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SHORT COMMUNICATION

Effects of dietary levels of calcium, phosphorus, and 1-alpha-hydroxycholecalciferol on digestibility, retention of calcium and phosphorus, and concentration of metabolizable energy in diets fed to sows in late-gestation

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Abstract: The apparent total tract digestibility and retention of calcium (Ca) and phosphorus (P) and concentrations of digestible energy and metabolizable energy in diets fed to late-gestating sows were not affected by Ca and P levels, but they were increased by dietary supplementation with 1-alpha-hydroxycholecalciferol. There was no interaction between dietary Ca and P and supplementation with 1-alpha-hydroxycholecalciferol.

Key words: calcium, digestibility, energy, phosphorus, sows, vitamin D supplementation.

Résumé : Les niveaux de calcium (Ca) et de phosphore (P) n'ont pas eu d'effet sur la digestibilité apparente du tractus intestinal total et la rétention du Ca et du P, ni des concentrations d'énergie digérable et d'énergie métabolisable dans les diètes données aux truies en gestation tardive, mais étaient augmentés par supplémentation alimentaire de 1-alpha-hydroxycholécalciférol. Il n'y avait pas d'interaction entre le Ca et P alimentaires et la supplémentation avec 1-alpha-hydroxycholécalciférol. [Traduit par la Rédaction]

Mots-clés : calcium, digestibilité, énergie, phosphore, truies, suppléments de vitamine D.

Absorption of calcium (Ca) and phosphorus (P) from the small intestine and renal reabsorption of Ca and P is regulated by parathyroid hormone, calcitonin, and calcitriol (i.e., 1,25-dihydroxycholecalciferol or 1,25-dihydroxy-vitamin D_3), which is the active form of vitamin D (Crenshaw 2001). Vitamin D in commercial vitamin premixes is provided mostly by one of the vitamin D_3 forms (i.e., cholecalciferol). To be utilized in the body, cholecalciferol needs to be hydroxylated in two steps. In the first step, vitamin D_3 is hydroxylated at the 25-position to yield 25-hydroxycholecalciferol (25-OH-D_3). In the second step, 25-OH-D_3 is hydroxylated at the 1-position to yield 1,25-dihydroxycholecalciferol. One-alpha-hydroxycholecalciferol (1-α-OH-D_3) is a vitamin D_3 analog that does not require the second hydroxylation step for vitamin D_3 to be active. It is possible that supplementation of diets with 1-α-OH-D_3 increases the conversion efficiency to 1,25-dihydroxycholecalciferol compared with using traditional cholecalciferol, which may increase Ca and P balance by increasing intestinal absorption and renal reabsorption, but this hypothesis has not been experimentally verified.

The requirement for Ca and P by gestating sows increases in late gestation compared with early- and mid-gestation because of increased needs by the developing fetuses (Bikker and Blok 2017). It is possible that 1-α-OH-D_3 increases absorption and retention of Ca and P, but this may work differently with different levels of Ca and P in late gestating sows. However, data to demonstrate this have not been reported. Therefore, the objective of this experiment was to test the hypothesis that supplementation of 1-α-OH-D_3 to diets for gestating sows containing Ca and P at or below the requirement will increase apparent total tract digestibility (ATTD) and retention of Ca and P as well as the ATTD of gross energy. The second hypothesis was that there is an interaction between dietary Ca and P concentrations and supplementation with 1-α-OH-D_3 in diets fed to gestating sows.

The protocol for the animal work was reviewed and approved by the Institutional Animal Care and Use

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Committee at the University of Illinois. Animal procedures followed "Guide and Care of Laboratory Animals".

