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Effects of feeding 25-hydroxyvitamin D_3 with an acidogenic diet during the prepartum period in dairy cows: Mineral metabolism, energy balance, and lactation performance of Holstein dairy cows

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ABSTRACT

Our objective was to determine the effects of feeding 25-hydroxyvitamin D_3 [25(OH)D_3], or vitamin D_3 (cholecalciferol) on plasma, mineral, and metabolite concentrations, mineral balance, mineral excretion, rumination, energy balance, and milk production of dairy cows. We hypothesized that supplementing 3 mg/d of 25(OH)D_3 during the prepartum period would be more effective than supplementing vitamin D_3 at the National Research Council (2001) levels to minimize calcium imbalance during the transition period and improve milk production of dairy cows. Forty multiparous, pregnant nonlactating-Holstein cows were enrolled in this study. Body weight, body condition score, parity, and milk yield in the previous lactation (mean ± standard deviation) were 661 ± 59.2, 3.46 ± 0.35, 1.79 ± 0.87, and 33.2 ± 6.43 kg/d, respectively. Cows were enrolled into the blocks (n = 20 for each treatment) at 30 d of the expected day of calving to receive an acidogenic diet (373 g/kg of neutral detergent fiber and 136 g/kg of crude protein, dry matter basis; −110 mEq/kg) associated with the treatments: (1) control (CTRL), vitamin D_3 at 0.625 mg/d (equivalent to 25,000 IU of vitamin D_3/d) or (2) 25(OH)D_3 at 3 mg/d (equivalent to 120,000 IU of vitamin D_3/d). All cows were fed with the base ration for 49 d after calving. Blood samples were taken on d 7, 0, 1, 2, 21, and 42, relative to calving. No effect of treatment was observed for prepartum dry matter intake or body condition score. A trend for increase of ionized Ca was observed for the cows fed 25(OH)D_3, compared with the CTRL, but no effect of treatment was detected for total Ca or total P. Feeding 25(OH)D_3 increased colostrum yield. The plasmatic concentration of 25-hydroxyvitamin D_3 was increased with 25(OH)D_3 supplementation. 25-Hydroxyvitamin D_3 supplementation increased plasma glucose concentration at parturition. The postpartum dry matter intake was not influenced by treatments. Feeding 25(OH)D_3 increases milk yield, 3.5% fat-corrected milk, and energy-corrected milk and improves milk yield components in early lactation. Overall, these findings suggest that 25(OH)D_3 at 3 mg/d can improve the energy metabolism and lactation performance, compared with the current-feeding practice of supplementing vitamin D_3 at 0.625 mg/d.

Key words: calcium homeostasis, glucose, prepartum diet, transition period

INTRODUCTION

The transition period is the most challenging phase for dairy cows due to all the metabolic and physiological changes that occur over late pregnancy and early lactation (Bell, 1995; Huzzey et al., 2005; Glosson et al., 2020). The negative energy balance (**NEB**) faced by cows plays a critical role in many changes that occur during the transition period, such as an increase in plasma concentrations of ketone bodies (Cardoso et al., 2020) and nonesterified fatty acids (**NEFA**; Wankhade et al., 2017), impaired neutrophil function (Hammon et al., 2006), fatty liver (McArt et al., 2012), and hypocalcemia (Horst et al., 1994).

Calcium metabolism plays an important role during the transition period and into the subsequent lactation period (Golder et al., 2021). Impaired health and reproduction were observed in cows that developed clinical and subclinical hypocalcemia (Martinez et al., 2012a;

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Rodríguez et al., 2017). Feeding acidogenic prepartum diets with a negative DCAD results in stimulation of Ca mobilization before calving and decreases clinical and subclinical hypocalcemia postpartum (Diehl et al., 2018; Rodney et al., 2018; Glosson et al., 2020).

Vitamin D_3 (cholecalciferol) plays a central role in mineral metabolism, especially Ca and P (Bronner, 1987). More specifically, parathyroid hormone (**PTH**) stimulates renal conversion of 25-hydroxyvitamin D_3 [**25(OH)D_3**], also known as calcidiol, to the active form, 1,25-dihydroxyvitamin D_3 [**1,25(OH)$_2D_3$**]. The 1,25(OH)$_2D_3$ increases concentrations of Ca and P in blood (Goff et al., 1991; Golder et al., 2021).

The NRC (2001) recommends 25,000 IU/d of vitamin D for a 680-kg mature dairy cow during the transition period; however, some studies have shown that vitamin D_3 supplementation was not enough to prevent subclinical hypocalcemia (Wilkens et al., 2012; Martinez et al., 2018b). Additionally, there are not sufficient data to indicate whether 25,000 IU/d of vitamin D_3 is adequate for optimal Ca homeostasis. According to Martinez et al. (2018b), replacing vitamin D_3 with 25(OH)D_3 (at 3 mg/d) during the prepartum period can improve postpartum lactation performance of Holstein cows. Poindexter et al. (2020) also concluded that feeding 25(OH)D_3 (at 1 or 3 mg/d) increased serum 25(OH)D_3 more effectively than supplemental vitamin D_3, resulting in increased serum mineral concentrations. Additionally, supplementing vitamin D_3 at 3 mg/d increased mammary immunity when compared with supplementing 1 mg/d (Poindexter et al., 2020). The use of an acidogenic diet associated with an active form of vitamin D would be more effective in improving Ca homeostasis reflecting a more integrated bone and mineral metabolism (Rodney et al., 2018a). However, these studies were performed under temperate settings, with greater milk production (+2.5 kg/d) and lower acidogenic salt addition to achieve a greater negative DCAD (−130 mEq/kg of DM; Martinez et al., 2018a) needed to promote a reduction in the blood pH. In addition, the greater K content of tropical forages (McNeill et al., 2002) presents a greater challenge in formulating diets that induce a compensated metabolic acidosis (Wildman et al., 2007).

