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Effects of 25-hydroxycholecalciferol (25-OH-D_3) and 1-hydroxycholecalciferol (1-OH-D_3) on serum bone biomarkers and calcium and phosphorus balance and concentrations of energy in diets without or with microbial phytase fed to sows in late gestation

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Abstract

The objective was to test the hypothesis that supplementation of diets for gestating sows with 25-hydroxycholecalciferol (**25-OH-D_3**) or 1-hydroxycholecalciferol (**1-OH-D_3**) affects serum biomarkers for bone and increases Ca and P balance and the apparent total tract digestibility (**ATTD**) of gross energy (**GE**), and the concentrations of digestible energy (**DE**) and metabolizable energy (**ME**) in diets without or with microbial phytase. Sixty multiparous sows were allotted to 1 of 6 diets. 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). Sows were housed individually in metabolism crates and feces and urine were collected quantitatively. Results indicated that there was no difference in the ATTD of dry matter (**DM**) and GE and concentration of DE among the 3 diets containing microbial phytase, but the ATTD of DM and GE and concentration of DE was greater ($P < 0.05$) in diets containing 1-OH-D_3 compared with the diet without a vitamin D metabolite if phytase was not used (interaction; $P < 0.05$). In diets without microbial phytase, ME was greater in diets containing either one of the 2 vitamin D metabolites than in the diet without a vitamin D metabolite, but among diets with microbial phytase, the ME of the 1-OH-D_3 diet was less than of the 25-OH-D_3 diet (interaction; $P < 0.05$). No effect of microbial phytase on concentrations of DE and ME was observed. There was no interaction between supplementation of microbial phytase and vitamin D metabolites for Ca and P balances, and regardless of metabolite supplementation, use of microbial phytase increased ($P < 0.05$) the ATTD and retention of Ca and P. Regardless of dietary phytase, the ATTD and retention of Ca and P increased ($P < 0.05$) for sows fed a diet containing one of the vitamin D metabolites compared with sows fed the diet without a vitamin D metabolite. Serum biomarkers for bone resorption or bone tissue synthesis were not affected by experimental diets. In conclusion, the ATTD of DM and GE, concentrations of DE and ME, and Ca and P balance in phytase-free diets fed to sows in late gestation were increased by supplementation with 1-OH-D_3 or 25-OH-D_3, but no differences between the 2 vitamin D metabolites were observed. Supplementation of diets with microbial phytase increased Ca and P balance, but did not affect DE and ME of diets.

Lay Summary

The role of vitamin D is to increase absorption of calcium and phosphorus in the gastrointestinal tract and maintain serum concentrations of calcium, but dietary vitamin D needs to be converted to an active form by 2-hydroxylation steps that take place in the liver and the kidneys. The conversion efficiency to active vitamin D may be increased if pre-hydroxylated metabolites rather than vitamin D are provided, which also increases calcium and phosphorus utilization. In a previous experiment it was also demonstrated that a vitamin D metabolite increases energy absorption in gestating sows. It is possible that use of a vitamin D metabolite and phytase have additive effects and the hypothesis, therefore, was that supplementation of a vitamin D metabolite increases calcium and phosphorus balance and energy digestibility in diets fed to gestating sows without or with microbial phytase. Results indicated that in diets without phytase, the 2 vitamin D metabolites increased energy concentration in diets by increasing apparent energy digestibility. There was no interaction between supplementation of phytase and vitamin D metabolites for calcium and phosphorus balances. Use of phytase and vitamin D metabolites increased calcium and phosphorus digestibility and retention.

Key words: alfacalcidol, calcifediol, calcium, phosphorus, vitamin D, vitamin D metabolites

Abbreviations: ATTD: apparent total tract digestibility, BAP: bone-specific alkaline phosphatase, CTX-I: carboxyterminal cross-linked telopeptide of type I collagen, DE: digestible energy, DM: dry matter, GE: gross energy, ME: metabolizable energy, OC: osteocalcin, 1-OH-D_3: 1-hydroxycholecalciferol, 25-OH-D_3: 25-hydroxycholecalciferol

Introduction

Concentration of blood Ca is 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.,

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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, conversion of the metabolite to calcitriol may be faster compared with vitamin D_3. Likewise, because the 25-OH-D_3 is already hydroxylated at the 25-position, only the second hydroxylation 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. Indeed, use of supplemental 1-OH-D_3 increased the balance of Ca and P and also increased digestibility of energy by sows in late gestation (Lee and Stein, 2022). It is possible that the 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.