Thirty-six gestating sows were allotted to one of four diets in a randomized complete block design with three blocks of 12 sows. Within each block, three sows were fed each diet for a total of nine replicate sows per diet. Sows were fed experimental diets from days 91 to 105 of gestation and were housed individually in metabolism crates during the experimental period. A feeder, a nipple drinker, and fully slatted T-bar floors were installed in the metabolism crates. Feces was collected quantitatively from a screen floor, and a urine pan was installed under each metabolism crate. The initial 5 d of each period in the metabolism crates were considered the adaptation period to the diets followed by 4 d of fecal collection using the marker to marker procedure. 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. To collect urine, buckets containing 50 mL of 3 N HCl were placed under the urine pans from the morning of day 6 to day 10. The weight of the collected urine was recorded daily, and 10% of the urine was stored at −20 °C.

Diets were formulated using a 2 × 2 factorial arrangement (Table 1) with two levels of Ca and P [i.e., 100% or 75% of the requirement; National Research Council (NRC) 2012] without or with supplemental 1-α-OH-D_3 (Alpha D_3, Premex Inc., Durham, NC, USA). Calcium to P ratio in all diets was 1.3:1.0. The 1-α-OH-D_3 premix (dietary level: 12.5 mg·kg^{-1} diet) contained 410 mg·kg^{-1} of 1-α-OH-D_3 and the calculated concentration of 1-α-OH-D_3 in the complete diets was, therefore, 5.12 μg·kg^{-1}, which is the commercially recommended dose. Analyzed 1-α-OH-D_3 in the two diets containing the premix were 4.96 and 3.46 μg·kg^{-1}, respectively. The calculated level of vitamin D_3 in all diets was 1660 IU·kg^{-1}. All vitamins and minerals except Ca and P were included in all diets to meet or exceed nutrient requirements (NRC 2012).

Daily feed allotments were provided in one daily meal that was fed at 0700 throughout the experiment. The daily feed allowance was calculated as 1.5× the maintenance energy requirement for late gestating sows based on the initial body weight of sows [i.e., 100 kcal metabolizable energy (ME)·kg^{-1} body weight$^{0.75}$; NRC 2012]. Water was available at all times.

Urine subsamples were collected at the end of the experiment after mixing all collected urine from each animal. Liquid urine samples were used to analyze Ca and P, and lyophilized urine samples were used to analyze gross energy. Collected fecal samples were stored at −20 °C as soon as collected, but at the conclusion of the experiment, fecal samples were dried at 55 °C in a forced-air oven and ground through a 1 mm screen using a Wiley mill (Model 4; Thomas Scientific, Swedesboro, NJ, USA). Subsamples of the ground feces were collected for analyses.

The concentration of 1-α-OH-D_3 in experimental diets was analyzed using liquid chromatography with tandem mass spectrometry (Aronov et al. 2008). Calcium and P in diet, fecal, and urine samples were analyzed using an inductively coupled plasma spectroscopy [American Association of Official Analytical Chemists (AOAC) Int. 2007; method 985.01 A, B, and C]. Samples were prepared by wet ashing [AOAC Int. 2007; method 975.03 B(b)]. Diets were analyzed for phytic acid (Ellis et al. 1977). All diets and fecal samples were analyzed for dry matter (AOAC Int. 2007; method 930.15), and ash was analyzed in all diets (AOAC Int. 2007; method 942.05). Gross energy in diet, fecal, and urine samples was also analyzed (Model 6400, Parr Instruments, Moline, IL, USA). Crude protein was calculated by multiplying analyzed N by 6.25, and N was analyzed by combustion (AOAC Int. 2007; method 990.03) using a LECO FP628 apparatus (LECO Corp., Saint Joseph, MI, USA).

The ATTD of dry matter, Ca, P, and gross energy was calculated using the analyzed dry matter, Ca, P, and gross energy in diet and fecal sample as described below:

$$\text{ATTD} = \frac{\text{intake} - \text{output}}{\text{intake}} \times 100,$$

where ATTD is in %, and intake and output of dry matter, Ca, P, and gross energy are in g·d^{-1} or kcal·d^{-1}. Retention of Ca and P was calculated by subtracting the analyzed Ca and P in feces and urine from Ca and P intake. Concentrations of digestible energy (DE) and ME were calculated using analyzed gross energy in diet, feces, and urine samples.

