J. Dairy Sci. 98:1–13
http://dx.doi.org/10.3168/jds.2014-9188

Effect of feeding 25-hydroxyvitamin D_3 with a negative cation-anion difference diet on calcium and vitamin D status of periparturient cows and their calves

W. P. Weiss,*[1] E. Azem,† W. Steinberg,† and T. A. Reinhardt‡
*Department of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster 44691
†DSM Nutritional Products Ltd., 4002 Basel, Switzerland
‡Ruminant Diseases and Immunology Research Unit, National Animal Disease Center, USDA Agricultural Research Service, Ames, IA 50010

ABSTRACT

Holstein cows (>1 gestation) were fed 1 of 3 diets during the last 13 d of gestation (ranged from 22 to 7 d). The control diet (16 cows) was formulated to provide 18,000 IU/d of vitamin D_3 and had a dietary cation-anion difference (DCAD) of 165 mEq/kg (DCAD = Na + K – Cl – S). The second diet (DCAD + D) provided the same amount of vitamin D_3 but had a DCAD of −139 mEq/kg (17 cows). The third diet (DCAD + 25D) had no supplemental vitamin D_3 but provided 6 mg/d of 25-(OH) vitamin D_3 [25-(OH)D_3] with a DCAD of −138 mEq/kg (20 cows). Diets were fed until parturition and then all cows were fed a common lactation diet that contained vitamin D_3. Negative DCAD diets reduced urine pH, with the greatest decrease occurring with the DCAD + D treatment. Urinary Ca excretion was greatest for cows fed DCAD + 25D followed by cows fed DCAD + D. Urinary pH was negatively correlated with urinary excretion of Ca for cows fed DCAD + D. No such correlation was observed with the DCAD + 25D treatment because substantial excretion of urinary Ca occurred at moderate urinary pH values for that treatment. Cows fed DCAD + 25D had greater serum concentrations of 25-(OH)D_3 than other treatments from 5 d after supplementation started through 7 d in milk. Concentrations of 1,25-$(OH)_2D_3$ in serum were greatest in DCAD + 25D cows starting at 2 d before calving and continued through 7 d in milk. Serum Ca concentrations 5 d before calving were greatest for cows fed DCAD + 25D, but at other time points before and after parturition treatment did not affect serum Ca. Incidence of clinical hypocalcemia was not statistically different between treatments, but cows fed DCAD + 25 had the highest incidence rate (12.5, 0, and 20% for control, DCAD + D, and DCAD + 25D). Calves born from cows fed DCAD + 25D had greater concentrations of 25-(OH)D_3 in serum at birth than calves from other treatments (before colostrum consumption), but concentrations were similar by 3 d of age. Concentrations of 25-(OH)D_3 in colostrum and transition milk were increased by feeding DCAD + 25D, but by 28 d in milk treatment effects no longer existed. Overall, feeding 25-OH vitamin D with a negative DCAD diet increased vitamin D status of the cow and her newborn calf but had minimal effects on calcium status and did not have positive effects on the incidence of hypocalcemia.
Key words: vitamin D, hypocalcemia, dietary cation-anion difference

INTRODUCTION

Peripartum hypocalcemia is common in dairy cows (Reinhardt et al., 2011), is a risk factor for numerous health disorders, and is associated with lower milk yields (Curtis et al., 1985; Gröhn et al., 1990; Massey et al., 1993; Chapinal et al., 2011; Chapinal et al., 2012; Chamberlin et al., 2013; Martinez et al., 2014). Feeding diets with a negative DCAD before partition is an established method of reducing clinical (Lean et al., 2006) and subclinical (Oetzel et al., 1988; Crnkic et al., 2010; Sakha et al., 2014) hypocalcemia.

The biologically active form of vitamin D [1,25-$(OH_2)D_3$] enhances Ca absorption by the intestine and in concert with parathyroid hormone (**PTH**) stimulates mobilization of Ca from bone (Horst et al., 1994). Dietary vitamin D_3 must first be hydroxylated to 25-(OH)D_3, which is then converted to 1,25-(OH_2) D_3. Concentrations of 25-(OH)D_3 are used to indicate vitamin D status, but a study with humans suggests that conversion of vitamin D_3 into 25-(OH)D_3 is reduced at high intakes of vitamin D_3 (Heaney et al., 2008). If this occurs in the bovine, higher concentrations of circulating 25-(OH)D_3 may be obtained with direct supplementation of 25-(OH)D_3 compared with supplementing vitamin D_3. Synthesis of 1,25-$(OH)_2D_3$ from 25-(OH)D_3 is regulated, but direct supplementation of 25-(OH)D_3 compared with vitamin D_3 may increase 1,25-$(OH)_2D_3$ concentrations to a greater extent. Intramuscular in-

Received December 3, 2014.
Accepted April 13, 2015.
[1]Corresponding author: Weiss.6@osu.edu

jections of 4 or 8 mg of 25-(OH)D_3 reduced clinical hypocalcemia in dairy cows, but timing of the injection before parturition was critical (Olson et al., 1973). Dietary supplementation of 25-(OH)D_3 would eliminate the injection timing issue. Supplementing peripartum dairy cows with 25-(OH)D_3 when fed an anionic diet increased plasma Ca concentrations in the peripartum period (Wilkens et al., 2012), but had no effect when supplemented with a diet containing a positive DCAD (Taylor et al., 2008). Anionic diets increase the conversion of 25-(OH)D_3 to 1,25$(OH)_2D_3$ (Goff et al., 1991); however, in the Wilkens et al. (2012) study, only a mild compensated acidosis was achieved based on urine pH. We hypothesized that supplementing 25-(OH)D_3 to cows fed an anionic diet that induced a substantial metabolic acidosis would enhance Ca status of peripartum cows and reduce the prevalence of clinical and subclinical hypocalcemia. In addition, supplementing 25-OHD_3 should increase concentrations of 25-(OH)D_3 and 1,25$(OH)_2D_3$ in plasma and colostrum, which may improve vitamin D status of the young calf.

MATERIALS AND METHODS

Cows and Diets

Dietary treatments and all procedures conducted on the cows in the experiment were approved by The Ohio State University Institutional Animal Care and Use Committee. Fifty-four dry Holstein cows (>1 gestation) were blocked into groups of 3 based on gestation number and anticipated calving date. While in the dry cow pen, all cows were fed a common diet that was formulated to meet NRC (2001) requirements, including vitamin D (18,000 IU of supplemental vitamin D_3/d). Cows were moved from the dry cow pen into individual boxstalls 10 d before anticipated calving and fed 1 of 3 prepartum diets (Tables 1 and 2). Treatments were (1) control (supplemental vitamin D_3), (2) supplemental vitamin D_3 with supplemental anions (**DCAD + D**), and (3) 6 mg/d of supplemental 25-(OH)D_3 (Rovimix, Hy-D, 1.25%, DSM Nutritional Products, Basel, Switzerland) plus supplemental anions (**DCAD + 25D**). The DCAD (Na + K − Cl − S) was 165 mEq/kg for control and −138 and −139 mEq/kg for the DCAD + D and DCAD + 25D, respectively. Average duration of the prepartum experimental period was 13 d (Table 3).

During the prepartum phase, all cows were fed 3.5 kg of concentrate DM (contained treatments) and 4.7 kg of a mix of 30:30:40 corn:grass:alfalfa silages DM daily. To ensure consumption of the concentrate mix, the concentrate and silage blend were mixed and offered to the cows at approximately 0800 h. A weighed amount of hay was offered at approximately 1100 h. Additional hay (measured) was offered at approximately 1600 h based on appetite. Daily feed refusal was measured to calculate daily DMI during the prepartum period. After calving, cows remained in the boxstalls for 3 d and then moved into individual tiestalls and fed a common lactation diet until 28 DIM that contained supplemental vitamin D_3 but no supplemental anions or 25-(OH)D_3. Cows were weighed when moved into the boxstalls and when moved to the tiestalls. Daily milk yields and DMI were measured when cows were in tiestalls. Health treatments were at the discretion of the herd manager and veterinarian. Calcium (i.v., oral gels, or oral bolus) was given when deemed necessary and a blood sample was taken immediately before treatment when practicable (in 2 cases blood was not sampled before Ca administration).

Calves were removed from their dam immediately after birth and not allowed to consume colostrum. The cow was milked and 2 L of fresh colostrum was fed to their calf and another 2 L was fed 12 h later. Calves were fed only colostrum or transition milk from their dams through 6 feedings (2 L/feeding). One calf from a cow fed DCAD + 25D was fed frozen colostrum from another cow fed DCAD + 25D because her dam did not produce sufficient colostrum.