Exogenous phytase increases the digestibility of both Ca and P in pigs (Selle et al., 2009; Létourneau-Montminy et al., 2012; Rojas and Stein, 2012; Lee et al., 2019b). It is 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. However, because phytase makes more Ca and P available for absorption and vitamin D affects absorption of Ca and P, it is likely that the effects of the 2 additives are additive, but to our knowledge, this has not been experimentally verified in sows. Therefore, the objective of this experiment was to test the hypothesis that supplementation of 25-OH-D_3 or 1-OH-D_3 affects serum bone biomarkers, digestibility and retention of Ca and P, digestibility of gross energy (**GE**), and concentration of metabolizable energy (**ME**) in diets without or with microbial phytase fed to sows in late 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.

Animals, housing, and sample collection

Sixty multiparous gestating sows (Camborough 22, Pig Improvement Company, Hendersonville, TN, USA) were allotted to 1 of 6 diets in 6 blocks. Two blocks had 6 sows (1 sow per diet) and 4 blocks had 12 sows (2 sows per diet) for a total of 10 replicate sows for each treatment. Sows were housed individually in metabolism creates from days 91 to 105 of gestation and fed the 6 experimental diets that were formulated based on corn, soybean meal, and sugar beet pulp (Tables 1 and 2). Metabolism crates were equipped with a feeder, a nipple drinker, and fully slatted T-bar floors.

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

Item, %	Corn	Soybean meal	Sugar beet pulp
Dry matter	85.73	90.17	89.35
Gross energy, kcal/kg	3,751	4,099	3,293
Acid hydrolyzed ether extract	3.41	2.71	2.33
Ash	1.03	6.69	10.78
Ca	0.004	0.50	1.73
P	0.23	1.14	0.11
Phytate	0.61	1.46	< 0.14
Phytate-P[1]	0.17	0.41	< 0.04
Non phytate-P[2]	0.06	0.73	< 0.07
Total dietary fiber	12.6	19.6	63.9
Soluble dietary fiber	3.2	2.9	17.8
Insoluble dietary fiber	9.4	16.7	46.1

[1]Phytate-P was calculated by multiplying the analyzed phytate by 0.282 (Tran and Sauvant, 2004).
[2]Non phytate-P was calculated as total P minus phytate-P.

A screen floor and a urine pan were installed below the T-bar floors to allow for separate collection of feces and urine. 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 fecal collection using the marker to marker procedure (Adeola, 2001). Fecal collection started when the first visual marker (i.e., indigo carmine) appeared in the feces and ceased when the second visual marker (i.e., chromic oxide) appeared (Adeola, 2001). Urine was collected for 4 d starting in the morning of day 6, and urine was collected in buckets placed under the urine pans with 50 mL of 3*N* HCl. Buckets were emptied daily, the weight of the collected urine was recorded, and 10% was stored at −20°C until subsampling.

Following fecal and urine collections, sows were fasted for 24 h and a blood sample was collected from the vena cava. Blood samples were immediately centrifuged and serum was collected and stored at −20 °C.

Dietary treatments

Diets were formulated using a 3 × 2 factorial arrangement with 3 inclusions of supplemental vitamin D metabolite (no metabolite, supplementation with 25-OH-D_3 (Hy-D®; DSM, Parsippany, NJ, USA), or supplementation with 1-OH-D_3 (Savint; Iluma Alliance, Durham, NC, USA) and 2 inclusion levels of microbial phytase [0 or 1,000 units of phytase (Quantum Blue®; AB Vista, Marlborough, UK)]. All vitamins and minerals except Ca and P were included in all diets to meet or exceed current nutrient requirements (NRC, 2012). All diets contained 90% of the requirement for Ca and P for gestating sows (NRC, 2012) and contained vitamin D_3 as cholecalciferol from the vitamin–mineral premix. The analyzed concentration of vitamin D_3 in the vitamin–mineral premix was 13.9 µg/g, which provided the complete diet with a concentration of 69.6 µg/kg. 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.

