation that use of phytase decreases the basal endogenous loss of Ca from gestating sows indicates that phytate in corn may bind endogenous Ca to form a Ca-phytate complex that is indigestible. However, the present data indicate that when phytase is added to the diet, there is less phytate bound to Ca with a subsequent increased absorption and reduced excretion of endogenous Ca. The total endogenous loss of Ca from growing pigs was not affected by phytase if diets were based on canola meal (González-Vega et al., 2013). Because sows have greater endogenous loss than growing pigs, more endogenous Ca is available for binding to the phytate molecule in sows, which may be the reason phytase reduces the basal endogenous loss of Ca from sows, but not from growing pigs.

The observation that values for ATTD of P in sows fed Ca-free diets were greater compared with values from sows fed diets containing corn and Ca carbonate illustrates that there is an interaction between Ca and P with a reduced ATTD of P if Ca in diets increases as has been previously demonstrated in growing pigs (Stein et al., 2011). The fact that sows absorbed P even if there was no Ca in the diet also illustrates that there is no downregulation of P absorption if bone tissue cannot be synthesized due to a lack of Ca, which has also been reported for growing pigs (Stein et al., 2006). Absorbed P can be retained in the body only if both Ca and P are available at the same time (Crenshaw, 2001), which is the reason most of the absorbed P from sows fed the

Ca-free diets was excreted in the urine. Likewise, the reason retention of P was greater if sows were fed diets containing Ca carbonate compared with sows fed Ca-free diets, despite a lower absorption of P, is that most absorbed P could be used for bone tissue synthesis if diets containing Ca carbonate were fed.

Effects of Phytase on Digestibility and Retention of Ca and P

Chelation of both endogenous and dietary Ca^{++} in the intestine of pigs, which is a result of the negative charge of phytate (Nelson and Kirby, 1987; Selle et al., 2009), is the reason phytase increases both Ca and P digestibility in feed ingredients and diets fed to growing pigs (Almeida et al., 2013; Rodríguez et al., 2013; González-Vega et al., 2015a). Supplementation with exogenous phytase of diets fed to sows in midgestation resulted in increased ATTD of Ca and P (Jongbloed et al., 2004), an increase in ATTD of P only (Nyachoti et al., 2006; Jongbloed et al., 2013; Jang et al., 2014), or no effect on ATTD of Ca or P (Kemme et al., 1997; Liesegang et al., 2005). In the case of late-gestation, phytase increased ATTD of P (Kemme et al., 1997; Jongbloed et al., 2004; Nyachoti et al., 2006) or ATTD of Ca and P (Hanczakowska et al., 2009; Jongbloed et al., 2013). In this experiment, phytase had limited effects on the ATTD and STTD of Ca and on the ATTD of P in the P-sufficient-corn-based diet. It is not clear why different responses to phytase fed to gestating sows have been observed, but it is possible that phytase efficacy is reduced in P-sufficient diets (Rodehutscord, 2016) and because the ATTD of Ca and P is much lower than in growing pigs fed diets without phytase (Lee et al., 2018), there likely are factors other than formation of Ca-P-phytate complexes that limit absorption of Ca and P. Digestibility of Ca and P by growing pigs decreases as the concentration of phytate increases (Almaguer et al., 2014; Lee et al., 2018), but phytate levels did not affect the ATTD of Ca and P in sows in midgestation (Lee et al., 2018), which further demonstrates that differences between growing pigs and gestating sows exist. Thus, more research to elucidate factors that affect absorption of Ca and P in gestating sows is warranted.

Effect of Gestation Period on Digestibility and Retention of Ca and P and Basal Endogenous Loss of Ca

To our knowledge, no data for the STTD of Ca in calcium carbonate by sows in different periods

of gestation have been published. However, the observation that sows in midgestation had reduced ATTD of Ca and P compared with sows in late gestation is in agreement with previous data (Kemme et al., 1997; Jongbloed et al., 2004; Liesegang et al., 2005; Nyachoti et al., 2006; Jongbloed et al., 2013). Providing Ca and P above requirements may reduce the digestibility and retention of Ca and P in gestating sows (Kemme et al., 1997; Nyachoti et al., 2006; Bikker and Blok, 2017). In this experiment, the same diets were fed to sows in all periods of gestation. The analyzed Ca and P were approximately 0.88% and 0.55%, respectively, which is close to the requirements in late gestation, but greater than the requirement estimates for sows in early gestation or midgestation (NRC, 2012). The observation that retained Ca and P, expressed in g/d, were not different between early gestation and midgestation indicates that the requirements for Ca and P are similar between these periods. However, the fact that ATTD of Ca and P increased in late gestation indicates that sows in late gestation require more Ca and P than sows in earlier periods. Indeed, very little Ca and P is needed for fetus development in early gestation to midgestation compared with late gestation (Bikker and Blok, 2017). The data for Ca and P retention that were calculated in this experiment also indicate that more Ca and P are needed in late gestation compared with previous periods. It is possible that differences in plasma estrogen during gestation influence 1,25-dihydroxyvitamin D_3 metabolism, which affects the digestibility of Ca and P (Heaney, 1990; Ross et al., 2011; Harmon et al., 2016). Estrogen increases during late gestation (Kensinger et al., 1982), which may contribute to the increased ATTD of Ca and P by sows in late gestation compared with in early gestation or midgestation.

CONCLUSION

Apparent total tract digestibility, basal endogenous loss, STTD, and retention of Ca in gestating sows are influenced by the trimester of gestation. To accurately predict Ca and P absorption in gestating sows, it therefore appears that it is necessary to assume different digestibility values for Ca in calcium carbonate and P in corn and monosodium phosphate in the late-gestation period compared with early- or midgestation periods. Use of microbial phytase decreases the basal endogenous loss of Ca, but the response to microbial phytase on STTD of Ca and ATTD of P in Ca and P-adequate-corn-based diets fed to gestating sows is less consistent. This may be

due to an oversupply of both Ca and P by phytase in nutrient adequate diets, and there is, therefore, a need to further clarify the Ca and P requirements of sows during the different trimesters of gestation.

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