Sampling and Analyses

On average, cows calved 3 d later than anticipated; therefore, the first day of the experiment was designated −13 d. Urine was collected from cows via vulva stimulation on −13 d (before treatment diets were fed), after cows were fed treatment diets for 5 d (averaged 8 d before actual calving), and at 7 DIM. Urine pH was measured immediately using a portable pH meter (Oakton pHTestr 10, Oakton Instruments, Vernon Hills, IL). A subsample of urine was frozen and analyzed later for minerals by inductively coupled plasma spectrometry after microwave digestion and creatinine (Creatinine Urinary Assay Kit No. 500701, Cayman Chemical Co., Ann Arbor, MI). Blood was sampled from the tail vein of cows when moved to the boxstalls, 5 d after diets were initiated and at −2 d before anticipated calving. If cows did not calve on the anticipated date, blood was sampled repeatedly until calving. The blood samples closest to 5 and 2 d before actual calving were used for analysis. Blood was also sampled within 6 h after parturition and at 2 and 7 DIM. Blood was allowed to clot and serum was harvested and frozen in multiple vials until analyzed for Ca (Calcium Liquicolor No. 0150, Stanbio Laboratory, Boerne, TX), P (Phosphorus Liqui-UV No. 0830, Stanbio), Mg (Magnesium Liquicolor No. 0130, Stanbio), BHBA (β-Hydroxybutyrate Liquicolor No. 2440, Stanbio), NEFA [NEFA-HR(2), Wako Chemicals,

Table 1. Composition of prefresh diets and the common lactation diet (% of DM)

	Prepartum diet[1]			
Item	Control	DCAD + D	DCAD + 25D	Lactation
Mature grass hay	37.7	36.7	36.9	—
Midmaturity grass silage	10.4	10.6	10.5	—
Corn silage	10.2	10.6	10.4	30.0
Alfalfa silage	14.4	14.8	14.8	22.0
Corn grain	15.90	16.00	16.10	18.16
Soybean meal	5.62	—	—	8.2
Soybean hulls	3.439	2.379	2.45	5.3
Animal-vegetable fat	0.27	0.27	0.27	0.9
Bio-chlor[2]	—	6.35	6.29	—
Magnesium sulfate	0.14	0.14	0.14	—
Calcium chloride	—	0.71	0.67	—
Limestone	0.68	0.24	0.27	1.25
Prepartum supplement[3]	1.21	1.21	1.21	—
Lactation supplement[4]	—	—	—	14.14
Vitamin D premix[5]	0.051	0.051	—	—
25-(OH)D Premix[6]	—	—	0.0037	—

[1]The control diet contained vitamin D_3 and had a DCAD of 165 mEq/kg. The DCAD + D diet contained vitamin D_3 and DCAD = −139 mEq/kg. The DCAD + 25D diet contained 25-(OH)D_3 and DCAD = −138 mEq/kg.

[2]Arm & Hammer Animal Nutrition (Church & Dwight Co. Inc., Princeton, NJ).

[3]Contained 182 g of NaCl, 116 g of MgO, 820 mg of Cu ($CuSO_4$), 1,520 mg of Zn ($ZnSO_4$), 30 mg of Se (Na_2SeO_4), 730 kIU of vitamin A, 8,900 IU of vitamin E (Rovimix E-50, DSM Nutritional Products, LLC, Parsippany, NJ), 140 mg of biotin (Rovimix, DSM Inc.), and 1.7 g of monensin (Elanco Animal Health, Inc., Indianapolis IN) per kilogram.

[4]Contained 168 g of soybean meal, 791 g of dried distillers grains, 34 g of NaCl, 148 mg of Cu ($CuSO_4$), 184 mg of Zn (from Zn-met, Zinpro Inc., Eden Prairie, MN),50 mg of Se (Na_2SeO_4), 560 kIU of vitamin A, 205 kIU of vitamin D, 3,260 IU of vitamin E, and 125 mg of biotin/kg.

[5]Contained 3,000 IU of vitamin D/g (as vitamin D_3). Formulated to provide 18,000 IU/d.

[6]Rovimix HyD-1.25% (DSM Inc.) containing 12.5 g of 25-(OH)D_3/kg of premix. The diet was formulated to provide 6 mg of 25-(OH)D_3/d.

Richmond, VA], and PTH (Bovine Intact PTH ELISA Kit, Immutopics Inc., San Clemente, CA) using 96-well plates and a Bio-tek Microplate Reader (Bio-tek Instruments, Winooski, VT) with KC4 software. Vitamin D compounds in serum were assayed using RIA (Hollis et al., 1993; Hollis et al., 1996).

First-milking colostrum, sixth-milking transition milk, and milk on 28 DIM was sampled and frozen until

Table 2. Nutrient composition of diets (DM basis)

	Prepartum diet[1]			
Itme	Control	DCAD + D	DCAD + 25D	Lactation
CP, %	14.6	14.5	14.5	17.6
NDF, %	41.8	41.8	41.6	30.0
Ca, %	0.88	1.00	0.90	1.28
P, %	0.30	0.32	0.32	0.36
Mg, %	0.31	0.36	0.37	0.25
K, %	2.00	1.92	1.91	1.42
Na, %	0.13	0.32	0.33	0.43
S, %	0.24	0.36	0.37	0.28
Cl, %	0.89	1.91	1.89	0.51
Cu, mg/kg	15	16	14	16
Mn, mg/kg	64	81	73	52
Zn, mg/kg	60	73	96	65
DCAD,[2] mEq/kg	165	−139	−138	230

[1]The control diet contained vitamin D_3 and no supplemental anions. The DCAD + D (dietary cation, anion difference) treatment contained vitamin D_3 and supplemental anions. The DCAD + 25D diet contained 25-(OH)D_3 and supplemental anions.

[2]Calculated as: (K + Na) − (S + Cl) expressed as milliequivalents per kilogram of diet DM.

Table 3. Effect of treatment on production measures

	Treatment[1]				*P*-value[2]	
Item	Control	DCAD + D	DCAD + 25D	SEM	DCAD	VD
Prepartum period (cows fed different treatments)						
Days fed treatment	12.4	12.9	13.8	1.03	<0.73	<0.51
Precalving BW, kg	765	743	750	14.0	<0.27	<0.71
Calf birth weight[3], kg	44.6	44.5	45.2	1.44	<0.97	<0.73
DMI (−10 to −1 d), kg/d	12.2	11.6	11.6	0.43	<0.40	<0.99
Postpartum period (all cows fed a common diet)						
Postcalving BW, kg	697	682	678	13.7	<0.44	<0.82
DMI (3 to 28 d), kg/d	20.4	19.8	19.8	0.84	<0.63	<0.99
Milk (3 to 28 d), kg/d	45.2	41.7	41.7	1.54	<0.11	<0.99

[1]During the prepartum period, control diet contained vitamin D_3 and no supplemental anions. The DCAD + D treatment contained vitamin D_3 and supplemental anions. The DCAD + 25D diet contained 25-(OH)D_3 and supplemental anions. Diets were fed starting 10 d before anticipated calving until calving. A common diet was fed after parturition to all cows.

[2]DCAD = effect of feeding supplemental anions with vitamin D (control vs. DCAD + D); VD = effect of type of supplemental vitamin D (DCAD + D vs. DCAD + 25D).

[3]Sex of calf affected BW (male > female), but a treatment × sex interaction was not observed ($P > 0.75$).

analyzed for cholecalciferol (vitamin D_3) and 25-(OH) D_3 by Analytical Research Center (DSM Nutritional Products, Ltd.). A blood sample was taken from the jugular vein of all calves immediately after birth (before colostrum was consumed) and again after the calf was fed 6 feedings of colostrum or milk from their dam. Serum was harvested and samples were frozen until assayed for vitamin D metabolites and Ca as described for cow serum.

Feeds were sampled weekly and composited by month. The composited silage samples were lyophilized and ground through a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA). Feed samples were analyzed for DM (100°C for 24 h), ash (600°C muffle oven overnight), NDF (Ankom Fiber Analyzer, Ankom Technology, Fairport, NY) with sodium sulfite and amylase (Ankom #FAA), CP (Kjeldahl N × 6.25), minerals (Ca, P, Mg, K, Na, Cu, Mn, and Zn) by inductively coupled plasma spectrometry after nitric-perchloric acid digestion (concentrates) or oven-ashing (forages), Cl (potentiometric titration, Brinkman Metrohm Titrino 848 Plus, Brinkman Instruments, Westbury, NY), and S (Leco S-144DR Sulfur Combustion Analyzer, Leco Corp., St. Joseph, MI).