To our knowledge, no studies have evaluated 25(OH)D_3, in association with an acidogenic diet, fed to Holstein dairy cows under tropical conditions in the transition period. We hypothesized that supplementing 3 mg/d of 25(OH)D_3 to Holstein cows during the transition period would increase the serum Ca concentration and, thus, increase the urine mineral excretion compared with the traditional feeding practice of supplementing 0.625 mg/d of vitamin D_3 (NRC, 2001). We also hypothesized that supplementation with 25(OH)D_3 could improve the homeostatic mechanisms of Ca, decreasing the negative energy status and increasing the productive performance of dairy cows (Rodney et al., 2018b). Our objective was to evaluate the effects of feeding vitamin D_3 at 25,000 IU/d (0.625 mg/d) or 25-hydroxyvitamin D_3 at 120,000 IU/d (3 mg/d) during the last 30-d prepartum period associated with an acidogenic diet on vitamin D and mineral metabolism, colostrum yield, and composition and lactation performance of Holstein dairy cows.

MATERIALS AND METHODS

The experiment was conducted at the "José Henrique Bruschi" Experimental Field, Embrapa Dairy Cattle, Coronel Pacheco, Minas Gerais, Brazil (21°33′22″ S, 43°06′15″ W). According to Köppen's classification, the climate of the region is a humid subtropical-climate, mesothermic, with hot and rainy summer and cold and dry winter seasons. All the procedures were approved by the Embrapa Dairy Cattle Animal Care and Use Committee (protocol #7946051218).

Animals, Treatment Diets, and Housing

Forty multiparous-Holstein cows were enrolled in the study. Body weight, BCS, lactation number, and milk yield in the previous lactation (mean ± SD) were 661 ± 59.2 kg, 3.46 ± 0.361, 1.79 ± 0.865, and 33.2 ± 6.43 kg/d, respectively. Sample size was calculated with the POWER procedure of SAS (version 9.3, SAS Institute Inc.) to detect differences in blood concentrations of total Ca, ionized Ca, and vitamin D metabolite as described by Rodney et al. (2018a). The experiment was a randomized complete-block design with cows as the experimental unit. Cows were blocked according to the expected calving date (n = 40 cows; 5 blocks, 8 cows/block, and 20 cows/treatment) to receive the acidogenic diet (373 g/kg of NDF and 136 g/kg of CP, DM basis; DCAD of −110 mEq/kg of DM) containing the treatments during 30 d prepartum. Treatments were (1) control (**CTRL**): vitamin D_3 at 0.625 mg/d (equivalent to 25,000 IU of vitamin D_3/d; Rovimix D_3 500, DSM Nutritional Products) or (2) 25-hydroxyvitamin D_3 [25(OH)D_3] at 3 mg/d (equivalent to 120,000 IU of vitamin D_3/d; Rovimix Hy-D 1.25%, DSM Nutritional Products), in addition to the acidogenic base ration (NRC, 2001). The dose of vitamin D_3 in the CTRL was based on NRC (2001). The dose of vitamin D_3 in 25(OH)D_3 treatment was based on Martinez et al.

(2018a). Cows remained in the treatment diets for 27.3 ± 8.48 d.

Cows were housed in a freestall barn equipped with electronic devices (feeders, water drinkers, and weighing devices; Intergado) and a cooling system (DeLaval) consisting of automatic fans and sprinklers located in the feed line.

During the 30 d prepartum, cows were fed the acidogenic ration containing the treatments (Table 1) once daily at 0800 h. Upon calving, all cows were fed the base lactation ration twice daily at 0800 and 1530 h during 49 DIM. All diets were fed as TMR (Table 1), formulated to meet the nutrient requirements specified by the NRC (2001). Refusals were weighed daily before the morning feeding, and feed allowances were calculated daily with a goal of 5% refusals.

Chemical Analysis and Measurement of Dry Matter Intake

Feed samples (ingredients, diet offered, and orts) were collected daily, dried in a forced ventilation oven at 55°C for 72 h, ground (Wiley mill; A. H. Thomas) through a 1-mm screen sieve, and composited (DM basis) by week for each cow and then for each treatment. Samples were analyzed for DM, OM, total N, and ether extract according to methods 930.15, 942.05, 984.13, and 920.39, respectively (AOAC International, 2005). To estimate the CP, a conversion factor of 6.25 was used to convert N values into CP. Neutral detergent fiber and ADF were determined sequentially using Ankom 220 Fiber Analyzer (Ankom Technology). For NDF analysis, a thermostable α-amylase was used without the addition

Table 1. Ingredient and chemical composition of the experimental diets

Item	Prepartum diet[1]		Lactation diet
	CTRL	25(OH)D_3	
Ingredient, g/kg of DM			
Corn silage	744.5	744.5	300.0
Tifton hay	93.7	93.7	45.0
Soybean meal	136.7	136.7	205.0
Ground corn grain	—	—	305.0
Whole cottonseed	—	—	112.0
Prepartum mineral[2]	25.1	25.1	—
Lactation mineral[3]	—	—	33.0
Nutrient composition (DM basis; ± SD)			
DM, %	38.2 ± 3.1	38.5 ± 2.9	53.3 ± 1.7
NE_L[4]	1.59 ± 0.09	1.59 ± 0.08	1.51 ± 0.10
OM, %	93.2 ± 0.4	92.6 ± 1.3	92.9 ± 0.7
CP, %	13.4 ± 1.6	13.7 ± 2.1	16.8 ± 1.3
NFC,[5] %	38.5 ± 4.7	37.2 ± 4.4	41.8 ± 3.2
FDN, %	37.8 ± 4.0	36.8 ± 3.7	28.9 ± 2.8
Ca, %	0.53 ± 0.07	0.53 ± 0.13	0.68 ± 0.10
P, %	0.27 ± 0.09	0.27 ± 0.09	0.41 ± 0.14
Mg, %	0.23 ± 0.02	0.22 ± 0.01	0.27 ± 0.05
K, %	0.87 ± 0.09	0.87 ± 0.09	1.11 ± 0.14
Cl, %	0.48	0.48	—
Na, %	0.09	0.09	—
S, %	0.38	0.38	—
DCAD,[6] mEq/kg of DM	−110	−110	275.0

