Vitamin D and Vitamin D Metabolites Impact on Calcium and Phosphorus Balance in Gestating Sows

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Summary

One-hydroxycholecalciferol (1-OH-D_3) and 25-hydroxycholecalciferol (25-OH-D_3) are vitamin D metabolites that may be added to diets for pigs. Because 1-OH-D3 is already hydroxylated at the 1-position, only the first hydroxylation in the liver at the 25-position is needed to convert the metabolite to calcitriol, which is the active form of vitamin D_3. Likewise, because the 25-OH-D_3 is already hydroxylated at the 25-position, only the second hydroxylation in the kidney at the 1-position is needed if this metabolite is used. It is possible that supplementation of diets with 25-OH-D_3 or 1-OH-D_3 increases absorption and retention of Ca and P by increasing the conversion efficiency to calcitriol compared with the conversion of cholecalciferol to calcitriol. Effects of supplementation of 1-OH-D_3 and dietary Ca and P in diets fed to gestating sows have been determined, and the effects of supplementation of 25-OH-D_3 and 1-OH-D_3 in diets without or with phytase have also been reported. Results of these experiments have indicated that Ca and P balance and concentrations of digestible energy and metabolizable energy in diets fed to late-gestating sows were not affected by Ca and P levels, but were increased by dietary supplementation with 1-OH-D_3. There was no interaction between dietary Ca and P and supplementation with 1-OH-D_3. Supplementation of 25-OH-D_3, 1-OH-D_3, or microbial phytase increased digestibility and retention of Ca and P. Supplementation of phytase did not affect digestibility of energy, but supplementation of 1-OH-D_3 increased digestibility of energy and concentration of metabolizable energy in diets containing no microbial phytase.

Introduction

Concentrations of blood Ca are tightly regulated by calcitonin, parathyroid hormone, and calcitriol (i.e., 1,25-dihydroxycholecalciferol), which is the active form of vitamin D_3 (Crenshaw, 2001). The regulation may change absorption of Ca and P from the intestinal tract, reabsorption of Ca and P from the kidneys, and formation or resorption of bone tissues (Renkema et al., 2008). In most diets for pigs, vitamin D_3 (i.e., cholecalciferol) is provided in vitamin premixes (Quisirumbay-Gaibor, 2019), and dietary vitamin D_3 needs to be converted to the active form before it can be utilized by animals. Cholecalciferol is transformed to calcitriol by two steps of hydroxylation (Henry, 2011). The first step, which takes place in the liver, involves hydroxylation of cholecalciferol at the 25-position to yield 25-hydroxycholecalciferol (**25-OH-D_3**). The second step, which takes place in the kidney, hydroxylates 25-OH-D_3 at the 1-position to yield 1,25-dihydroxycholecalciferol (i.e., calcitriol).

One-hydroxycholecalciferol (**1-OH-D_3**) and 25-OH-D_3 are vitamin D metabolites that may be added to diets for pigs. Because 1-OH-D_3 is already hydroxylated at the 1-position, only the hydroxylation in the liver at the 25-position is needed to convert the metabolite to calcitriol. Likewise, because the 25-OH-D_3 is already hydroxylated at the 25-position, only the hydroxylation in the kidney at the 1-position is needed if this metabolite is used. Supplementation of diets with 25-OH-D_3 or 1-OH-D_3 may increase absorption and retention of Ca and P by increasing the conversion efficiency to calcitriol compared with the conversion of cholecalciferol to calcitriol. It is possible that effects on Ca and P balance differ between 25-OH-D_3 and 1-OH-D_3, but research to test this hypothesis has not been reported. It is also possible that effects of supplemental vitamin D metabolites (i.e., 1-OH-D_3 or 25-OH-D_3) are affected by the use of microbial phytase because both feed additives can change Ca and P metabolism in broilers (Han et al., 2009), but pig data to demonstrate this have not been reported. Therefore, the objective of this contribution is to summarize current knowledge and experiments about the effects of vitamin D metabolites on Ca and P balance in sows.

Table 1. Calcium and P balance, the apparent total tract digestibility (ATTD) of gross energy, and concentrations of digestible energy and metabolizable energy in diets containing different levels of Ca and P without 1-hydroxycholecalciferol (1-OH-D_3) or with 1-OH-D_3[1,2,3]