Statistical Analyses

The PTH data had an extremely skewed distribution and the transformation that resulted in the most normal distribution was $1/\sqrt{\text{PTH}}$. The data were back-transformed after statistical analyses. Cow and calf serum measures, DMI (standardized to include only the last 10 d of gestation), milk yield, and concentrations of vitamin D metabolites were analyzed using a model (PROC MIXED; SAS Institute, 2011) that included block (random), day (repeated measure), treatment, and treatment × day interaction. The SLICE option was used when treatment × day interaction was significant ($P < 0.10$). Serum (cow and calf), milk, and urine data were also analyzed within each time point using a model that included the main effect of treatment and the random effect of cow. Two contrasts were used to partition treatment effects within day: effect of DCAD with supplemental vitamin D (control vs. DCAD + D) and effect of feeding 25-(OH)D_3 in a DCAD diet (DCAD + D vs. DCAD + 25D). Almost all the colostrum and milk samples from cows on the DCAD + 25D treatment and all samples of milk at 28 DIM were below the quantification limit (<60 ng/mL) for cholecalciferol. Therefore, vitamin D concentrations in colostrum and transition milk were statistically evaluated using the repeated model, except only control and DCAD + D treatments were used. Relationships among various measures were evaluated using regression (PROC REG of SAS). Incidence of hypocalcemia (subclinical, defined as blood calcium ≤8 mg/dL with no clinical signs and clinical defined animals treated with i.v. Ca) was evaluated using PROC FREQ with Fisher's exact test (SAS Institute, 2011). A likelihood-ratio test was used to assess treatment differences in the variation in serum Ca concentrations using the GROUP option of the MIXED procedure.

RESULTS AND DISCUSSION

The number of cows that calved per treatment was 16 for control, 17 for DCAD + D, and 20 for DCAD + 25D. The original protocol called for 18/treatment; however, to ensure adequate numbers of animals for the primary treatment of interest (DCAD + 25D), 3

additional cows were enrolled in the DCAD + 25D treatment. This was because 1 cow fed DCAD + 25D died 2 d after calving and another cow fed DCAD + 25D developed severe hypocalcemia approximately 24 h before calving and Ca therapy had to be administered (no d-0 blood sample was taken). The number of cows that completed the experiment was 15 for control, 16 for DCAD + D, and 19 for DCAD + 25D. One control cow was euthanized on 17 DIM because of a severe injury that occurred when the cow fell. One cow on the DCAD + D treatment was euthanized at 20 DIM after developing an unresponsive abdominal infection following surgery for a displaced abomasum. One cow on DCAD + 25D was found dead in the boxstall at 2 DIM. No health issues were identified and the cow received no veterinary treatments before dying. However, when the d-0 blood sample was analyzed, the cow was classified as subclinical hypocalcemic (6.34 mg/dL). A necropsy was conducted but the cause of death could not be determined.

Treatment did not affect DMI prepartum and calculated average intake of 25-(OH)D_3 was 5.4 mg/d and intakes of supplemental vitamin D for control and DCAD + D treatments were 18,700 and 17,700 IU/d, respectively (Table 3). A meta-analysis (Charbonneau et al., 2006) found a negative relationship between dietary DCAD and DMI; however, DMI of multiparous cows was not affected by negative DCAD diets in several individual studies (Moore et al., 2000; Grünberg et al., 2011; Wilkens et al., 2012). Milk yield and DMI in the first 28 d of lactation were not affected by treatment (Table 3).

Urine Measures

Feeding DCAD diets reduced ($P < 0.01$) urine pH measured 5 d after supplementation began and cows fed DCAD + 25D tended ($P < 0.10$) to have higher urinary pH than cows fed DCAD + D (Table 4). Including 25-(OH)D_3 in diets with a negative DCAD compared with similar diets without 25-(OH)D_3 reduced urine pH in one study (Gibbens, 2012), but not in another (Wilkens et al., 2012). The increased urinary pH for cows fed DCAD + 25D could be caused by the increased Ca concentration in their urine (Constable et al., 2009). The greatly increased urinary Ca excretion when DCAD + D or DCAD + 25D were fed (Table 4) indicates increased Ca flux in those cows. Inducing metabolic acidosis by feeding negative DCAD diets increases bone resorption and reduces bone accretion. Approximately 45% of Ca in the negative DCAD diets was provided by $CaCl_2$ (no $CaCl_2$ was included in the control diet), and because the Ca from $CaCl_2$ is highly available (Goff and Horst, 1993) it may have increased Ca absorption by cows on those diets. Increasing serum concentrations of 1,25-$(OH)_2$ D also increases Ca absorption from the gut and bone resorption. Excluding cows fed DCAD + 25D, urinary Ca or creatine was negatively correlated with urinary pH (Figure 1; $r = -0.76$; $P < 0.01$), as reported previously (Grünberg et al., 2011). However, for cows fed DCAD + 25D, urinary pH was not correlated with Ca or creatinine ($P > 0.25$) and urinary excretion of Ca was high even when metabolic acidosis was not extreme. This suggests that for those cows Ca flux was mostly influenced by vitamin D, rather than metabolic acidosis. Assuming a constant urinary excretion of creatinine of 29 mg/kg of BW (Valadares et al., 1999), cows fed DCAD + 25D excreted about 1.7 g more Ca/d via urine than did cows fed DCAD + D (approximately 8 g/d more than control cows). Urine pH or urinary Ca excretion (measured at −5 d) were not correlated ($P > 0.3$) with serum Ca concentrations on −2 d, calving, or 2 DIM, and no apparent relationship between urine measures and prevalence of subclinical or clinical hypocalcemia were observed (discussed herein). By 7 DIM, urinary Ca excretion and urine pH did not differ among treatments (Table 4).

Serum Vitamin D Metabolites and PTH

Because of the high cow-to-cow variation, reaching statistically valid conclusions for the PTH data was difficult. Concentrations of PTH followed the same time profile for all treatments (time × treatment interaction; $P > 0.6$), with concentrations increasing until calving and then decreasing (Table 5). The time profile was similar to previous studies (Shappell et al., 1987; Wilkens et al., 2012). When all time points were included, cows fed either of the DCAD diets tended ($P < 0.13$) to have lower PTH concentrations than cows fed the control. No differences were observed between the 2 DCAD treatments. Wilkens et al. (2012) reported lower PTH concentrations in cows fed negative DCAD diets compared with those fed positive DCAD, with no effect of feeding 25-(OH)D_3. In Wilkens et al. (2012), treatment mean PTH concentrations were inversely related to plasma Ca concentrations as would be expected, but treatment differences in PTH concentrations were much greater than treatment differences in plasma Ca. We also observed very large numeric differences in PTH between control and cows fed DCAD (with or without 25-OH vitamin D) and only small and usually nonsignificant differences in serum Ca. One function of PTH is to induce activity of renal 1-α-hydroxylase, resulting in increased conversion of 25-(OH)D_3 to 1,25-$(OH)_2D_3$. Goff et al. (2014) showed that plasma 1,25-$(OH)_2D_3$ response to PTH depended on whether cows were fed a negative or positive DCAD diet; cows

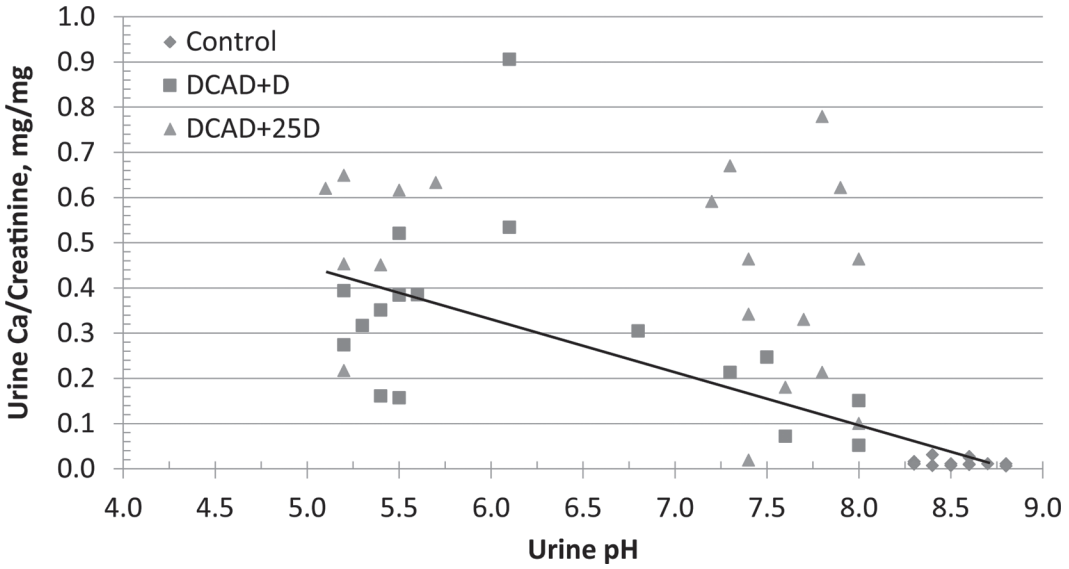

Figure 1. Relationship between urine pH and excretion of urinary Ca measured 5 d after start of treatments. The regression line includes data from cows fed control and DCAD + vitamin D (DCAD + D) diets (Y = 1.03 – 0.117X; $r^2 = 0.58$). The Ca-to-creatinine ratio was not correlated ($P > 0.25$) to urine pH for cows fed the DCAD + 25 (OH)D diet (DCAD + 25D). Color version available online.

fed negative DCAD had much greater increase in plasma 1,25-$(OH)_2D_3$ following PTH administration. Our data generally support the idea that high DCAD reduces tissue sensitivity to PTH in that cows fed control had much higher PTH concentrations but similar serum 1,25-$(OH)_2D_3$ concentrations, ratios of serum 1,25-$(OH)_2D_3$ to 25-$(OH)D_3$, and Ca concentrations as cows fed the DCAD + D diet.