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

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

Item	Microbial phytase					
	0 unit/kg diet			1,000 unit/kg diet		
	Vitamin D_3 metabolite					
	No metabolite	25-OH-D_3	1-OH-D_3	No metabolite	25-OH-D_3	1-OH-D_3
Ingredients, %						
Corn	73.54	72.54	72.54	72.54	71.54	71.54
Soybean meal	14.00	14.00	14.00	14.00	14.00	14.00
Sugar beet pulp	8.00	8.00	8.00	8.00	8.00	8.00
25-OH-D_3 premix[1]	—	1.00	—	—	1.00	—
1-OH-D_3 premix[1]	—	—	1.00	—	—	1.00
Corn–phytase premix[2]	—	—	—	1.00	1.00	1.00
Calcium carbonate	0.78	0.78	0.78	0.78	0.78	0.78
Monocalcium phosphate	0.78	0.78	0.78	0.78	0.78	0.78
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00
Sodium chloride	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin–mineral premix[3]	0.50	0.50	0.50	0.50	0.50	0.50
Analyzed composition, %						
Dry matter	88.32	87.58	88.76	89.20	88.39	87.72
Gross energy, kcal/kg	3,935	3,866	3,925	3,941	3,838	3,835
Crude protein	11.86	12.19	10.99	12.97	12.17	12.13
Acid hydrolyzed ether extract	4.18	4.14	3.58	4.21	3.69	4.29
Ash	4.25	3.97	4.03	4.30	4.27	4.00
Ca	0.48	0.48	0.45	0.49	0.46	0.51
P	0.52	0.49	0.51	0.55	0.50	0.57
Phytate[4]	0.66	0.66	0.66	0.66	0.66	0.66
Phytate-P[4]	0.19	0.19	0.19	0.19	0.19	0.19
Non-phytate P[4]	0.33	0.30	0.32	0.36	0.31	0.38
Phytase, phytase unit/kg	<70	<70	<70	800	1,100	1,200
Total dietary fiber	16.7	17.7	17.3	14.8	16.7	15.5
Soluble dietary fiber	2.8	2.7	2.7	3.0	2.3	2.2
Insoluble dietary fiber	13.9	15.0	14.6	11.8	14.4	13.3
25-OH-D_3, µg/kg	<0.5	32.4	0.8	<0.5	45.0	1.6
1-OH-D_3, µg/kg	<0.64	<0.64	3.04	<0.64	<0.64	3.97

[1]The 25-OH-D_3 premix was prepared by mixing 175 g of 25-OH-D_3 concentrate (179.7 mg/kg of 25-OH-D_3; Hy-D; DSM Nutritional Products, Parsippany, NJ, USA) and 6,825 g ground corn. The 1-OH-D_3 premix was prepared by mixing 8.75 g of 1-OH-D_3 concentrate (302 mg/kg of 1-OH-D_3; Savint; Iluma Alliance, Durham, NC, USA) and 6,991.25 g ground corn. At 1% inclusion of the premixes, calculated concentrations of the metabolites in the complete diets were 44.925 µg/kg 25-OH-D_3 and 3.775 µg/kg 1-OH-D_3, respectively.
[2]Corn–phytase premix was prepared by mixing 140 g of phytase product (5,000 unit/g; Quantum Blue, AB Vista, Marlborough, UK; level of phytase = 0.02% in diet) and 6,860 g ground corn (level of corn used in corn-phytase premix = 0.98% in diet). At 1% inclusion, the phytase premix provided 1,000 units of microbial phytase to diets.
[3]The vitamin–micromineral premix was calculated to provide the following quantities of vitamins and micro minerals per kg of complete diet: vitamin A as retinyl acetate, 3,187 µg retinol; vitamin D_3 as cholecalciferol, 41.5 µg; vitamin E as $_{DL}$-alpha tocopheryl acetate, 59.4 mg $_{DL}$-alpha tocopherol; 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.
[4]Phytate and phytate-P were calculated based on analyzed phytic acid in ingredients; non phytate-P was calculated as total P minus phytate-P.