[1]Acidogenic prepartum diets containing the treatments were fed 30 d before the expected calving date. CTRL: vitamin D_3 at 0.625 mg/d, equivalent to 25,000 IU of vitamin D_3/d (Rovimix D_3 500, DSM Nutritional Products) or 25-hydroxyvitamin D_3 [25(OH)D_3] at 3 mg/d (Rovimix Hy-D 1.25%, DSM Nutritional Products).

[2]Prepartum mineral: custom blend containing (DM basis) 105 g of Ca, 25 g of P, 100 g of S, 40 g of Mg, 25.35 g of Na, 130 g of Cl, 12 mg of Co, 600 mg of Cu, 30 mg of Cr, 600 mg of Fe, 60 mg of I, 1,600 mg of Mn, 16 mg of Se, 2,400 mg of Zn, 480,000 IU of vitamin A, 12,000 IU of vitamin E, 80 mg of biotin, 1.5 $\times 10^9$ cfu of *Saccharomyces cerevisiae*, and 250 mg of F.

[3]Lactation mineral: custom blend containing (DM basis) 105 g of Ca, 35 g of P, 18 g of S, 36.5 g of Mg, 50 g of K, 107.25 g of Na, 11.8 mg of Co, 415 mg of Cu, 16.5 mg of Cr, 875 mg of Fe, 17.5 mg of I, 1,165 mg of Mn, 15 mg of Se, 2,330 mg of Zn, 170,000 IU of vitamin A, 60,000 IU of vitamin D_3, 1,000 IU of vitamin E, 66.5 mg of biotin, and 1.25 $\times 10^9$ cfu of *Saccharomyces cerevisiae*.

[4]Estimated using the equations proposed by the NRC (2001) based on the chemical analysis of dietary ingredients and DMI of 11.0 kg/d prepartum and 18 kg/d postpartum.

[5]Nonfiber carbohydrates were calculated using the equation NFC = 100 − [CP + NDF + fat + ash − (NDF insoluble protein)].

[6]Dietary cation-anion difference was calculated using the equation [(mEq of Na + mEq of K) − (mEq of Cl + mEq of S)].

of sodium sulfite. The NFC (g/kg of DM) contents were calculated by difference as

$$\text{NFC} = 100 - \text{CP} + \text{NDF} + \text{ether extract} + \text{ash} - (\text{NDF insoluble protein}).$$

The energetic density of the diets was estimated using chemical analysis of dietary ingredients, calculated for 11.0 and 20.0 kg of DMI for the prepartum and postpartum periods, respectively, using the NRC (2001).

Body Weight and Body Condition Score

Cows were weighed daily throughout the trial using the electronic weighing device (Intergado). The BCS was evaluated on the day of enrollment in the experiment and then weekly throughout the trial by the same trained evaluator, using a 1 to 5 scale (Ferguson et al., 1994) with increments of 0.25 units as described in the Elanco BCS chart (Elanco Animal Health, 2009).

Yield and Mineral Content of Colostrum

Cows were milked within the first 6 h after calving and colostrum yield was measured. Duplicate samples were collected and frozen at −20°C for later analysis of total Ca and Mg according to Bird et al. (1961).

Milk Yield and Composition

Cows were milked twice daily at 0700 and 1600 h, and the milk yield was recorded automatically using the Delpro Manager System software (Delpro, DeLaval), equipped with electronic milk measurer MM23, controls MPC 580/680, and an automatic extractor for sampling milk. Individual samples were collected 3 times per week in 2 sequential milkings (morning and afternoon) for measurements of concentrations of fat, protein, TS, SNF, and urea nitrogen by mid-infrared absorption spectrometry using Bentley model FCM (Bentley Instruments). Milk SCC was analyzed by flux cytometry (SomaCount FCM; Bentley Instruments). The 3.5% FCM, ECM, and net energy (**NE**) were calculated as follows: 3.5% FCM = 0.4324 × milk kg + (16.218 × milk fat kg); ECM = [(0.3246 × milk yield) + (12.86 × fat yield) + (7.04 × protein yield)]; NE = (0.0929 × fat %) + (0.0563 × protein %) + (0.0395 × lactose %).

Net Energy Balance

Energy balance was measured using the caloric intake obtained from the DMI and the energy content of the diets estimated according to NRC (2001), using the NE_L system. The energy requirement for maintenance was calculated according to NRC (2001), using the metabolic BW ($0.08 \times BW^{0.75}$). Calories required for gestation during the prepartum period were estimated at 3.7 Mcal of NE_L/d for a calf that would eventually be born with a BW of 43 kg (NRC, 2001). The calories secreted as milk were calculated according to yields of fat, protein, and lactose according to NRC (2001): calories secreted = milk yield × [(0.0929 × fat %) + (0.0563 × protein %) + (0.0395 × lactose %)].