Concentrations of 25-$(OH)D_3$ in serum were affected ($P < 0.001$) by treatment, sampling day, and the day by treatment interaction (Table 5). Time did not affect concentrations of 25-$(OH)D_3$ of cows fed control or DCAD + D, but for cows fed DCAD + 25D concentrations of 25-$(OH)D_3$ increased until about −2 DIM and were then maintained through calving and started to decline by 7 DIM. At calving, concentrations of 25-

Table 4. Effect of treatment on urine measures[1]

	Treatment[2]				*P*-value[3]	
Item	Control	DCAD + D	DCAD + 25D	SEM	DCAD	VD
pH						
−13 d	8.58	8.59	8.58	0.037	<0.91	<0.91
−5 d	8.54	6.23	6.79	0.231	<0.001	<0.09
7 DIM	8.36	8.31	8.14	0.096	<0.76	<0.21
Ca, mg/L						
−13 d	20.7	19.1	32.2	5.3	<0.82	<0.08
−5 d	18.9	342.1	437.9	42.9	<0.001	<0.11
7 DIM	53.3	62.3	105.6	30.3	<0.83	<0.30
Creatinine, mg/L						
−13 d	1162.3	1054.0	1262.4	80.2	<0.35	<0.07
−5 d	1142.3	1073.2	1104.3	89.5	<0.60	<0.80
7 DIM	805.5	796.7	705.2	68.7	<0.93	<0.33
Ca/Creatinine						
−13 d	0.018	0.019	0.026	0.039	<0.94	<0.21
−5 d	0.013	0.319	0.443	0.043	<0.001	<0.04
7 DIM	0.061	0.078	0.127	0.029	<0.70	<0.23

[1]At −13 d and 7 DIM cows were fed a common diet within day. The −5-d sample was taken 5 d after treatments started.

[2]Control diet contained vitamin D_3 and no supplemental anions; DCAD + D diet contained vitamin D_3 and supplemental anions; DCAD + 25D diet contained 25-$(OH)D_3$ and supplemental anions.

[3]DCAD = effect of feeding supplemental anions (control vs. DCAD + D); VD = effect of type of supplemental vitamin D (DCAD + D vs. DCAD + 25D).

Table 5. Effect of prepartum diet on serum concentrations of parathyroid hormone (PTH) and vitamin D metabolites in cows[1]

	Treatment[2]				*P*-value[3]	
Item	Control	DCAD + D	DCAD + 25D	SEM	DCAD	VD
PTH,[4] pEq/mL						
−13 DIM	NA	NA	NA	—	—	—
−5 DIM	962	378	625	211	<0.12	<0.32
−2 DIM	NA	NA	NA	—	—	—
Calving	1,568	808	961	441	<0.19	<0.76
2 DIM	1,309	518	883	336	<0.08	<0.22
7 DIM	793	512	635	158	<0.38	<0.62
25-(OH)D_3, ng/mL						
−13 DIM	75.5	76.9	72.1	4.3	<0.82	<0.43
−5 DIM	75.7	71.0	173.0	7.1	<0.66	<0.001
−2 DIM	91.1	75.8	270.0	12.4	<0.42	<0.001
Calving	82.1	65.8	274.3	13.1	<0.39	<0.001
2 DIM	74.1	66.9	261.0	10.6	<0.65	<0.001
7 DIM	74.9	59.6	249.5	13.4	<0.43	<0.001
1,25 $(OH)_2$ D, pg/mL						
−13 DIM	98.5	94.6	87.9	14.3	<0.85	<0.74
−5 DIM	111.5	104.6	122.9	15.9	<0.77	<0.41
−2 DIM	133.7	98.9	194.1	25.7	<0.37	<0.02
Calving	166.2	151.5	225.3	24.2	<0.67	<0.04
2 DIM	205.2	195.4	320.8	32.6	<0.84	<0.01
7 DIM	125.6	101.2	161.7	17.6	<0.33	<0.02

[1]At −13, 2, and 7 DIM all cows were fed a common diet within day. Time and time × treatment effects were significant ($P < 0.01$) for all variables. NA = not analyzed.

[2]Control diet contained vitamin D_3 and no supplemental anions; DCAD + D treatment contained vitamin D_3 and supplemental anions; DCAD + 25 D diet contained 25-(OH)D_3 and supplemental anions.

[3]*P*-values for contrasts: DCAD = effect of feeding supplemental anions (control vs. DCAD + D); VD = effect of type of supplemental vitamin D (DCAD + D vs. DCAD + 25D).

[4]Data were transformed as 1/(SQRT) before statistical analysis and then back transformed.

(OH)D_3 were more than 3 times greater in cows fed DCAD + 25D than for cows fed the other diets. Similar to 25-(OH)D_3, serum concentrations of 1,25-$(OH)_2D_3$ were affected ($P < 0.001$) by treatment, sampling day, and the day × treatment interaction (Table 5). Time affected concentrations of 1,25-$(OH)_2D_3$ for all treatments ($P < 0.01$), with peak concentrations at 2 DIM. Feeding DCAD did not affect 1,25-$(OH)_2D_3$, but starting at −2 DIM through 7 DIM cows fed DCAD + 25D had greater ($P < 0.05$) concentrations than did cows fed DCAD + D. Whereas concentrations of 25-(OH) D_3 were elevated by −5 DIM, 1,25-$(OH)_2D_3$ was not elevated until −2 DIM, and rather than a 3 to 4 times increase in concentrations as observed with 25-(OH)D_3, concentrations of 1,25-$(OH)_2D_3$ were about 1.5 times greater for DCAD + 25D than for cows fed DCAD + D.

The number of days individual cows were fed 25-(OH)D_3 ranged from 7 to 24 d and concentrations of 1,25-$(OH)_2D_3$ at −2 d (r = 0.84) and at calving (r = 0.84) were positively correlated ($P < 0.001$) with number of days they were fed 25-(OH)D_3 before the blood sample was taken. Serum concentrations of 25-(OH)D_3 were not correlated with duration of feeding ($P > 0.8$).

Although temporal patterns for serum 25-(OH)D_3 were similar to previous studies (Gibbens, 2012; Wilkens et al., 2012), peak concentrations in cows fed 25-(OH) D_3 were greater in our study, likely because 3 mg/d of 25-(OH)D_3 was fed by Wilkens et al. (2012) and Gibbens (2012) whereas cows in our study consumed about 6 mg/d. The temporal pattern for 1,25-$(OH)_2D_3$ in our study differed from previous studies. In both Wilkens et al. (2012) and Gibbens (2012), feeding 25-(OH)D_3 did not affect concentrations of 1,25-$(OH)_2D_3$ until after parturition, but in our study concentrations of 1,25-$(OH)_2D_3$ were higher ($P < 0.01$) for cows fed DCAD + 25D at −2 d and at parturition than for the other treatments. The differences between studies may be a result of the differing supplementation rates (3 vs. 6 mg/d). Peak concentrations of serum 1,25-$(OH)_2D_3$ occurred at 2 DIM independent of treatment in agreement with previous studies (Barton et al.,1981; Gibbens 2012; Wilkens et al., 2012). Although the amount of 25-(OH)D_3 supplemented was greater in our study, peak concentrations of 1,25-$(OH)_2D_3$ were similar to those in Wilkens et al. (2012) and Gibbens (2012). The ratio of serum 1,25-$(OH)_2D_3$ to 25-(OH) D_3 was lower ($P < 0.01$) for cows fed DCAD + 25D than for cows on other treatments at all time points after supplementation started (data not shown). The lower ratio of 1,25-$(OH)_2D_3$ to 25-(OH)D_3 for cows fed 25-(OH)D_3 may indicate that elevated concentrations

of 1,25 downregulated 1-α-hydroxylase (Engstrom et al., 1987). High concentrations of 1,25-$(OH)_2D_3$ can also upregulate 24-hydroxylase, which would increase conversion of 25-$(OH)D_3$ into 1,24,25-$(OH)_3$ and 24,25$(OH)_2D_3$ (not measured) which is thought to have limited or no metabolic activity.