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 and finely ground through a 1-mm screen before analysis using a Wiley mill (Model 4; Thomas Scientific, Swedesboro, NJ, USA).

Chemical analysis

The concentration of 1-OH-D_3 in experimental diets was analyzed using liquid chromatography with tandem mass spectrometry (Aronov et al., 2008). The concentration of 25-OH-D_3 was analyzed by a solid phase-extraction followed by high-pressure liquid chromatography clean-up and isolation; final quantification of 25-OH-D_3 was completed via liquid chromatography–mass spectrometry (Heartland Assays, Ames, IA, USA). Concentration of vitamin D_3 in the vitamin–micromineral premix was analyzed using liquid chromatography (Huang et al., 2014). Calcium and P in feed ingredients, diets, feces, and urine samples were analyzed (method 985.01 A, B, and C; AOAC

Int., 2019) by inductively coupled plasma spectroscopy (ICP-OES; Avio 200, PerkinElmer, Waltham, MA, USA). Sample preparation included dry ashing at 600 °C for 4 h (method 942.05; AOAC Int., 2019) and wet digestion with nitric acid (method 3050 B; U.S. Environmental Protection Agency, 2000). Corn, soybean meal, and sugar beet pulp were analyzed for phytic acid (Ellis et al., 1977), and phytic acid in diets was calculated based on analyzed phytic acid in ingredients. Phytase activity in diet samples was also analyzed (method 2000.12; AOAC Int., 2019). All ingredient, diet, and fecal samples were analyzed for dry mater (**DM**; method 930.15; AOAC Int., 2019), and ash was analyzed in ingredients and diets (method 942.05; AOAC Int., 2019). Diet, feed ingredient, fecal, and urine samples were analyzed for GE (Model 6400, Parr Instruments, Moline, IL, USA). Crude protein in diets was calculated as N × 6.25 and N was analyzed by combustion (method 990.03; AOAC Int., 2019) using a LECO FP628 Nitrogen Analyzer (LECO Corp., Saint Joseph, MI, USA). Acid hydrolyzed ether extract in ingredient and diet samples was analyzed by acid hydrolysis using 3*N* HCl (AnkomHCl; Ankom Technology, Macedon, NY, USA) followed by crude fat extraction using petroleum ether (AnkomXT15; Ankom Technology, Macedon, NY, USA). Insoluble and soluble dietary fiber were analyzed according to method 991.43 (AOAC Int., 2019) using the Ankom^{TDF} Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA). Concentration of total dietary fiber was calculated as the sum of insoluble and soluble dietary fiber.

Serum samples were analyzed for carboxyterminal cross-linked telopeptide of type I collagen (**CTX-I**; a biomarker for bone degradation) using a Pig Cross-Linked C-Telopeptide of Type I Collagen Enzyme-Linked Immunosorbent Assay Kit (Abbexa Ltd., Cambridge, UK). Concentration of osteocalcin (**OC**; a biomarker for bone synthesis) in serum was analyzed using an N-MID Osteocalcin Enzyme-Linked Immunosorbent Assay Kit (Immunodiagnostic Systems Ltd, The Boldons, UK), and bone-specific alkaline phosphatase (**BAP**; a biomarker for bone synthesis) was analyzed using an Ostase BAP Enzyme Immunoassay Kit (Immunodiagnostic Systems Ltd, The Boldons, UK).

Statistical analysis

Normality and homogeneity of data were verified using the UNIVARIATE and MIXED procedures (SAS Inst. Inc., Cary, NC, USA) and outliers were identified using Internally Studentized Residuals (Tukey, 1977). 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 vitamin D metabolite, phytase, and the interaction between vitamin D metabolite and phytase as fixed effects and block and parity as random effects. Because the interactions between vitamin D metabolite and phytase in Ca and P balance and concentrations of serum bone biomarkers were not significant, only main effects were included in the final model to analyze these parameters. 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 model was significant. Statistical significance and tendency were considered at $P < 0.05$ and $0.05 \leq P < 0.10$, respectively.