Normality of data and homogeneity were verified using the UNIVARIATE and MIXED procedures in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA), and outliers were identified using internally studentized residuals. Sow was the experimental unit for all analyses. Data were analyzed using MIXED procedures of SAS (SAS Institute Inc., Cary, NC, USA). The statistical model included levels of Ca and P, supplemental 1-α-OH-D_3, and the interaction between dietary Ca and P and supplemental 1-α-OH-D_3 as fixed effects and block and parity as random effects. Least squares means were calculated using the LSMeans statement in SAS, and means were separated using the PDIFF statement with Tukey's adjustment if the interaction was significant. Statistical significance was considered at $P < 0.05$, and a tendency was considered if the P value was between 0.05 and 0.10.

Because the analyzed Ca and P in diets varied slightly from the calculated Ca and P, which is likely due to analytical discrepancies, calculated Ca and P were used for all calculations. No interactions between dietary Ca and P levels and supplemental 1-α-OH-D_3 were observed for feed intake, fecal and urine excretion, or Ca balance (Table 2). Feed intake, fecal excretion, urine excretion, and the ATTD of dry matter by sows were not affected by the levels of Ca and P in the diets. Calcium intake and fecal Ca output were

Table 1. Composition of diets containing different levels of calcium and phosphorus with or without supplemental 1-alpha-hydroxycholecalciferol (1-α-OH-D_3) (as-is basis).

	Ca and P levels[a]	Normal		Low	
Item	1-α-OH-D_3, mg·kg^{-1} diet	0	12.5	0	12.5
Ingredients (%)					
Corn		72.91	69.91	73.73	70.73
Soybean meal		14.00	14.00	14.00	14.00
Sugar beet pulp		8.00	8.00	8.00	8.00
Corn–vitamin D_3 premix[b]		—	3.00	—	3.00
Calcium carbonate		1.01	1.01	0.83	0.83
Monocalcium phosphate		1.18	1.18	0.54	0.54
Soybean oil		2.00	2.00	2.00	2.00
Sodium chloride		0.40	0.40	0.40	0.40
Vitamin–mineral premix[c]		0.50	0.50	0.50	0.50
Analyzed nutrient composition					
Gross energy (kcal·kg^{-1})		3831	3848	3827	3861
Dry matter (%)		88.71	89.11	88.82	88.20
Ash (%)		4.82	4.48	3.97	3.99
Crude protein (%)		12.64	12.10	12.10	12.41
Ca (%)		0.77	0.71	0.59	0.48
P (%)		0.53	0.49	0.38	0.37
Phytate (%)		0.74	0.69	0.69	0.65
Phytate-P[d] (%)		0.21	0.19	0.19	0.18
Non phytate-P (%)		0.32	0.30	0.19	0.19
Ca to P ratio		1.46:1	1.43:1	1.56:1	1.31:1
1-α-OH-D_3 (μg·kg^{-1})		<0.64	4.96	<0.64	3.46

[a]Normal level of Ca and P = 100% of the requirement for late gestation sows; low level of Ca and P = 75% of the requirement for late gestation sows (NRC 2012).

[b]The corn-1-α-OH-D_3 premix provided 12.5 mg Alpha D_3 (concentration: 410 mg·kg^{-1} of 1-α-OH-D_3) per kilogram of complete diet; 1 kg of the premix was prepared by mixing 417 mg Alpha D_3 and 999.583 g ground corn.