Item	Ca and P levels[b]	Normal		Low		SEM	*P*-value		
	1-OH-D_3, mg/kg diet	0	12.5	0	12.5		Ca and P Levels	1-OH-D_3	Interaction
Feed intake, kg/d		2.98	2.93	2.99	2.92	0.05	0.997	0.223	0.901
Fecal excretion, kg/d		0.40	0.33	0.36	0.31	0.06	0.096	0.001	0.441
Urine excretion, kg/d		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									
ATTD of Ca, %		11.31	30.25	18.21	30.49	12.17	0.407	0.001	0.439
Ca retention, % of intake		9.80	27.48	15.41	24.07	11.97	0.796	0.005	0.292
P balance									
ATTD of P, %		15.57	34.70	23.38	29.63	11.16	0.731	0.003	0.114
P retention, % of intake		12.44	28.03	21.93	28.51	11.52	0.223	0.010	0.269
Energy concentrations									
ATTD of gross energy, %		84.6	87.3	85.6	87.5	2.3	0.291	0.001	0.654
Digestible energy, kcal/kg		3,240	3,361	3,275	3,388	90	0.227	< 0.001	0.871
Metabolizable energy, kcal/kg		3,163	3,268	3,197	3,291	98	0.357	0.003	0.861

[1]Data from Lee and Stein (2022).
[2]Each least squares mean for each treatment represents 9 observations, respectively, except for the 2 diets containing normal or low Ca and P levels with no supplemental 1-OH-D_3 ($\underline{n}$ = 8).
[3]Normal level of Ca and P = 100% of the requirement for late gestation sows (0.72% Ca and 0.55% P); low level of Ca and P = 75% of the requirement for late gestation sows (0.54% Ca and 0.41% P; NRC, 2012).

Effects of Dietary Supplementation of Vitamin D Metabolites in Gestating Sows

Effects of dietary Ca and P and 1-OH-D_3 on digestibility and retention of Ca and P in sows

This experiment was conducted to test the hypothesis that supplementation of 1-OH-D_3 to diets for gestating sows containing Ca and P at or below the requirement increases apparent total tract digestibility (**ATTD**) and retention of Ca and P as well as the ATTD of gross energy (**GE**; Lee and Stein, 2022). The second hypothesis was that there is an interaction between dietary Ca and P concentrations and supplementation with 1-OH-D_3 in diets fed to gestating sows.

Diets were formulated using a 2 × 2 factorial arrangement with 2 levels of Ca and P (i.e., 100% or 75% of the requirement; NRC, 2012) without or with supplemental 1-OH-D_3 (Savint, Savint, Iluma Alliance, Durham, NC). Calcium to total P ratio in all diets was 1.3:1.0. Analyzed 1-OH-D_3 in the 2 diets containing the premix were 4.96 and 3.46 μg/kg, respectively, which are close to the commercially recommended dose of 5 μg/kg. The calculated level of vitamin D_3 in all diets was 1,660 IU/kg. All vitamins and minerals except Ca and P were included in all diets to meet or exceed nutrient requirements (NRC, 2012). The 4 diets were fed to 36 multiparous sows from d 91 to 105 of gestation. Sows were housed individually in metabolism crates during the experimental period. Daily feed allotments were provided in one daily meal that was fed at 0700 h throughout the experiment. The daily feed allowance was calculated as 1.5 × the maintenance energy requirement for late gestating sows based on the initial (day 90) body weight of sows (i.e., 100 kcal ME/kg body weight$^{0.75}$; NRC, 2012). Water was available at all times. The initial 5 days of each period were considered the adaptation period and urine were collected during the following 4 days. A color marker was included in the meal fed on day 6 and again in the meal fed on day 10. Fecal collections started when the first marker appeared in the feces and concluded when the second marker appeared. At the conclusion of the experiment, fecal samples were dried at 50 °C in a forced air oven and dried samples were ground; a sub-sample was collected for analysis. Diets, urine and fecal samples were analyzed for Ca, P, and GE and diet and fecal samples were also analyzed for dry matter.

No interactions between dietary Ca and P levels and supplemental 1-OH-D_3 were observed for Ca and P balance, the ATTD of dry matter (**DM**) and GE, or concentrations of DE and ME (Table 1). Dietary Ca and P did not affect Ca and P balance, the ATTD of DM and GE, or concentrations of DE and ME. However, although feed intake was not different among treatments, fecal excretion was less (P = 0.001) from sows fed diets supplemented with 1-OH-D_3 compared with sows fed diets with no 1-OH-D_3, which resulted in greater (P = 0.003) ATTD of DM in sows fed diets supplemented with 1-OH-D_3 compared with sow fed no supplemental 1-OH-D_3. The ATTD of Ca and P and retention of Ca and P were greater ($P < 0.05$) if sows were fed diets supplemented with 1-OH-D_3 compared with sows fed no supplemental 1-OH-D_3. The ATTD of GE and concentrations of DE and ME increased ($P < 0.01$) by supplementing 1-OH-D_3 to the diets.