Results

There were no interactions between use of phytase and vitamin D metabolites and no effects of use of phytase and vitamin D metabolites were observed for feed intake, urine excretion, GE intake, or urine GE excretion (Table 3). There was no difference in the weight of feces excreted among sows fed the 3 diets containing microbial phytase, but among sows fed diets without phytase, fecal excretion was greater ($P < 0.05$) from sows fed the diet with no vitamin D metabolite compared with sows fed the diet supplemented with 1-OH-D_3 (interaction; $P = 0.007$). 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

Table 3. Apparent total tract digestibility (ATTD) of dry matter (DM) and gross energy (GE) and concentrations of digestible energy (DE) and metabolizable energy (ME) in diets fed to sows in late gestation[1]

Item	Microbial phytase						SEM	*P*-value		
	0 unit/kg diet			1,000 unit/kg diet						
	Vitamin D_3 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
Feed intake, kg/d	2.69	2.75	2.70	2.79	2.70	2.76	0.08	0.458	0.960	0.401
Fecal excretion, kg dry matter/d	0.34^{a}	0.30^{ab}	0.28^{b}	0.31^{ab}	0.27^{b}	0.32^{ab}	0.02	0.614	0.007	0.007
Urine excretion, kg/d	4.46	4.62	6.49	3.86	5.63	6.16	1.34	0.979	0.269	0.820
ATTD of DM, %	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
GE intake, Mcal/d	10.48	10.70	10.51	10.84	10.50	10.74	0.30	0.464	0.957	0.395
Fecal GE excretion, kcal/d	1,569^{a}	1,388^{ab}	1,289^{b}	1,469^{ab}	1,279^{b}	1,525^{ab}	82	0.855	0.012	0.007
Urine GE excretion, kcal/d	400	389	434	390	433	477	35	0.285	0.097	0.561
ATTD of GE, %	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
DE in diet, 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
ME in diet, 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

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

vitamin D metabolite (interaction; $P < 0.05$). 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.

Calcium and P intakes were not affected by supplementation of microbial phytase or vitamin D metabolites (Table 4). Regardless of metabolite supplementation, use of microbial phytase tended to decrease ($P = 0.069$) fecal Ca output and decreased ($P < 0.001$) fecal P output, but increased ($P < 0.05$) absorbed Ca and P, the ATTD of Ca and P, and Ca and P retention (gram per day and percentage). Regardless of dietary phytase, fecal Ca and P outputs were less ($P < 0.05$) from sows fed the 2 diets containing a vitamin D metabolite compared with the diet with no vitamin D metabolite. As a conseq uence, the ATTD of Ca and P and absorption of Ca and P were 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. Urine Ca and P output was greater ($P < 0.05$) from sows fed the diet containing 1-OH-D_3 than from sows fed the other 2 diets. Calcium and P retentions (gram per day and percentage) 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.

Serum biomarkers for bone formation or bone resorption were not affected by addition of phytase to the diets (Table 5). Likewise, addition of 1-OH-D_3 or 25-OH-D_3 to the diets did not affect OC, BAP, CPX-I, or the OC to CPX-I ratio.

Discussion

Analyzed concentrations in diets of crude protein, GE, Ca, and P were in agreement with calculated values. Analyzed vitamin D_3 in the vitamin–micro mineral premix was 13.9 μg/g, which provided 69.6 μg of vitamin D_3 in 1 kg of complete diet. Therefore, vitamin D_3 in the diet was greater than expected (41.5 μg/kg). Diet concentrations of 1-OH-D_3 and 25-OH-D_3 were as expected.

Values for the ATTD and retention of Ca and P were in agreement with previous values for gestating sows (Nyachoti et al., 2006; Jang et al., 2014; Lee et al., 2019a; Lee and Stein, 2022). The ATTD of GE and the resulting contributions of DE and ME from diets were greater than calculated values from growing pigs (NRC, 2012), which is likely a result of the greater digestibility of energy by gestating sows compared with growing pigs (Le Goff and Noblet, 2001; Casas and Stein, 2017).

The observation that supplementation of vitamin D metabolites increased the ATTD and retention of Ca and P was in agreement with previous data (Regassa et al., 2015; Zhang and Piao, 2021; Lee and Stein, 2022). 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 observation that inclusion of 1-OH-D_3 and no phytase in the diet increased ATTD of DM and GE was in agreement with results of our previous experiment (Lee and Stein, 2022).