[c]The vitamin–micromineral premix provided the following quantities of vitamins and micro minerals per kilogram of complete diet: vitamin A as retinyl acetate, 10 622 IU; vitamin D_3 as cholecalciferol, 1660 IU; vitamin E as selenium yeast, 66 IU; vitamin K as menadione nicotinamide bisulfate, 1.40 mg; thiamin as thiamine mononitrate, 1.08 mg; riboflavin, 6.49 mg; pyridoxine as pyridoxine hydrochloride, 0.98 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.2 mg; niacin, 43.4 mg; folic acid, 1.56 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 123 mg as iron sulfate; I, 1.24 mg as ethylenediamine dihydriodide; Mn, 59.4 mg as manganese hydroxychloride; Se, 0.27 mg as sodium selenite and selenium yeast; and Zn, 124.7 mg as zinc hydroxychloride.

[d]Phytate-P was calculated by multiplying the analyzed phytate by 0.282.

greater ($P < 0.001$) in sows fed normal level of Ca and P compared with sows fed diets containing low Ca and P, but absorbed Ca and the ATTD of Ca were not affected by dietary Ca and P. Urine Ca output was less ($P < 0.05$) if sows were fed diets containing more Ca and P compared with sows fed diets containing lower Ca and P, but dietary Ca and P levels did not affect Ca retention by sows.

Although feed intake was not different among treatments, fecal excretion was less ($P < 0.05$) from sows fed diets supplemented with 1-α-OH-D_3 compared with sows fed diets with no 1-α-OH-D_3, which resulted in greater ATTD of dry matter in sows fed diets supplemented with 1-α-OH-D_3 compared with sow fed no supplemental 1-α-OH-D_3. Fecal Ca output was less ($P < 0.01$), resulting in greater ($P < 0.01$) absorbed Ca and ATTD of Ca in sows fed diets supplemented with 1-α-OH-D_3 compared with sows fed no supplemental 1-α-OH-D_3. Supplementation of 1-α-OH-D_3 increased ($P < 0.01$) urine Ca outputs from sows, but Ca retention was greater ($P < 0.01$) in sows fed diets with supplemental 1-α-OH-D_3 compared with sows fed no supplemental 1-α-OH-D_3 because of the increase in absorbed Ca.

Table 2. Calcium balance for sows in late gestation fed experimental diets containing different levels of calcium and phosphorus with or without supplemental 1-alpha-hydroxycholecalciferol (1-α-OH-D_3).[a]

Item Ca and P levels[b]	Normal		Low			*P* value		
1-α-OH-D_3, mg·kg^{-1} diet	0	12.5	0	12.5	SEM	Ca and P levels	Vit D_3	Interaction
Feed intake (kg·d^{-1})	2.98	2.93	2.99	2.92	0.05	0.997	0.223	0.901
Fecal excretion (kg·d^{-1})	0.40	0.33	0.36	0.31	0.06	0.096	0.001	0.441
Urine excretion (kg·d^{-1})	10.51	11.26	12.10	13.91	3.40	0.327	0.554	0.804
ATTD of dry matter (%)	85.04	87.72	86.80	88.31	2.17	0.076	0.003	0.365
Ca balance								
Ca intake (g·d^{-1})	22.00	21.61	15.90	15.55	0.28	<0.001	0.194	0.929
Fecal Ca output (g·d^{-1})	19.44	15.00	13.04	10.89	2.25	<0.001	0.001	0.184
Absorbed Ca (g·d^{-1})	2.55	6.63	2.82	4.66	2.27	0.331	0.002	0.206
ATTD of Ca (%)	11.31	30.25	18.21	30.49	12.17	0.407	0.001	0.439
Urine Ca output (g·d^{-1})	0.32	0.59	0.44	0.99	0.13	0.020	0.001	0.201
Ca retention (g·d^{-1})	2.23	6.02	2.37	3.67	2.24	0.212	0.007	0.161
Ca retention (% of intake)	9.80	27.48	15.41	24.07	11.97	0.796	0.005	0.292
P balance								
P intake (g·d^{-1})	15.18	14.90	11.07	10.83	0.19	<0.001	0.192	0.923
Fecal P output (g·d^{-1})	12.77a	9.71b	8.52bc	7.67c	1.44	<0.001	0.001	0.049
Absorbed P (g·d^{-1})	2.41	5.19	2.53	3.16	1.47	0.103	0.005	0.066
ATTD of P (%)	15.57	34.70	23.38	29.63	11.16	0.731	0.003	0.114
Urine P output (g·d^{-1})	0.48b	0.99a	0.16c	0.12c	0.09	<0.001	0.001	<0.001
P retention (g·d^{-1})	1.93	4.20	2.37	3.04	1.52	0.541	0.016	0.176
P retention (% of intake)	12.44	28.03	21.93	28.51	11.52	0.223	0.010	0.269
Energy concentrations								
Gross energy intake (Mkcal·d^{-1})	11.4	11.3	11.4	11.3	0.2	0.923	0.514	0.832
Fecal gross energy output (kcal·d^{-1})	1728	1420	1654	1381	264	0.443	0.001	0.818
Urine gross energy output (kcal·d^{-1})	222	263	232	293	40	0.560	0.136	0.778
ATTD of gross energy (%)	84.6	87.3	85.6	87.5	2.3	0.291	0.001	0.654
DE (kcal·kg^{-1})	3240	3361	3275	3388	90	0.227	<0.001	0.871
ME (kcal·kg^{-1})	3163	3268	3197	3291	98	0.357	0.003	0.861