Blood Sample Collection

Blood samples were collected 2 h after the morning feeding (between 0800 and 1000 h) by puncture of the coccygeal blood vessels into 10-mL evacuated tubes (Vacutainer, Becton Dickinson) containing no anticoagulant agents for serum separation or into tubes containing K_3-EDTA or sodium fluoride for plasma separation. After collection, samples were immediately placed on ice until processing. The tubes were centrifuged for 15 min at 2,500 × *g* (LS-3 Plus, CELM) at environmental temperature (25°C), for plasma and serum separation. Plasma and serum samples were transferred into multiple aliquots of 2.5 mL and stored in a freezer at −20°C for later analyses. Serum ionized Ca (**iCa**), total Ca (**tCa**), total Mg (**tMg**), and total P (**tP**) were analyzed immediately after collection. Blood samples used for plasma vitamin D analysis were centrifuged for 10 min at 1,800 × *g* at environmental temperature (25°C) for plasma separation and stored at −80°C until analysis.

Measurements of Serum Minerals

Serum iCa, tCa, tMg, and tP were determined on d 7 relative to calving and on d 0, 1, 2, and 21 after calving, using the ion-selective technique with an automatic ion analyzer (MHlab-ise; MH Equipamentos e Materiais para Laboratório Ltda.).

Vitamin D and Parathyroid Hormone in the Plasma

Plasma samples were collected on d −7 and 0, relative to calving, and on d 21, after calving, and analyzed for $25(OH)D_3$, using HPLC coupled with mass spectrometry (LC-MS/MS) detection by the LabFor Análises Laboratoriais Ltda. Parathyroid hormone was analyzed by chemiluminescence (VetLab).

Urine Collection and Analyses

Urine samples were collected twice per week before calving and on d 21 and 42 after calving, by massaging

the perineal area, until a copious stream of urine was obtained. Samples were stored in plastic tubes on ice and the pH was measured within 10 min after collection using a portable pH meter (MPA 210, MS Tecnopon). Creatinine concentration was analyzed by the calorimetric method (ref. 35, Labtest). Calcium and Mg concentrations in the urine samples were performed by spectrophotometry using an automatic analyzer (ref. 35, Labmax Plenno, Labtest).

Creatinine was used as a marker to estimate daily urinary volume using the constant value of 24.04 mg of creatinine per kg of BW per day (Chizzotti et al., 2008). The estimate of daily urine volume was calculated as follows:

$$\frac{\text{BW} \times 24.04}{\text{urinary concentrations of creatinine (mg/L)}}.$$

Daily urinary excretion of Ca and Mg were calculated as a product of urinary volume, and the concentrations of those minerals in the urine sample.

Estimated Mineral Balance

Calcium and Mg balances were calculated based on the differences in Ca and Mg intake and urinary losses of Ca and Mg during the last week of gestation, as well as the Ca and Mg concentrations in the colostrum on the day of calving. Estimated absorptions of Ca and Mg and fetal tissues accretion were computed according to diet and calf BW at birth (NRC, 2001). The mineral digestion was performed using 1.0 g of sample, burned at 525°C by 8 h, then, the residue was dissolved in 6.0 mL of nitric acid (HNO_3) with 25 g/L of perchloric acid ($HClO_4$; nitroperchloric digestion; Sarruge and Haag, 1974). The determination of mineral concentration (Ca, Mg, and K) in the diets was made by atomic absorption using a spectrophotometer (model AA-6800, Shimadzu Scientific Instruments). The P concentration analysis was performed using a spectrophotometer (UV Genesys 10s Vis, Thermo Scientific).

Physiological Responses

Seven days before calving and on d 0, 7, and 21 after calving, blood samples were collected for determination of BHB, NEFA, insulin, and glucose concentration. The glucose concentrations in the plasma were analyzed by glucose oxidation with glucose oxidase kits (Glucose PAP Liquiform, Labtest), using a microplate spectrophotometer and the software of interphase Gen5 (Eon Biotek). Nonesterified fatty acids and BHB were analyzed using colorimetric enzymatic assays (Randox Laboratories Ltd.). Insulin concentration was analyzed by enzyme immunoassays (Bovine Insulin ELISA, Mercodia Inc.). The threshold (iCa $\leq$1.0 mM) was selected to define subclinical hypocalcemia using whole blood (Oetzel et al., 1988). Hyperketonemia was defined as serum BHB concentrations >1.20 mM (Martinez et al., 2012).

Rumination Monitoring

Total time spent ruminating was measured using an Hr-Tag rumination monitoring system (SCR Engineers Ltd.). The system consisted of neck collars with rumination loggers built in, stationary readers, and software for processing the electronic data (Data Flow software, SCR Engineers Ltd.). A neck collar positioned the logger on the left side of the neck. The logger contained a microphone to record the distinctive sounds of regurgitation and rumination. Data were calculated and summarized at 2-h intervals and stored in the memory of the logger. For data analysis, the rumination time was taken during wk 1, 2, 3, 4, 5, and 6 relative to calving.

Statistical Procedures

The experiment was a randomized complete block design with cows used as the experimental unit in each block. The prepartum and postpartum periods were analyzed separately. The normality of the residues and the homogeneity of variance were examined for each continuous dependent variable analyzed after model fitting. Variables that violated the assumptions of normality were subjected to transformation, according to the Box-Cox procedure (Box and Cox, 1964), using the TRANSREG procedure of SAS version 9.3 (SAS/STAT, SAS Institute Inc.). All data were analyzed using the MIXED procedure of SAS. The Kenward-Roger method was used to calculate the approximate denominator degrees of freedom. The statistical model included the fixed-effect treatment of vitamin D_3 (CTRL), or 25-hydroxyvitamin D_3 25(OH)D_3, experimental day (or week for milk yield and composition), and its interaction. Block was included as a random effect, as well as cows nested within the interaction of treatment and day. All results were reported as least squares means. Significance was set at $P \leq 0.05$ and tendencies were determined if $P > 0.05$ and ≤ 0.10.