Table 4. Apparent total tract digestibility (ATTD) of Ca and P and retention of Ca and P in diets fed to sows in late-gestation[1, 2]

Item	Microbial phytase, unit/kg diet		SEM	Vitamin D_3 metabolite			SEM	*P*-value	
	0	1,000		—	25-OH-D_3	1-OH-D_3		Phytase	Vitamin D_3 metabolite
Ca balance									
Ca intake, g/d	13.03	13.21	0.31	13.16	13.09	13.11	0.32	0.417	0.959
Fecal Ca output, g/d	9.86	9.04	0.63	10.82[a]	9.21[b]	8.33[b]	0.67	0.069	<0.001
Absorbed Ca, g/d	3.27	4.30	0.41	2.43[b]	4.03[a]	4.89[a]	0.48	0.037	<0.001
ATTD of Ca, %	24.50	32.30	3.87	17.73[b]	30.38[a]	37.10[a]	4.27	0.036	<0.001
Urine Ca output, g/d	0.75	0.72	0.09	0.52[b]	0.58[b]	1.10[a]	0.10	0.756	<0.001
Ca retention, g/d	2.49	3.53	0.47	1.88[b]	3.39[a]	3.75[a]	0.52	0.030	0.004
Ca retention, % of intake	18.43	26.53	4.24	13.56[b]	25.47[a]	28.41[a]	4.59	0.026	0.003
P balance									
P intake, g/d	14.27	14.46	0.33	14.40	14.32	14.35	0.35	0.415	0.960
Fecal P output, g/d	8.56	7.17	0.42	9.28[a]	7.48[b]	6.84[b]	0.44	<0.001	<0.001
Absorbed P, g/d	5.71	7.31	0.32	5.15[b]	6.86[a]	7.52[a]	0.37	<0.001	<0.001
ATTD of P, %	40.28	51.02	2.25	35.56[b]	48.51[a]	52.88[a]	2.51	<0.001	<0.001
Urine P output, g/d	0.84	1.67	0.16	0.84[b]	0.94[b]	1.99[a]	0.18	<0.001	<0.001
P retention, g/d	4.91	5.69	0.33	4.33[b]	5.99[a]	5.58[a]	0.37	0.033	0.001
P retention, % of intake	34.30	39.40	2.59	29.65[b]	41.94[a]	38.97[a]	2.85	0.036	<0.001

[1]Each least squares mean represents 10 observations except that there were only 9 observations for the 2 diets containing 25-OH-D_3.
[2]Only main effects are shown because there were no interactions between phytase and inclusion of vitamin D metabolite.
[a-b]Within a row, means without a common superscript differ ($P < 0.05$).

Table 5. Concentrations of bone formation and bone resorption biomarkers in serum samples from late-gestating sows fed experimental diets[1,2]

Item	Microbial phytase, unit/kg diet		SEM	Vitamin D_3 metabolite			SEM	*P*-value	
	0	1,000		—	25-OH-D_3	1-OH-D_3		Phytase	Vitamin D_3 metabolite
Bone formation marker									
OC, µg/L	13.21	13.68	1.63	13.47	13.20	13.67	1.71	0.638	0.929
BAP, µg/L	6.99	7.97	1.25	7.77	6.74	7.93	1.29	0.137	0.294
Bone resorption marker									
CTX-I, µg/L	2.44	2.66	0.35	3.06	2.16	2.42	0.43	0.656	0.329
OC to CTX-I ratio	9.90	10.29	2.87	10.81	8.42	11.06	3.51	0.924	0.844

[1]Each least squares mean represents 10 observations.
[2]OC = osteocalcin; BAP = bone-specific alkaline phosphatase; CTX-I = carboxyterminal cross-linked telopeptide of type I collagen.