Considering the high 25-$(OH)D_3$ concentrations observed in the treated cows, one might think that 25-$(OH)D_3$ itself might be directly signaling Ca homeostasis via binding to vitamin D receptor (**VDR**). However, 25-$(OH)D_3$ is a poor ligand for VDR, with an approximate 1% cross reactivity with VDR compared with 1,25-$(OH)_2D_3$ when both are in their free state (Reinhardt et al., 1982). In addition, only about 0.03% of serum 25-$(OH)D_3$ is free for cell entry (Schwartz et al., 2014), and, based on that assumption, the concentration of free 25-$(OH)D_3$ in serum, even for cows fed DCAD + 25D, would not be a high enough to act as VDR ligand. Although it cannot be completely ruled out, the high serum 25-$(OH)D_3$ concentrations observed are unlikely directly activating VDR in this instance.

Serum Minerals and Energy Metabolites

Treatment had little effect on serum Mg, but did influence serum Ca and P concentrations (Table 6). Cows fed diets with negative DCAD prepartum often have greater concentrations of serum Ca than cows fed diets with positive DCAD (Block, 1984; Charbonneau et al., 2006; DeGroot et al., 2010; Grünberg et al., 2011), but no effect was observed in our experiment. Average serum Ca concentrations measured for control cows were in the normal range at all time points, which likely reduced potential responses to supplemental anions. Cows fed DCAD + 25D had greater ($P < 0.01$) concentrations of serum Ca than cows fed DCAD + D at −5 d, but no differences were observed at other time points. Wilkens et al. (2012) and Gibbens (2012) reported that feeding a negative DCAD diet with 3 mg/d of 25-$(OH)D_3$ increased serum Ca concentrations at parturition and for up 24 h postpartum, but we observed no effect of treatment on postpartum Ca concentrations. Perhaps the higher daily dose of 25-$(OH)D_3$ (6 mg/d) used in our study increased concentrations of 1,25-$(OH)_2D_3$ for a long enough period of time and to a great enough extent to cause a self-induced reduction in tissue responsiveness to the hormone (Reinhardt and Horst, 1989), thereby eliminating the positive effects observed in previous studies.

Treatment did not affect mean concentrations of serum Ca at calving or at 2 DIM; however, treatment did affect variation among cows in serum Ca concentrations. Serum Ca concentrations were more variable for cows fed DCAD + 25D at 2 DIM than cows fed the other 2 treatments ($P < 0.05$). At calving, serum Ca concentrations were more variable for cows fed DCAD + 25D than cows fed DCAD + D ($P < 0.05$), but variation was similar between cows fed DCAD + 25D and the control diet. Duration of 25-$(OH)D_3$ supplementation, serum concentrations of 1,25-$(OH)_2D$ and 25-$(OH)D_3$, urine pH, and lactation number were not significant sources of variation in serum Ca concentrations within the DCAD + 25D treatment. The cause of this greater variation is unknown but may explain why an apparent increase was observed in the prevalence of clinical milk fever but a decrease in subclinical hypocalcemia was noted in cows fed DCAD + 25D compared with cows fed the control diet.

Subclinical hypocalcemia was defined as cows that had serum Ca concentrations ≤8 mg/dL without clinical signs (Reinhardt et al., 2011). A case of clinical milk fever occurred when cows required i.v. Ca therapy (therapy was initiated based on clinical observations, not low blood Ca). Treatment did not affect prevalence of subclinical or clinical hypocalcemia ($P > 0.3$). For control, DCAD + D, and DCAD + 25D, 50.0, 41.2, and 35.0% of cows had subclinical hypocalcemia, and 12.5 (1 case confirmed by low blood Ca and no blood sample was taken from the other case), 0, and 20% of cows (3 cases confirmed by low blood Ca and no blood sample was taken for 1 case) had clinical milk fever, respectively. Subclinical hypocalcemia was identified in 22 (42%) cows sometime during the experiment (Figure 2). These rates are similar to prevalence rates observed in the field (Reinhardt et al., 2011).

Feeding DCAD + D did not affect serum P, except at 2 DIM when concentrations were higher ($P < 0.05$) than control (Table 6). Cows fed DCAD + 25D had elevated ($P < 0.08$) serum P concentrations compared with the DCAD + D treatment, similar to previous studies (Gibbens, 2012; Wilkens et al., 2012). Phosphorus absorption from the intestine is stimulated by 1,25-$(OH)_2D_3$, which could explain the elevated serum P concentrations.

Treatment did not affect concentrations of serum NEFA or BHBA (data not shown). Prepartum NEFA concentrations for all treatments were low (average = 208 μEq/L). Postpartum concentrations of NEFA ≥0.6 mEq/L are associated with increased disease risk (Ospina et al., 2010). Averages for all treatments and days (excluding the day of calving) were less than this threshold, except for cows fed DCAD + 25D on 2 DIM (0.78 mEq/L). This was likely caused by the greater number of cows with hypocalcemia (Reinhardt et al., 2011). At −2 ($r = -0.66$; $P < 0.001$) and 2 ($r = -0.45$; $P < 0.01$) DIM, serum Ca and NEFA were negatively correlated. Subclinical ketosis was defined as BHBA concentrations >1,200 μM (Duffield et al., 1998) and is

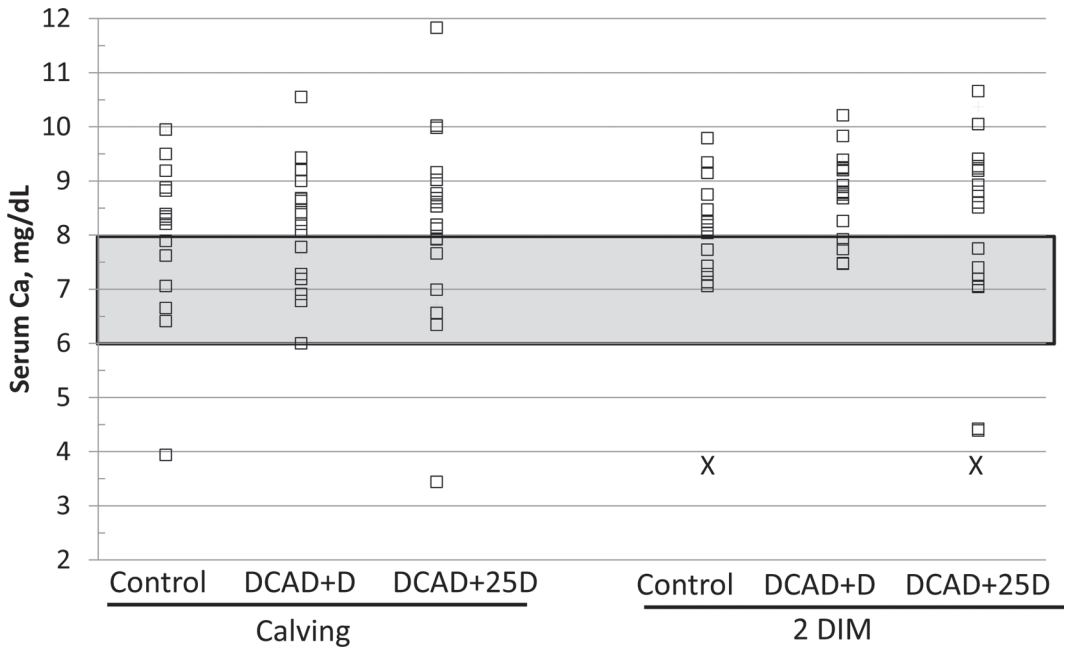

Figure 2. Concentrations of serum Ca at calving (<6 h postcalving) and at 2 DIM for cows fed control, DCAD + vitamin D (DCAD + D), and DCAD + 25-(OH)D_3 (DCAD + 25D). The shaded box indicates subclinical hypocalcemia. X = cows that had clinical milk fever and were treated with Ca and no blood sample was taken.