RESULTS

Prepartum Dry Matter Intake and Energy Balance

Feeding 25(OH)D_3 tended to increase DMI ($P = 0.08$) and caloric intake ($P = 0.09$) compared with the

Table 2. Dry matter intake, energy balance, BW, and BCS of multiparous pregnant Holstein dairy cows fed a prepartum acidogenic diet supplemented with CTRL or 25(OH)D_3

	Treatment[1]			
Item	CTRL	25(OH)D_3	SEM	*P*-value
DMI, kg/d	10.7	11.7	0.49	0.08
Caloric intake, Mcal/d	17.0	18.6	0.63	0.09
NE balance, Mcal/d	3.6	3.7	0.68	0.88
BW, kg	679.1	694.5	13.42	0.42
BW change, kg/d	1.02	1.33	0.035	<0.0001
BCS[2]	3.45	3.53	0.08	0.45

[1]Acidogenic prepartum diets containing the treatments were fed 30 d before the expected calving date. CTRL: vitamin D_3 at 0.625 mg/d, equivalent to 25,000 IU of vitamin D_3/d (Rovimix D_3 500, DSM Nutritional Products) or 25-hydroxyvitamin D_3 [25(OH)D_3] at 3 mg/d (Rovimix Hy-D 1.25%, DSM Nutritional Products).
[2]Using 1 to 5 scale according to Ferguson et al. (1994).

CTRL (11.7 vs. 10.7 kg/d and 18.6 vs. 17.0 Mcal/d, respectively; Table 2). Net energy balance ($P = 0.88$), BW ($P = 0.42$), and BCS ($P = 0.45$) were not affected by the treatments (Table 2). However, feeding 25(OH)D_3 increased the positive BW change ($P < 0.0001$) compared with the CTRL (1.33 vs. 1.02 kg/d, respectively; Table 2).

Colostrum Yield and Mineral Content

Feeding 25(OH)D_3 increased ($P = 0.02$) colostrum yield compared with the CTRL (5.44 vs. 7.50 kg, respectively). Treatments did not affect ($P \geq 0.13$) the concentration (g/L) and secretion (g/d) of Ca and Mg in colostrum (Table 3).

Concentration of Ionized and Total Ca, Total P, Total Mg, PTH, and 25-Hydroxyvitamin D_3

Treatments did not affect the prepartum (d −7) serum iCa concentrations ($P = 0.40$; Table 4). However, feeding 25(OH)D_3 tended ($P = 0.053$) to increase postpartum serum iCa concentration compared with the CTRL (1.31 vs. 1.24 m*M*, respectively; Figure 1A).

No treatment × day interactions were observed for serum concentration of tCa, tP, tMg, and PTH ($P \geq 0.26$; Table 4). However, a treatment × day interaction was detected for plasma 25(OH)D_3 ($P < 0.01$; Table 4). Plasma 25-hydroxyvitamin D_3 was greater for cows fed 25(OH)D_3 compared with the CTRL ($P < 0.001$) from d −7 to 21 relative to calving (Figure 1D). An effect of day ($P < 0.01$) was observed for serum concentration of tCa and tP (Figure 1B), and PTH (Figure 1C). Serum concentration of tCa was less on d 2 and 21 compared with d −7 ($P < 0.05$). Total serum concentration of P was greater on d 2 compared with d −7, 0, and 21 ($P < 0.05$). Serum concentration of PTH was greater on d 21 compared with d −7 ($P < 0.05$; Figure 1C).

Table 3. Colostrum yield and Ca and Mg secretion in colostrum of multiparous pregnant Holstein dairy cows fed a prepartum acidogenic diet supplemented with CTRL or 25(OH)D_3

	Treatment[1]			
Item	CTRL	25(OH)D_3	SEM	*P*-value
Colostrum yield, kg	5.44	7.50	0.71	0.02
Calcium, g/L	3.36	3.37	0.19	0.95
Calcium, g/d	18.3	25.3	3.53	0.13
Magnesium, g/L	0.530	0.533	0.03	0.93
Magnesium, g/d	2.88	3.99	0.56	0.13

[1]Acidogenic prepartum diets containing the treatments were fed 30 d before the expected calving date. CTRL: vitamin D_3 at 0.625 mg/d, equivalent to 25,000 IU of vitamin D_3/d (Rovimix D_3 500, DSM Nutritional Products) or 25-hydroxyvitamin D_3 [25(OH)D_3] at 3 mg/d (Rovimix Hy-D 1.25%, DSM Nutritional Products).

Blood Metabolites

No treatment or treatment × day interactions were observed for plasma concentration of insulin ($P \geq 0.44$; Table 5), but an effect of day was observed ($P = 0.004$; Table 5). The concentration of insulin was less on d 7 compared with d −7 and 21 ($P < 0.05$), but not compared with d 0 ($P = 0.27$; Figure 2B).

A treatment × day interaction was observed for glucose and NEFA ($P \geq 0.004$; Table 5). The concentration of glucose was greater for cows fed 25(OH)D_3 on d 0 compared with the CTRL (Figure 2A: $P \leq 0.05$), and NEFA was less for cows fed 25(OH)D_3 on d 7 postpartum compared with the CTRL (Figure 2C; $P \leq 0.05$).

No treatment, day, or treatment × day interactions were observed for plasma concentrations of BHB ($P \geq 0.10$, Table 5).