Effects of 25-OH-D_3 and 1-OH-D_3 on digestibility and retention of Ca and P in sows

A follow-up experiment was conducted to test the hypothesis that supplementation of diets for gestating sows with 25-OH-D_3 or 1-OH-D_3 increases Ca and P balance, the ATTD of GE and concentrations of ME in diets without or with microbial phytase (Lee et al., 2022). Diets were formulated using a 3 × 2 factorial with 3 inclusions of supplemental vitamin D metabolite (no metabolite, 25-OH-D_3, or 1-OH-D_3) and 2 inclusion levels of microbial phytase (0 or 1,000 units; Quantum Blue; AB Vista, Marlborough, UK). Diets were fed to 60 multiparous sows. All diets contained 90% of the requirement for Ca and P (NRC, 2012) and contained 1,660 IU/kg of vitamin D_3 as cholecalciferol from the vitamin-mineral premix. The daily feed allowance was 1.5 times the maintenance energy requirement for gestating sows based on the initial body weight of sows (i.e., 100 kcal ME/kg body weight$^{0.75}$; NRC, 2012). Water was available at all times. Total feces and urine samples during the collection period were collected and prepared for further analyses of Ca, P, and GE.

Results indicated that there was no difference in the ATTD of DM and GE among the 3 diets containing microbial phytase, but among diets without phytase, the ATTD of DM and GE was greater ($P < 0.05$) in diets containing 1-OH-D_3 compared with the diet without a vitamin D metabolite (interaction; $P < 0.05$; Table 2). If no phytase was added to diets, the DE was greater in the diet containing 1-OH-D_3 compared with the diet without a vitamin D metabolite, but if phytase was added to the diets, no difference among diets was observed (interaction; $P < 0.05$). In diets without microbial phytase, the ME was greater in diets containing either one of the 2 vitamin D metabolites than in the diet without one of the metabolites, but among diets with microbial phytase, the ME in the diet containing the 1-OH-D_3 metabolite was less than in the diet with 25-OH-D_3 (interaction; $P < 0.05$). No effects of microbial phytase on ATTD of DM or GE, or on concentrations of DE and ME were observed. Because the interactions between vitamin D metabolite and phytase in Ca and P balance were not significant, only main effects were included in the final model to analyze these parameters. Regardless of metabolite supplementation, use of microbial phytase increased ($P < 0.05$) the ATTD of Ca and P and Ca and P retention (Table 3). Regardless of dietary phytase, the ATTD of Ca and P was greater ($P < 0.05$) for sows fed a diet containing one of the vitamin D metabolites compared with sows fed a diet without a vitamin D metabolite. Calcium and P retentions were greater ($P < 0.05$) for sows fed a diet containing one of the 2 vitamin D metabolites compared with sows fed a diet without one of the metabolites.

Discussion

In both experiments, it was observed that supplementation of vitamin D metabolites increased the ATTD and retention of Ca and P in pigs, which was in agreement with previous data (Regassa et al., 2015; Zhang and Piao, 2021). This indicates that both of the vitamin D metabolites were effective in increasing digestibility of Ca and P and further implies that there is a beneficial effect of providing metabolites in which the first or the second hydroxylation has taken place, even if sows are provided diets that contain vitamin D_3 well above the requirement.

The increase in Ca and P balance by supplemental 1-OH-D_3 and 25-OH-D_3 in the second experiment may be a result of sows being fed below the requirements for Ca

Table 2. Apparent total tract digestibility (ATTD) of gross energy, and concentrations of digestible energy and metabolizable energy in diets fed to sows in late gestation[1]

Item	Microbial phytase:	0 unit/kg diet			1,000 unit/kg diet			SEM	*P*-value		
	Vitamin D3 metabolite:	-	25-OH-D_3	1-OH-D_3	-	25-OH-D_3	1-OH-D_3		Phytase	Vit D_3	Phytase × Vit D_3
ATTD of dry matter, %		85.45^{b}	87.57^{ab}	88.32^{a}	87.41^{ab}	88.48^{a}	86.77^{ab}	0.71	0.345	0.021	0.009
ATTD of gross energy, %		84.91^{b}	87.02^{ab}	87.65^{a}	86.35^{ab}	87.73^{a}	85.78^{ab}	0.77	0.852	0.023	0.021
Digestible energy, kcal/kg		3,303^{b}	3,385^{ab}	3,410^{a}	3,359^{ab}	3,413^{a}	3,337^{ab}	30	0.852	0.023	0.021
Metabolizable energy, kcal/kg		3,156^{c}	3,244^{ab}	3,249^{a}	3,219^{abc}	3,255^{a}	3,166^{bc}	32	0.889	0.088	0.029

[a-c]Within a row, means without a common superscript differ ($P < 0.05$).
[1]Each least squares mean represents 10 observations except that there were only 9 observations for the 2 diets containing 25-OH-D_3.