Table 6. Effect of treatment on serum minerals (mg/dL) in cows[1]

	Treatment[2]				*P*-value[3]	
Item	Control	DCAD + D	DCAD + 25D	SEM	DCAD	VD
Ca						
−13 DIM	9.52	9.68	9.56	0.15	<0.47	<0.56
−5 DIM	9.18	9.27	9.76	0.13	<0.62	<0.01
−2 DIM	9.11	9.30	9.64	0.24	<0.56	<0.35
Calving	8.07	8.24	8.23	0.36	<0.74	<0.98
2 DIM	8.14	8.76	8.50	0.33	<0.22	<0.58
7 DIM	9.49	9.18	9.32	0.19	<0.25	<0.57
P (inorganic)						
−13 DIM	6.13	6.34	6.05	0.12	<0.22	<0.08
−5 DIM	6.78	6.38	7.48	0.20	<0.19	<0.001
−2 DIM	6.40	6.98	8.10	0.28	<0.16	<0.01
Calving	4.60	4.41	5.16	0.30	<0.66	<0.08
2 DIM	5.15	6.12	6.02	0.32	<0.05	<0.82
7 DIM	5.41	5.68	6.06	0.35	<0.60	<0.44
Mg						
−13 DIM	2.49	2.50	2.45	0.044	<0.80	<0.35
−5 DIM	2.46	2.29	2.18	0.049	<0.02	<0.11
−2 DIM	2.33	2.32	2.23	0.042	<0.87	<0.11
Calving	2.62	2.56	2.44	0.064	<0.48	<0.17
2 DIM	2.44	2.56	2.65	0.09	<0.35	<0.42
7 DIM	2.26	2.24	2.13	0.07	<0.81	<0.24

[1]At −13, 2, and 7 DIM all cows were fed a common diet within day. Time and time × treatment effects were significant for Ca and P ($P < 0.01$).

[2]Control diet contained vitamin D_3 and no supplemental anions; DCAD + D diet contained vitamin D_3 and supplemental anions; DCAD + 25D diet contained 25-(OH)D_3 and supplemental anions.

[3]DCAD = effect of feeding supplemental anions (control vs. DCAD + D); VD = effect of type of supplemental vitamin D (DCAD + D vs. DCAD + 25D).

Table 7. Effect of prefresh diet of dam on vitamin D metabolites in their milk and in serum from their calves[1]

	Treatment[2]				*P*-value[3]	
Item	Control	DCAD + D	DCAD + 25D	SEM	DCAD	VD
Milk						
Vitamin D_3, pg/mL						
First milking colostrum	471	340	ND	55.1	0.09	—
Sixth milking	324	295	ND	75.4	0.77	—
25-$(OH)D_3$, pg/mL						
First milking colostrum	1,021	891	3690	163.2	0.72	0.001
Sixth milking	458	340	1088	167.7	0.64	0.001
Milk, 28 DIM	358	473	607	168.7	0.37	0.20
Serum of calves						
25-$(OH)D_3$, ng/mL						
Birth	45.7	42.3	80.2	44.4	0.51	0.001
6th feeding	37.1	35.5	57.6	44.3	0.68	0.001
1,25-$(OH)_2D$, pg/mL						
Birth	149.0	136.8	160.1	30.5	0.77	0.59
Sixth feeding	192.3	212.1	257.2	33.6	0.35	0.68
Calcium, mg/dL						
Birth	11.7	11.5	11.3	0.18	0.49	0.26
Sixth feeding	12.0	11.6	11.4	0.19	0.16	0.53

[1]Birth samples were taken before consuming colostrum and sixth milking sample was taken within 2 h after calves consumed their sixth feeding of milk. Calves were only fed colostrum or milk from their dams. For milk measures and serum 25-$(OH)D_3$, time and time × treatment were significant ($P < 0.01$), but only time was significant for 1,25-$(OH)_2$-D_3 ($P < 0.01$). Milk from cows fed DCAD + 25 D did not have measurable concentrations of vitamin D_3 and that treatment was excluded from the statistical analysis.

[2]Control diet contained vitamin D_3 and no supplemental anions; DCAD + D diet contained vitamin D_3 and supplemental anions; DCAD + 25D diet contained 25-$(OH)D_3$ and supplemental anions.

[3]DCAD = effect of feeding supplemental anions (control vs. DCAD + D); VD = effect of type of supplemental vitamin D (DCAD + D vs. DCAD + 25D).

a risk factor for several health disorders (Ospina et al., 2010). At 7 DIM, incidence of subclinical ketosis was 50, 35, and 20% of cows that were fed control, DCAD + D, and DCAD + 25D (DCAD + 25D < control; $P < 0.06$).

Milk Vitamin D and Vitamin D Status of Calves

No treatment (only control and DCAD + D were included in this analysis), time (colostrum vs. sixth milking), or time × treatment interactions effects were observed for vitamin D concentration in milk (Table 7). The concentrations of 25-$(OH)D_3$ in colostrum and transition milk (i.e., sixth milking) were greater for cows fed DCAD + 25D ($P < 0.001$), but by 28 DIM concentrations were similar among treatments. Concentrations of 25-$(OH)D_3$ in colostrum and sixth milking milk were correlated ($P < 0.001$) with serum concentrations of 25-$(OH)D_3$ (Figure 3). The slope relating concentrations of 25-$(OH)D_3$ in serum and milk at 2 DIM was essentially the same as reported by Hollis et al. (1986) for humans; the slope relating 25-$(OH)D_3$ concentrations in serum (sampled at −2 DIM) to colostrum was much greater, indicating different mechanisms regulate concentrations of 25-$(OH)D_3$ in colostrum compared with milk. For cows fed DCAD + 25D, concentrations of 25-$(OH)D_3$ in colostrum (r = 0.60; $P < 0.01$) and sixth milking milk (r = 0.57; $P < 0.01$) were positively correlated with number of days the supplement was fed. Duration of supplementation of 25-$(OH)D_3$ to the dam was also correlated (r = 0.46; $P < 0.05$) with concentration of 25-$(OH)D_3$ in serum of calves at birth.

Calves from cows fed DCAD + 25D had greater ($P < 0.01$) concentrations of 25-$(OH)D_3$ in serum at birth (before colostrum was fed) and after their sixth feeding (Table 7). Concentrations of 25-$(OH)D_3$ decreased ($P < 0.03$) with age for all treatments; whereas concentrations of 1,25-$(OH)_2D_3$ increased for all treatments (Table 7), but treatment had no effect on concentrations at either age. Rajaraman et al. (1997) reported similar changes with age when calves were fed milk from cows not supplemented with 25-$(OH)D_3$. Concentrations of serum 25-$(OH)D_3$ in calves at birth were correlated (r = 0.79; $P < 0.001$) with concentrations of 25-$(OH)D_3$ in the serum of their dams (Figure 4), similar with previous results (Goff et al., 1982). The optimal concentration of 25-$(OH)D_3$ in serum of calves with respect to immune function have not been determined, but probably range between 30 and 100 ng/mL (Nelson et al., 2012). Elevating serum 25-$(OH)D_3$ concentrations is generally associated with improved immune function in vitro and in vivo in bovine and human models (Liu et al., 2006; Nelson et al., 2010). The greater concentrations of 25-$(OH)D_3$ in neonatal calves