Milk Yield and Composition

Effect of treatment ($P \leq 0.05$; Table 6) and week ($P < 0.01$; Figure 3), but not treatment × week interaction

Table 4. Serum concentration of ionized Ca (iCa), total Ca (tCa), total P (tP), total Mg (tMg), parathyroid hormone (PTH), and 25-hydroxyvitamim D_3 of multiparous pregnant Holstein dairy cows fed a prepartum acidogenic diet supplemented with CTRL or 25(OH)D_3; all minerals expressed in m*M*

	Treatment[1]			*P*-value		
Item	CTRL	25(OH)D_3	SEM	Treatment	Day[2]	Treatment × day
Prepartum period						
iCa	1.31	1.34	0.026	0.40	—	—
tCa	2.54	2.62	0.073	0.34	—	—
tP	2.22	2.32	0.140	0.56	—	—
tMg	0.93	0.97	0.039	0.15	—	—
Postpartum period						
iCa	1.24	1.31	0.031	0.05	0.09	0.38
tCa	2.41	2.47	0.04	0.22	<0.01	0.62
tP	2.22	2.17	0.07	0.57	<0.01	0.41
tMg	1.07	0.99	0.07	0.41	0.32	0.26
PTH, pg/mL	12.2	11.6	1.39	0.16	<0.0001	0.53
25(OH)D_3, ng/mL	66.6	245.3	14.1	<0.0001	<0.0001	<0.0001

[1]Acidogenic prepartum diets containing the treatments were fed 30 d before the expected calving date. CTRL: vitamin D_3 at 0.625 mg/d, equivalent to 25,000 IU of vitamin D_3/d (Rovimix D_3 500, DSM Nutritional Products) or 25-hydroxyvitamin D_3 [25(OH)D_3] at 3 mg/d (Rovimix Hy-D 1.25%, DSM Nutritional Products).

[2]Blood samples for mineral analyses were performed at d −7, 0, 1, 2, and 21 relative to calving; collection of blood samples for analyses of PTH and 25(OH)D_3 was performed at d −7, 0, and 21 relative to calving.

($P \geq 0.42$; Table 6) were observed for milk yield, 3.5% FCM, ECM, protein (kg/d), fat (kg/d), and lactose (kg/d). Feeding 25(OH)D_3 increased milk yield [CTRL = 29.7 vs. 25(OH)D_3 = 32.5 kg/d; $P = 0.03$], 3.5%FCM [CTRL = 29.1 vs. 25(OH)D_3 = 33.9 kg/d; $P = 0.03$], ECM [CTRL = 29.3 vs. 25(OH)D_3 = 32.4 kg/d; $P = 0.03$], fat [CTRL = 1.05 vs. 25(OH)D_3 = 1.17 kg/d; $P = 0.054$], protein [CTRL = 0.88 vs. 25(OH)D_3 = 0.96 kg/d; $P = 0.04$], and lactose [CTRL = 1.36 vs. 25(OH)D_3 = 1.49 kg/d; $P = 0.04$] compared with the CTRL (Table 6).

Treatments did not affect the percentage of protein, fat, and lactose, or urea ($P \geq 0.78$; Table 6).

Postpartum Dry Matter Intake, Energy Balance, and Rumination Time

A tendency of treatment × week interaction was observed for NE intake and NEB ($P = 0.09$; Table 7; Figure 4C). Cows fed 25(OH)D_3 had lower energy intake only during the first week of lactation, but CTRL had lower NE intake during the 4 wk after parturition ($P < 0.05$; Figure 4A).

Treatments did not affect postpartum DMI, BW, BW change, or BCS ($P \geq 0.15$; Table 7). Net energy secreted as milk tended to the greater for cows fed 25(OH)D_3 compared with the CTRL [CTRL = 19.9 vs. 25(OH)D_3 = 21.7 Mcal/d; $P = 0.08$; Table 7; Figure 4B].

An effect of week was observed for DMI, NE secreted as milk, BW, BW change, and BCS ($P < 0.003$; Table 7). Overall all these variables increased with the progress of lactation (data not shown).

No treatment ($P = 0.66$) effect was observed, but week ($P < 0.0001$) and treatment × week interaction ($P = 0.03$) effects were detected for rumination time (Table 7). Cows fed CTRL had greater rumination time on wk 3, 5, and 6 relative to calving compared with 25(OH)D_3 ($P < 0.05$; Figure 4), with no differences observed between the treatments on week −1, 1, 2, and 4 relative to calving ($P > 0.05$; Figure 5).

Prepartum and Postpartum Urine Excretion of Minerals and Mineral Balance

Treatments did not affect prepartum urinary volume, creatinine concentration, urinary concentration of Mg, or urine pH ($P \geq 0.25$; Table 8). However, cows fed 25(OH)D_3 had greater prepartum urinary Ca concentration (mg/L) and excretion (g/d) than CTRL [CTRL = 345 mg/L or 8.01 g/d vs. 25(OH)D_3 = 448.3 mg/L or 12.83 g/d; $P \leq 0.03$; Table 8].

No effects of treatment, day, or treatment × day interaction were observed for postpartum urinary volume, creatinine, and Mg excretion (g/d; $P \geq 0.23$; Table 8). A treatment × day interaction was detected for urinary concentration of Mg ($P = 0.02$; Table 8). The urinary concentration of Mg (mg/L) was higher on d 42 for cows fed CTRL than 25(OH)D_3 ($P \leq 0.05$), and for cows fed 25(OH)D_3 the opposite occurred ($P = 0.019$; data not shown).

Prepartum Ca balance was lesser for cows fed 25(OH)D_3 than CTRL [CTRL = 24.0 vs. 25(OH)D_3 = 11.9 g/d; $P < 0.0001$; Table 9]. Treatments did not affect prepartum Mg balance ($P = 0.64$; Table 9).