Table 3. Apparent total tract digestibility (ATTD) of Ca and P and retention of Ca and P in diets fed to sows in late-gestation[1]

Item	Microbial phytase, unit/kg diet		SEM	*P*-value	Vitamin D_3 metabolite			SEM	*P*-value
	0	1,000			-	25-OH-D_3	1-OH-D_3		
ATTD of Ca, %	24.50	32.30	3.87	0.036	17.73[b]	30.38[a]	37.10[a]	4.27	< 0.001
Ca retention, % of intake	18.43	26.53	4.24	0.026	13.56[b]	25.47[a]	28.41[a]	4.59	0.003
ATTD of P, %	40.28	51.02	2.25	< 0.001	35.56[b]	48.51[a]	52.88[a]	2.51	< 0.001
P retention, % of intake	34.30	39.40	2.59	0.036	29.65[b]	41.94[a]	38.97[a]	2.85	< 0.001

[a-b]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Each least squares mean represents 10 observations except that there were only 9 observations for the 2 diets containing 25-OH-D_3.

and P. The ATTD of P in lactating sows was not affected by adding 25-OH-D_3 to diets containing 100% of the Ca and P required by lactating sows (Zhang et al., 2019), but no interaction was observed between dietary Ca and P levels and supplementation of 1-OH-D_3 in the first experiment. It therefore appears that supplementation of late-gestation diets with 1-OH-D_3 positively affects Ca and P balance regardless of dietary Ca and P.

Effects of using both phytase and 1-OH-D_3 in P-deficient diets fed to broilers were additive (Snow et al., 2004), but this was not the case in diets for pigs (Biehl and Baker, 1996). Results from the second experiment demonstrated that effects of addition of phytase and vitamin D metabolites were not additive in diets for sows and the results, therefore, are in agreement with the data by Biehl and Baker (1996). Although there were no interactions between phytase and vitamin D metabolites for Ca and P balance, interactions were observed for the ATTD of DM and GE and concentration of DE and ME in diets, which to our knowledge has not been previously demonstrated. The mechanisms for these interactions are, however, not clear and additional research is needed to elucidate the reasons for this observation.

The level of vitamin D_3 in all diets was well above the presumed requirement for sows in gestation (i.e. 20 µg/kg; NRC, 2012), but the fact that the ATTD of Ca and P and retention of Ca and P increased by adding one of the vitamin D metabolites to the diets indicates that sows are not able to convert sufficient quantities of vitamin D_3 to calcitriol to maximize Ca and P balance. It is not clear why the vitamin D metabolites are so effective in sows fed diets containing vitamin D_3 in excess of the requirement. It appears that sows have difficulty hydroxylating vitamin D_3 to 1,25-dihydroxycholecalciferol, whereas use of one of the vitamin D metabolites results in increased synthesis of 1,25-dihydroxycholecalciferol. Conversion of 25-OH-D_3 to 1,25-dihydroxycholecalciferol in women in wk 12 of pregnancy is two-fold greater than in non-pregnant women (Hollis and Wagner, 2017), and it is, therefore, possible that pregnancy increases the need for calcitriol, but because we did not include non-pregnant females in this experiment we cannot confirm this hypothesis. It is also possible that the increase in 1,25-dihydroxycholecalciferol synthesis in sows fed a vitamin D metabolite is beneficial to their progeny because supplementation of diets for sows with 25-OH-D_3 resulted in increases in blood vitamin D, growth performance, and bone mineralization of their offspring (Witschi et al., 2011; Flohr et al., 2016). However, more research is needed to confirm this hypothesis.

To be converted to the calcitriol, 25-OH-D_3 skips the hydroxylation step in the liver and 1-OH-D_3 skips the hydroxylation step in the kidneys. The observation that the 2 vitamin D metabolites are equally effective in increasing ATTD of DM, GE, Ca, and P indicates that it is the double hydroxylation that is problematic for sows, whereas it appears to be less important which hydroxylation step needs to be completed if a vitamin D metabolite is provided.
The increases in DE and ME in diets containing 1-OH-D_3 were a result of increased ATTD of DM. This was observed in both experiments (Lee and Stein, 2022; Lee et al., 2022) and confirms that 1-OH-D_3 increases energy concentrations in diets by increasing the ATTD of DM when no phytase was used.

Conclusions

In conclusion, there were no interactions between levels of dietary Ca and P and supplemental 1-OH-D_3 and between use of vitamin D metabolites and microbial phytase on Ca and P balance in sows in late gestation. 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 25-OH-D_3 and 1-OH-D_3 increased the ATTD and retention of Ca and P. Supplementation of 1-OH-D_3 increased the ATTD of DM and GE and concentrations of DE and ME in diets containing no microbial phytase. No effect of phytase was observed for the ATTD of GE and concentrations of DE and ME in diets.

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