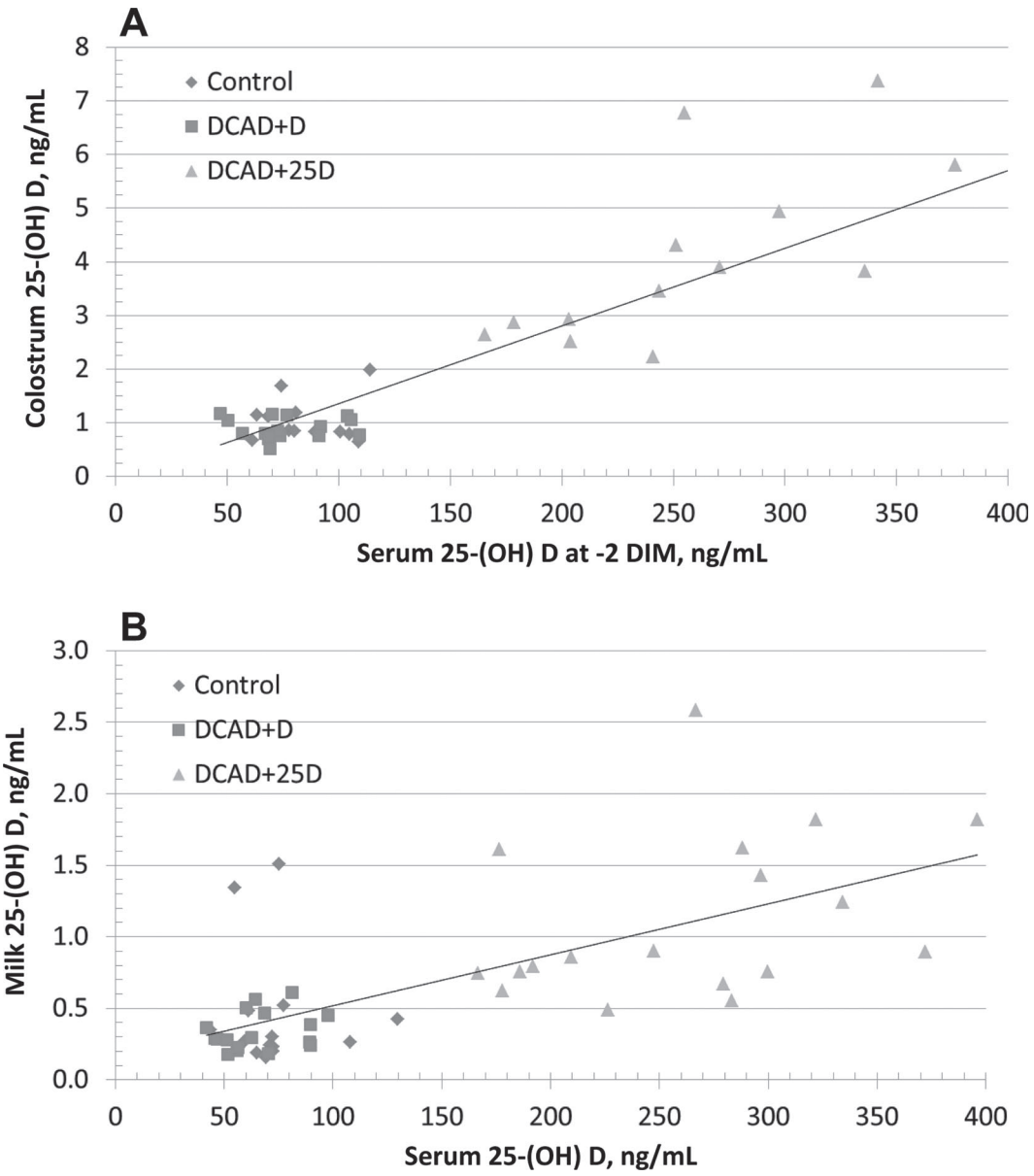

Figure 3. (A) Relationship between 25-(OH)D_3 in serum of cows 2 d before calving and concentration of 25-(OH)D_3 in colostrum. The regression line includes all treatments ($Y = -0.085 + 0.0145X$; $r^2 = 0.74$). (B) Relationship between 25-(OH)D_3 in serum of cows and concentration of 25-(OH)D_3 in milk both sampled 2 d after calving. The regression line includes all treatments ($Y = 0.16 + 0.0036X$; $r^2 = 0.46$). DCAD + D = DCAD + vitamin D; DCAD + 25D = DCAD + 25-(OH)D_3. Color version available online.

from cows fed DCAD + 25D may confer some measure of improved immune function.

CONCLUSIONS

Supplementing cows with 25-(OH)D_3 in combination with a negative DCAD diet for the last 13 d of gestation enhanced vitamin D status of the cows based on serum concentrations of 25-(OH)D_3 and 1,25-$(OH)_2D_3$. This translated into increased concentrations of 25-(OH)D_3 in colostrum and milk and in the serum of the neonatal calf. A short-lived increase in serum Ca before calving occurred with this treatment, but no effect on serum Ca was observed at calving and in the early postpartum period. Although no statistical differences were observed in the incidence of clinical milk fever, feeding 6 mg/d of 25-(OH)D_3 resulted in the highest incidence rate and it did not reduce subclinical hypocalcemia over DCAD + D diets alone.

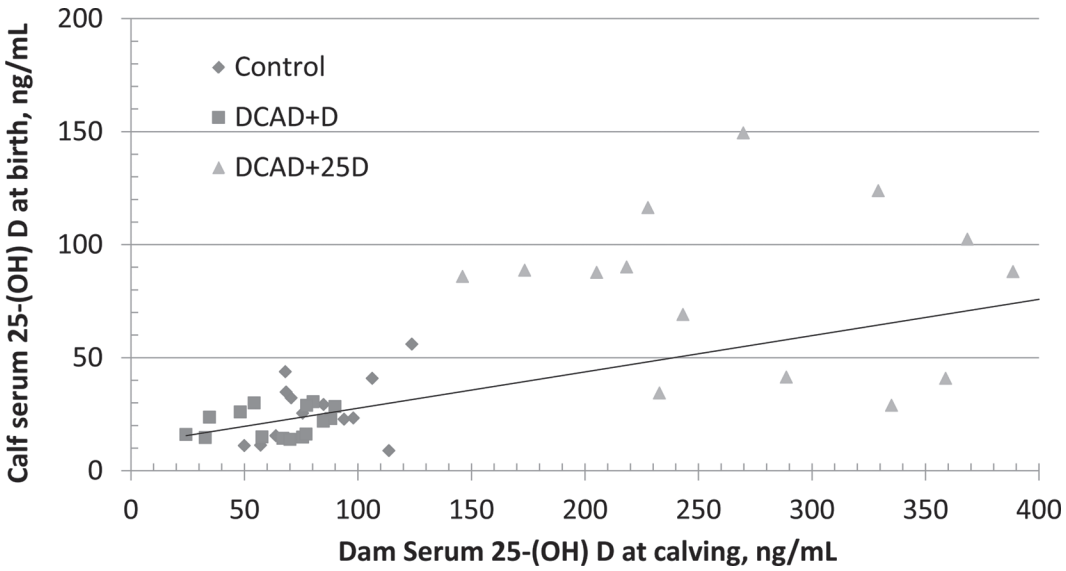

Figure 4. Relationship between concentrations of 25-(OH)D_3 in serum of cows and their calves at birth. Regression includes all treatments. Y = 11.6 + 0.16X (r^2 = 0.63). DCAD + D = DCAD + vitamin D; DCAD + 25D = DCAD + 25-(OH)D_3. Color version available online.

ACKNOWLEDGMENTS

Salaries and research support provided by state and federal funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University. Additional research support provided by DSM Nutritional Products Ltd., Basel, Switzerland.

REFERENCES

Barton, B. A., R. L. Horst, N. A. Jorgensen, and H. F. DeLuca. 1981. Concentration of calcium, phosphorus, and 1,25-dihydroxyvitamin D in plasma of dairy cows during the lactation cycle. J. Dairy Sci. 64:850–852.

Block, E. 1984. Manipulating dietary anions and cations for prepartum dairy cows to reduce incidence of milk fever. J. Dairy Sci. 67:2939–2948.

Chamberlin, W. G., J. R. Middleton, J. N. Spain, G. C. Johnson, M. R. Ellersieck, and P. Pithua. 2013. Subclinical hypocalcemia, plasma biochemical parameters, lipid metabolism, postpartum disease, and fertility in postparturient dairy cows. J. Dairy Sci. 96:7001–7013.

Chapinal, N., M. Carson, T. F. Duffield, M. Capel, S. Godden, M. Overton, J. E. P. Santos, and S. J. LeBlanc. 2011. The association of serum metabolites with clinical disease during the transition period. J. Dairy Sci. 94:4897–4903.

Chapinal, N., M. E. Carson, S. J. LeBlanc, K. E. Leslie, S. Godden, M. Capel, J. E. P. Santos, M. W. Overton, and T. F. Duffield. 2012. The association of serum metabolites in the transition period with milk production and early-lactation reproductive performance. J. Dairy Sci. 95:1301–1309.

Charbonneau, E., D. Pellerin, and G. R. Oetzel. 2006. Impact of lowering dietary cation-anion difference in nonlactating dairy cows: A meta-analysis. J. Dairy Sci. 89:537–548.

Constable, P. D., C.-C. Gelfert, M. Fürll, R. Staufenbiel, and H. R. Stämpfli. 2009. Application of strong ion difference theory to urine and the relationship between urine pH and net acid excretion in cattle. Am. J. Vet. Res. 70:915–925.

Crnkic, C., S. Muratovic, S. Piplica, A. Kavazovic, and S. Kutlaca. 2010. Blood plasma mineral profile and health status in postpartum cows fed an anionic diet before parturition. Turk. J. Vet. Anim. Sci. 34:255–260.

Curtis, C. R., H. N. Erb, C. J. Sniffen, R. D. Smith, and D. S. Kronfeld. 1985. Path analysis of dry period nutrition, postpartum metabolic and reproductive disorders, and mastitis in Holstein cows. J. Dairy Sci. 68:2347–2360.

DeGroot, M. A., E. Block, and P. D. French. 2010. Effect of prepartum anionic supplementation on periparturient feed intake, health, and milk production. J. Dairy Sci. 93:5268–5279.

Duffield, T. F., D. Sandals, K. E. Leslie, K. Lissemore, B. W. McBride, H. Lumsden, P. Dick, and R. Bagg. 1998. Efficacy of monesin for the prevention of subclinical ketosis in lactating dairy cows. J. Dairy Sci. 81:2866–2873.

Engstrom, G. W., J. P. Goff, R. L. Horst, and T. A. Reinhardt. 1987. Regulation of calf renal 25-hydroxyvitamin D-hydroxylase activities by calcium-regulating hormones. J. Dairy Sci. 70:2266–2271.