Table 5. Plasma glucose, insulin, nonesterified fatty acids (NEFA), and BHB concentrations of multiparous pregnant Holstein dairy cows fed a prepartum acidogenic diet supplemented with CTRL or 25(OH)D_3

	Treatment[1]			*P*-value		
Item	CTRL	25(OH)D_3	SEM	Treatment	Day[2]	Treatment × day
Glucose, m*M*	3.18	3.63	1.68	0.0017	<0.0001	0.004
Insulin, ng/mL	0.37	0.34	0.031	0.44	0.004	0.52
NEFA, m*M*	0.43	0.32	0.016	<0.0001	<0.0001	<0.0001
BHB, m*M*	0.72	0.70	0.022	0.36	0.10	0.76

[1]Acidogenic prepartum diets containing the treatments were fed 30 d before the expected calving date. CTRL: vitamin D_3 at 0.625 mg/d, equivalent to 25,000 IU of vitamin D_3/d (Rovimix D_3 500, DSM Nutritional Products) or 25-hydroxyvitamin D_3 [25(OH)D_3] at 3 mg/d (Rovimix Hy-D 1.25%, DSM Nutritional Products).

[2]Blood samples for metabolites were performed at d −7, 0, 1, 2, and 21 relative to calving.

The Ca balance in the postpartum period was lesser for cows fed 25(OH)D_3 than CTRL [CTRL = 13.2 vs. 25(OH)D_3 = –16.3 g/d; $P = 0.001$; Table 9]. Postpartum Mg balance was not affected by treatment ($P = 0.11$; Table 9).

DISCUSSION

The current study aimed to evaluate the 3 mg/d of 25(OH)D_3 versus the current recommendations of vitamin D_3 (0.625 mg/d) for dairy nutrition under tropical

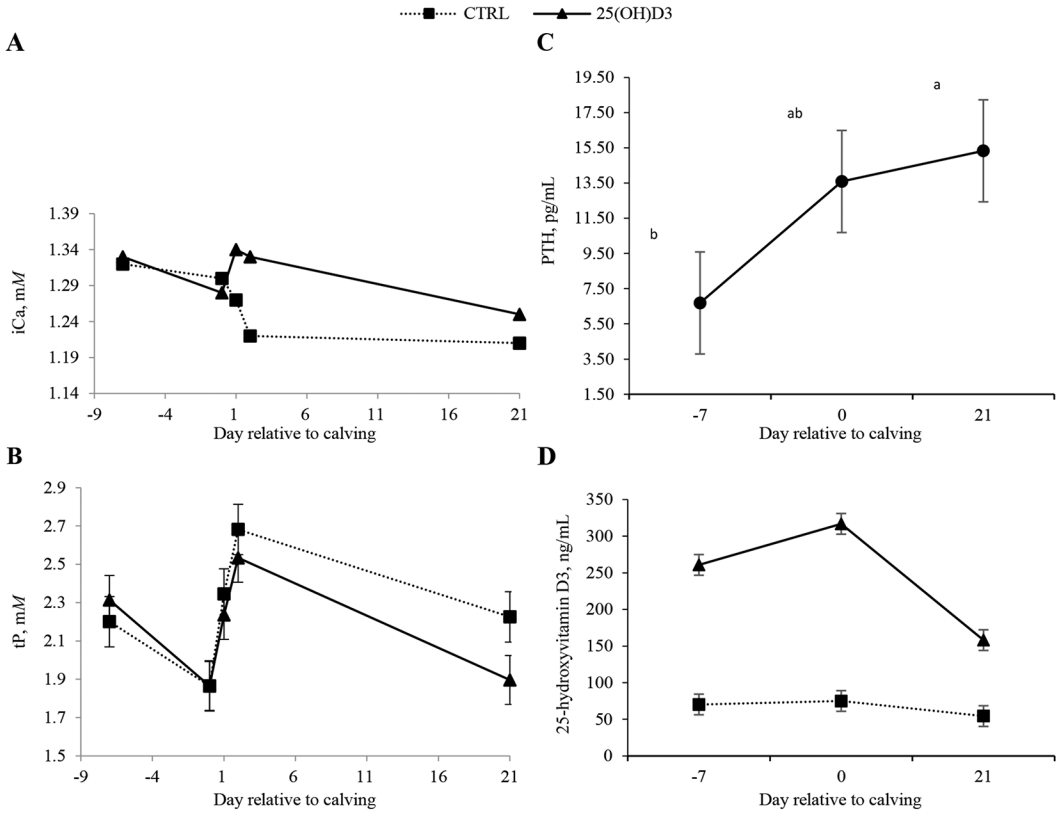

Figure 1. Serum concentration of total ionized Ca (iCa) and total P (tP), parathyroid hormone (PTH), and 25-hydroxyvitamim D_3 of multiparous pregnant Holstein dairy cows fed a prepartum acidogenic diet supplemented with control (CTRL) or 25(OH)D_3. Acidogenic prepartum diets containing the treatments were fed 30 d before the expected calving date. CTRL: vitamin D_3 at 0.625 mg/d, equivalent to 25,000 IU of vitamin D_3/d (Rovimix D_3 500, DSM Nutritional Products) or 25-hydroxyvitamin D_3 [25(OH)D_3] at 3 mg/d (Rovimix Hy-D 1.25%, DSM Nutritional Products). Panel A: effect of treatment ($P = 0.06$), effect of the day ($P = 0.02$), and interaction between treatment and day ($P = 0.28$). Panel B: effect of treatment ($P = 0.38$), effect of the day ($P < 0.0001$), and interaction between treatment and day ($P = 0.34$). Panel C: effect of treatment ($P \geq 0.16$), effect of the day ($P < 0.0001$), and interaction between treatment and day ($P \geq 0.41$). Means with different letters (a, b) are different ($P < 0.05$). Panel D: effect of treatment ($P < 0.0001$), effect of the day ($P < 0.0001$), and interaction between treatment and day ($P < 0.0001$). Error bars denote SEM.