Note: Within a row, means without a common lowercase letter differ ($P < 0.05$). ATTD, apparent total tract digestibility; DE, digestible energy; ME, metabolizable energy; SEM, standard error of the mean.

[a]Each least squares mean for each treatment represents nine observations, respectively, except for the two diets containing normal or low Ca and P levels with no supplemental 1-α-OH-D_3 ($n = 8$).

[b]Normal level of Ca and P = 100% of the requirement for late gestation sows; low level of Ca and P = 75% of the requirement for late gestation sows (NRC 2012).

No interactions between Ca and P levels and use of supplemental 1-α-OH-D_3 were observed for P balance except for fecal and urine P outputs. Supplementation of 1-α-OH-D_3decreased ($P < 0.05$) fecal and urine P output from sows fed diet containing normal level of dietary Ca and P, but there was no difference between the two diets with the low level of dietary Ca and P (interaction; $P < 0.05$).

Phosphorus intake and fecal P output were greater ($P < 0.001$) in sows fed normal level of Ca and P compared with sows fed diets containing low Ca and P, but absorbed P and the ATTD of P were not affected by dietary Ca and P. Likewise, dietary Ca and P levels did not affect P retention by sows. Regardless of dietary Ca and P levels, absorbed P, ATTD of P, and P retention were greater ($P < 0.05$) in sows fed diets supplemented with 1-α-OH-D_3 compared with sows fed no supplemental 1-α-OH-D_3.

No interactions between main effects were observed for gross energy intake, ATTD of gross energy, or concentrations of DE, and ME in diets. There was no effect of Ca and P level on those variables, but the ATTD of gross energy and concentrations of DE and ME increased ($P < 0.01$) by supplementing 1-α-OH-D_3 to the diets.

Values for the ATTD and retention of Ca and P were in agreement with previous values for gestating sows (Nyachoti et al. 2006; Lee et al. 2020). Values for the ATTD of gross energy and concentrations of DE and ME in diets were greater than values calculated from growing pig data (NRC 2012), which is likely due to greater digestibility of energy in gestating sows compared with growing pigs (Le Goff and Noblet 2001).