Gibbens, N. 2012. Influence of 25-hydroxyvitamin D and anionic salts on the calcium status of dairy cattle. MS Thesis, University of Pretoria, Pretoria, South Africa.

Goff, J. P., and R. L. Horst. 1993. Oral administration of calcium salts for treatment of hypocalcemia in cattle. J. Dairy Sci. 76:101–108.

Goff, J. P., R. L. Horst, and E. T. Littledike. 1982. Effect of the maternal vitamin D status at parturition on the vitamin D status of the neonatal calf. J. Nutr. 112:1387–1393.

Goff, J. P., R. L. Horst, F. J. Mueller, J. K. Miller, G. A. Kiess, and H. H. Dowlen. 1991. Addition of chloride to a prepartal diet high in cations increases 1,25-dihydroxy vitamin D response to hypocalcemia preventing milk fever. J. Dairy Sci. 74:3863–3871.

Goff, J. P., A. Liesegang, and R. L. Horst. 2014. Diet-induced pseudohypo para-thyroidism: A hypocalcemia and milk fever risk factor. J. Dairy Sci. 97:1520–1528.

Gröhn, Y., H. N. Erb, C. E. McCulloch, and H. S. Saloniemi. 1990. Epidemiology of reproductive disorders in dairy cattle: Associations among host characteristics, disease and production. Prev. Vet. Med. 8:25–39.

Grünberg, W., S. S. Donkin, and P. D. Constable. 2011. Periparturient effects of feeding a low dietary cation-anion difference diet on acid-base, calcium, and phosphorus homeostasis and on intravenous glucose tolerance test in high-producing dairy cows. J. Dairy Sci. 94:727–745.

Heaney, R. P., L. A. Armas, J. R. Shary, N. H. Bell, N. Binkley, and B. W. Hollis. 2008. 25-hydroxylation of vitamin D_3: Relation to

circulating vitamin D_3 under various input conditions. Am. J. Clin. Nutr. 87:1738–1742.

Hollis, B. W., J. Q. Kamerud, A. Kurkowski, J. Beaulieu, and J. L. Napoli. 1996. Quantification of circulating 1,25-dihydroxyvitamin D by radioimmunoassay with an ^{125}I-labeled tracer. Clin. Chem. 42:586–592.

Hollis, B. W., J. Q. Kamerud, S. R. Selvaag, J. D. Lorenz, and J. L. Napoli. 1993. Determination of vitamin D status by radioimmunoassay with an ^{125}I-labeled tracer. Clin. Chem. 39:529–533.

Hollis, B. W., W. B. Pittard III, and T. A. Reinhardt. 1986. Relationships among vitamin D, 25-hydroxyvitamin D, and vitamin D-binding protein concentrations in the plasma and milk of human subjects. J. Clin. Endocrinol. Metab. 62:41–44.

Horst, R. L., J. P. Goff, and T. A. Reinhardt. 1994. Calcium and vitamin D metabolism in the dairy cow. J. Dairy Sci. 77:1936–1951.

Lean, I. J., P. J. DeGaris, D. M. McNeil, and E. Block. 2006. Hypocalcemia in dairy cows: Meta-analysis and dietary cation anion difference theory revisited. J. Dairy Sci. 89:669–684.

Liu, P. T., S. Stenger, H. Li, L. Wenzel, B. H. Tan, S. R. Krutzik, M. T. Ochoa, J. Schauber, K. Wu, C. Meinken, D. L. Kamen, M. Wagner, R. Bals, A. Steinmeyer, U. Zügel, R. L. Gallo, D. Eisenberg, M. Hewison, B. W. Hollis, J. S. Adams, B. R. Bloom, and R. L. Modlin. 2006. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 311:1770–1773.

Martinez, N., L. D. P. Sinedino, R. S. Bisinotto, E. S. Ribeiro, G. C. Gomes, F. S. Lima, L. F. Greco, C. A. Risco, K. N. Galvão, D. Taylor-Rodriguez, J. P. Driver, W. W. Thatcher, and J. E. P. Santos. 2014. Effect of induced subclinical hypocalcemia on physiological responses and neutrophil function in dairy cows. J. Dairy Sci. 97:874–887.

Massey, C. D., C. Wang, G. A. Donovan, and D. K. Beede. 1993. Hypocalcemia at parturition as a risk factor for left displacement of the abomasum in dairy cows. J. Am. Vet. Med. Assoc. 203:852–853.

Moore, S. J., M. J. Vandehaar, B. K. Sharma, T. E. Pilbeam, D. K. Beede, H. F. Bucholtz, J. S. Liesman, R. L. Horst, and J. P. Goff. 2000. Effects of altering dietary cation-anion difference on calcium and energy metabolism in peripartum cows. J. Dairy Sci. 83:2095–2104.

Nelson, C. D., T. A. Reinhardt, J. D. Lippolis, R. E. Sacco, and B. J. Nonnecke. 2012. Vitamin D signaling in the bovine immune system: a model for understanding human vitamin D requirements. Nutrients 4:181–196.

Nelson, C. D., T. A. Reinhardt, T. C. Thacker, D. C. Beitz, and J. D. Lippolis. 2010. Modulation of the bovine innate immune response by production of 1α,25-dihydroxyvitamin D_3 in bovine monocytes. J. Dairy Sci. 93:1041–1049.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Oetzel, G. R., J. D. Olson, C. R. Curtis, and M. J. Fettman. 1988. Ammonium chloride and ammonium sulfate for prevention of parturient paresis in dairy cows. J. Dairy Sci. 71:3302–3309.

Olson, W. G., N. A. Jorgensen, L. H. Schultz, and H. F. DeLuca. 1973. 25-Hydroxycholecalciferol (25-OHD_3) II. Efficacy of parenteral administration in prevention of parturient paresis. J. Dairy Sci. 56:889–895.

Ospina, P. A., D. V. Nydam, T. Stokol, and T. R. Overton. 2010. Evaluation of nonesterified fatty acids and β-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. J. Dairy Sci. 93:546–554.

Rajaraman, V., B. J. Nonnecke, and R. L. Horst. 1997. Effects of replacement of native fat in colostrum and milk with coconut oil on fat-soluble vitamins in serum and immune function in calves. J. Dairy Sci. 80:2380–2390.

Reinhardt, T. A., and R. L. Horst. 1989. Self-induction of 1,25-dihydroxyvitamin D_3 metabolism limits receptor occupancy and target tissue responsiveness. J. Biol. Chem. 264:15917–15921.

Reinhardt, T. A., R. L. Horst, E. T. Littledike, and D. C. Beitz. 1982. 1,25-Dihydroxyvitamin D_3 receptor in bovine thymus gland. Biochem. Biophys. Res. Commun. 106:1012–1018.

Reinhardt, T. A., J. D. Lippolis, B. J. McCluskey, J. P. Goff, and R. L. Horst. 2011. Prevalence of subclinical hypocalcemia in dairy herds. Vet. J. 188:122–124.

Sakha, M., M. Mahmoudi, and M. G. Nadalian. 2014. Effects of dietary cation-anion difference on milk fever, subclinical hypocalcemia and negative energy balance in transition dairy cows. Res. Opin. Anim. Vet. Sci. 4:69–73.

SAS Institute. 2011. SAS/STAT 9.3 User's Guide. SAS Institute Inc., Cary, NC.

Schwartz, J. B., J. Lai, B. Lizaola, L. Kane, S. Markova, P. Weyland, N. A. Terrault, N. Stotland, and D. Bikle. 2014. A comparison of measured and calculated free 25(OH) vitamin D levels in clinical populations. J. Clin. Endocrinol. Metab. 99:1631–1637.

Shappell, N. W., J. H. Herbein, L. J. Deftos, and R. J. Aiello. 1987. Effects of dietary calcium and age on parathyroid hormone, calcitonin and serum and milk minerals in the periparturient dairy cows. J. Nutr. 117:201–207.

Taylor, M. S., K. F. Knowlton, M. L. McGilliard, W. M. Seymour, and J. H. Herbein. 2008. Blood mineral, hormone, and osteocalcin responses of multiparous jersey cows to an oral dose of 25-hydroxyvitamin D_3 or vitamin D_3 before parturition. J. Dairy Sci. 91:2408–2416.

Valadares, R. F. D., G. A. Broderick, S. C. Valadares, and M. K. Clayton. 1999. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. J. Dairy Sci. 82:2686–2696.

Wilkens, M. R., I. Oberheide, B. Schröder, E. Azem, W. Steinberg, and G. Breves. 2012. Influence of the combination of 25-hydroxyvitamin D_3 and a diet negative in cation-anion difference on peripartal calcium homeostasis of dairy cows. J. Dairy Sci. 95:151–164.