The increase in Ca and P balance by supplemental 1-OH-D_3 and 25-OH-D_3 may be a result of sows being fed below the requirements for Ca 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., 2019b), but no interaction was observed between dietary Ca and P levels and supplementation of 1-OH-D_3 in our previous experiment with gestating sows (Lee and Stein, 2022). 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 for growing pigs (Biehl and Baker, 1996). Results of the present experiment demonstrated that effects of addition of phytase and vitamin D metabolites were not additive for the ATTD of DM and GE in diets for sows. 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 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, but the response obtained in this experiment agrees with our previous data where we did not have excess vitamin D_3 (Lee and Stein, 2022). 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, which subsequently increase Ca and P balance. A possible explanation for the increased Ca and P balance includes possible calcification of soft tissue, but because we did not measure calcification this is only a speculation and additional research is needed to elucidate the reasons for this observation. Conversion of 25-OH-D_3 to 1,25-dihydroxycholecalciferol in women in week 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.

Because microbial phytase increases Ca and P release in the intestinal tract and vitamin D metabolites upregulate the transcellular absorption of Ca and P from the intestinal tract, the additive effects of using microbial phytase and vitamin D metabolites that were observed were expected. 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 increase in ME in diets containing one of the vitamin D metabolites were a result of reduced fecal output and increased ATTD of DM. This observation is in accordance with previous data with 1-OH-D_3 (Lee et al., 2022) and confirms that vitamin D metabolites increase energy concentrations in diets by increasing the ATTD of DM. It is however, not clear which mechanisms are responsible for this increase and it is not known if this is something that is unique to gestating sows or if this effect will also be obtained in early gestating sows, lactating sows, and growing pigs. More research to elucidate these effects is, therefore, warranted.

Concentrations of OC and BAP, which are the bone formation biomarkers, were within the range of previous values (Lee et al., 2020). Blood biomarkers may reflect bone turnover in the body. Osteoblasts produces OC when new bone tissues are synthesized and BAP enables blood phosphate to be utilized as a building block for bone tissue synthesis (Vasikaran et al., 2011; Lee et al., 2020). Addition of vitamin D metabolites or exogenous phytase to diets was expected to increase serum concentrations of BAP and OC or the OC to CTX-I ratio because these compounds may increase retained Ca and P, which increases bone formation. However, the observation that serum biomarkers were not affected by vitamin D metabolites or microbial phytase was in contrast with the hypothesis, but the response was in agreement with previous data (Zhang et al., 2019a; Zhao et al., 2022). Bone formation takes up to 3 month (Seibel, 2005) and this may explain the lack of phytase or vitamin D metabolite effects on serum concentrations of OC and BAP because sows were fed the experimental diets for only 12 d.

Serum concentration of CTX-I, which is the bone resorption marker, was greater than previous values (van Riet et al., 2016; Lee et al., 2020; Karst et al., 2021). The reason for this observation may be that gestating sows used in this experiment were fed

diets that were deficient in Ca and P, which are the main minerals needed for synthesis of bone tissue. Therefore, there likely was a net breakdown of bone in the sows. Addition of vitamin D metabolites or exogenous phytase to diets was expected to decrease serum concentration of CTX-I, but that was not the case. There are no data demonstrating effects of vitamin D metabolites or microbial phytase on serum concentration of CTX-I, but concentration of CTX-I decreased as concentration of Ca increased from 25% to 100% of the requirement in diets fed to late-gestation sows (Lee et al., 2020). However, in the present experiment dietary Ca and P were less deficient, which may be the reason no changes in CTX-I was observed.

Conclusion

There were no interactions between use of a vitamin D metabolite and use of microbial phytase on Ca and P balance and bone biomarkers. Supplementation of 1-OH-D_3 increased the ATTD of DM and GE and concentrations of DE and ME by approximately 90 kcal per kg in diets containing no microbial phytase. Regardless of use of microbial phytase, the ATTD and retention of Ca almost doubled and the ATTD and retention of P increased by around 50 and 30%, respectively, if diets were supplemented with one of the vitamin D metabolites. This demonstrates that the responses to phytase and the vitamin D metabolites are additive. Supplementation of microbial phytase increased Ca and P balance, but no effect of phytase was observed for the ATTD of GE and concentrations of DE and ME in diets. Concentrations of blood biomarkers were not affected by supplementation of microbial phytase or vitamin D metabolites.

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Conflict of Interest Statement

The authors have no real or perceived conflicts of interest.

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