To our knowledge, no data demonstrating the effects of 1-α-OH-D_3 supplementation on digestibility and retention

of Ca and P in sows have been published. However, results from this experiment demonstrating that the ATTD and retention of Ca and P are increased by 1-α-OH-D_3 supplementation indicate that it may be beneficial to supply vitamin D in a form that does not need the second hydroxylation. Absorption of Ca by active transport is regulated by calcitriol, which is the active form of vitamin D (Crenshaw 2001). The increased ATTD of Ca and P indicates that 1-α-OH-D_3 upregulates absorption of Ca and P from the intestinal tract, whereas the increased excretion of Ca in urine indicates that recovery of Ca in the kidneys was not increased by 1-α-OH-D_3. We are not aware of any side effects of feeding 1-α-OH-D_3 in pigs.

The increases in ATTD of Ca and P were also a result of reduced fecal dry matter excretion, which resulted in an increase in the ATTD of dry matter and gross energy, and therefore, the increases in concentrations of DE and ME when 1-α-OH-D_3 was added to the diets. The ATTD of Ca and P are not affected by increasing Ca and P intake by sows (Lee et al. 2018), and results from this experiment are in agreement with previous data. This is likely a result of the fixed Ca:P ratio that was used in formulating the diets because an increased Ca:P ratio reduces ATTD of P (Lee et al. 2020). It is, however, not clear if addition of 1-α-OH-D_3 will have a different influence on ATTD of Ca and P if the Ca to P ratio is changed.

In conclusion, the ATTD and retention of Ca and P and concentrations of DE and ME in diets fed to sows in late gestation were not affected by dietary Ca and P, but supplementation of 1-α-OH-D_3 increased the ATTD of Ca and P by almost 100%. The increased ATTD of dry matter and gross energy resulted in increased DE and ME of approximately 100 kcal·kg^{-1} in diets containing 1-α-OH-D_3 compared with diets not containing 1-α-OH-D_3.

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References

Association of Official Analytical Chemists (AOAC) Int. 2007. Official methods of analysis of AOAC Int. 18th ed. rev. 2nd ed. AOAC Int., Gaithersburg, MD, USA.

Aronov, P.A., Hall, L.M., Dettmer, K., Stephensen, C.B., and Hammock, B.D. 2008. Metabolic profiling of major vitamin D metabolites using Diels-Alder derivatization and ultra-performance liquid chromatography-tandem mass spectrometry. Anal. Bioanal. Chem. **391**: 1917–1930. doi:10.1007/s00216-008-2095-8. PMID:18437365.

Bikker, P., and Blok, M. 2017. Phosphorus and calcium requirements of growing pigs and sows. Wageningen Livestock Research. CVB Documentation Report 59.

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

Ellis, R., Morris, E.R., and Philpot, C. 1977. Quantitative determination of phytate in presence of high inorganic-phosphate. Anal. Biochem. **77**: 536–539. doi:10.1016/0003-2697(77)90269-X. PMID:842837.

Le Goff, G., and Noblet, J. 2001. Comparative total tract digestibility of dietary energy and nutrients in growing pigs and adult sows. J. Anim. Sci. **79**: 2418–2427. doi:10.2527/2001.7992418x. PMID:11583429.

Lee, S.A., Casas, G.A., and Stein, H.H. 2018. The level of feed intake does not influence digestibility of calcium and phosphorus in diets fed to gestating sows, but gestating sows have reduced digestibility of calcium and phosphorus compared with growing gilts. Can. J. Anim. Sci. **98**: 589–592. doi:10.1139/cjas-2017-0144.

Lee, S.A., Lagos, L.V., Bedford, M.R., and Stein, H.H. 2020. 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. J. Anim. Sci. **98**: skaa076. doi:10.1093/jas/skaa076. PMID:32150262.

National Research Council (NRC). 2012. Nutrient requirements of swine, 11th rev. ed. Natl. Acad. Press, Washington, DC, USA.

Nyachoti, C.M., Sands, J.S., Connor, M.L., and Adeola, O. 2006. Effect of supplementing phytase to corn- or wheat-based gestation and lactation diets on nutrient digestibility and sow and litter performance. Can. J. Anim. Sci. **86**: 501–510. doi:10.4141/A04-500.