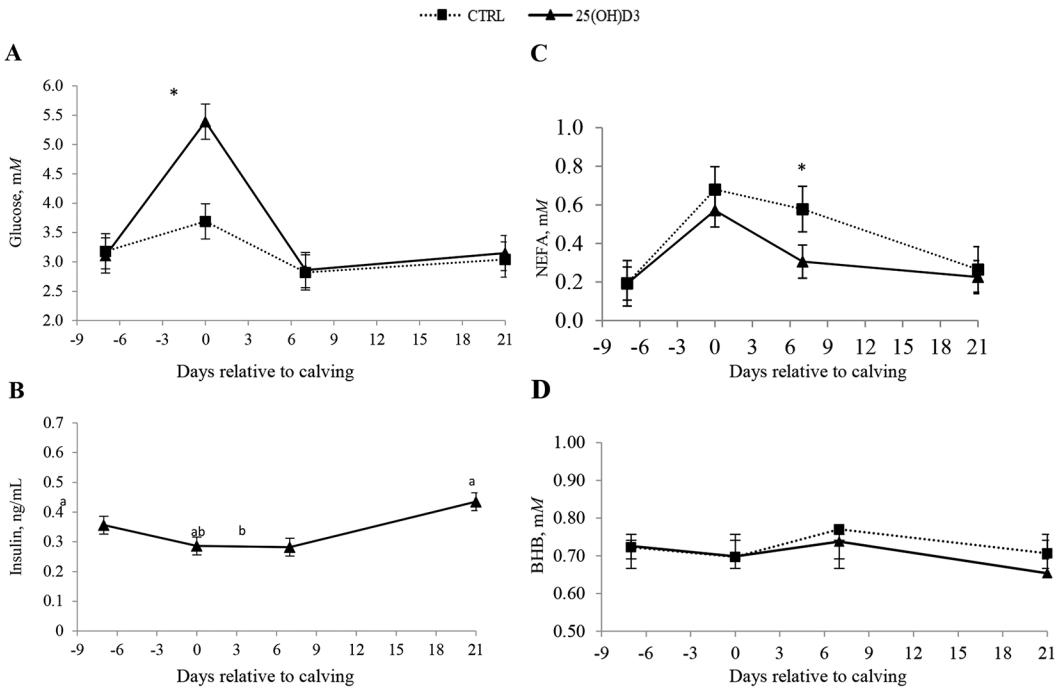

Figure 2. Plasma concentration of glucose (A), insulin (B), nonesterified fatty acids (NEFA; C), and BHB (D) of multiparous pregnant Holstein dairy cows fed a prepartum acidogenic diet supplemented with CTRL or 25(OH)D_3. Acidogenic prepartum diets containing the treatments were fed 30 d before the expected calving date. CTRL: vitamin D_3 at 0.625 mg/d, equivalent to 25,000 IU of vitamin D_3/d (Rovimix D_3 500, DSM Nutritional Products) or 25-hydroxyvitamin D_3 [25(OH)D_3] at 3 mg/d (Rovimix Hy-D 1.25%, DSM Nutritional Products). Panel A: effect of treatment ($P = 0.0017$), effect of the day ($P < 0.001$), and interaction between treatment and day ($P = 0.0036$). Panel B: effect of treatment ($P = 0.43$), effect of day ($P < 0.004$), and interaction between treatment and the day ($P = 0.52$). Panel C: treatment effect ($P = 0.0001$), effect of day ($P < 0.0001$), and interaction between treatment and day of collection ($P = 0.0001$). Panel D: effect of treatment ($P = 0.36$), effect of day ($P < 0.10$), and interaction between treatment and day ($P = 0.76$). Means with different letters (a,b) are different ($P < 0.05$). Error bars denote SEM; asterisks denote interaction between vitamin D source and day of collection.

conditions. Under tropical conditions, the incidence of sunlight is higher than in temperate conditions, so the cows in the tropics may synthetize vitamin D naturally, which can influence the effects of vitamin D supplementation for dairy cows, although hypocalcemia is still persistent in tropical settings (Paiano et al., 2020). Additionally, the greater K content of tropical forages presents a greater challenge in formulating diets that induce a compensated metabolic acidosis. Therefore, the use of a more effective vitamin D metabolite

Table 6. Milk yield, 3.5% FCM, ECM, and milk composition of multiparous pregnant Holstein dairy cows fed a prepartum acidogenic diet supplemented with CTRL or 25(OH)D_3

	Treatment[1]			*P*-value		
Item	CTRL	25(OH)D_3	SEM	Treatment	Week[2]	Treatment × week
Milk yield, kg/d	29.7	32.5	0.89	0.03	<0.01	0.57
3.5% FCM, kg/d	29.1	33.9	0.99	0.03	<0.01	0.49
ECM, kg/d	29.3	32.4	0.92	0.03	<0.01	0.49
Fat, kg/d	1.05	1.17	0.04	0.05	<0.01	0.62
Protein, kg/d	0.88	0.96	0.03	0.04	<0.01	0.50
Lactose, kg/d	1.36	1.49	0.04	0.04	<0.01	0.62
Fat, %	3.56	3.59	0.14	0.84	—	—
Protein, %	2.96	2.98	0.04	0.82	—	—
Lactose, %	4.58	4.59	0.03	0.78	—	—
Urea, mg/dL	16.7	16.5	0.77	0.80	—	—

[1]Acidogenic prepartum diets containing the treatments were fed 30 d before the expected calving date. CTRL: vitamin D_3 at 0.625 mg/d, equivalent to 25,000 IU of vitamin D_3/d (Rovimix D_3 500, DSM Nutritional Products) or 25-hydroxyvitamin D_3 [25(OH)D_3] at 3 mg/d (Rovimix Hy-D 1.25%, DSM Nutritional Products).

[2]Week relative to calving (1, 2, 3, 4, 5, and 6) was used as a repeated measure for the performance and composition yield.

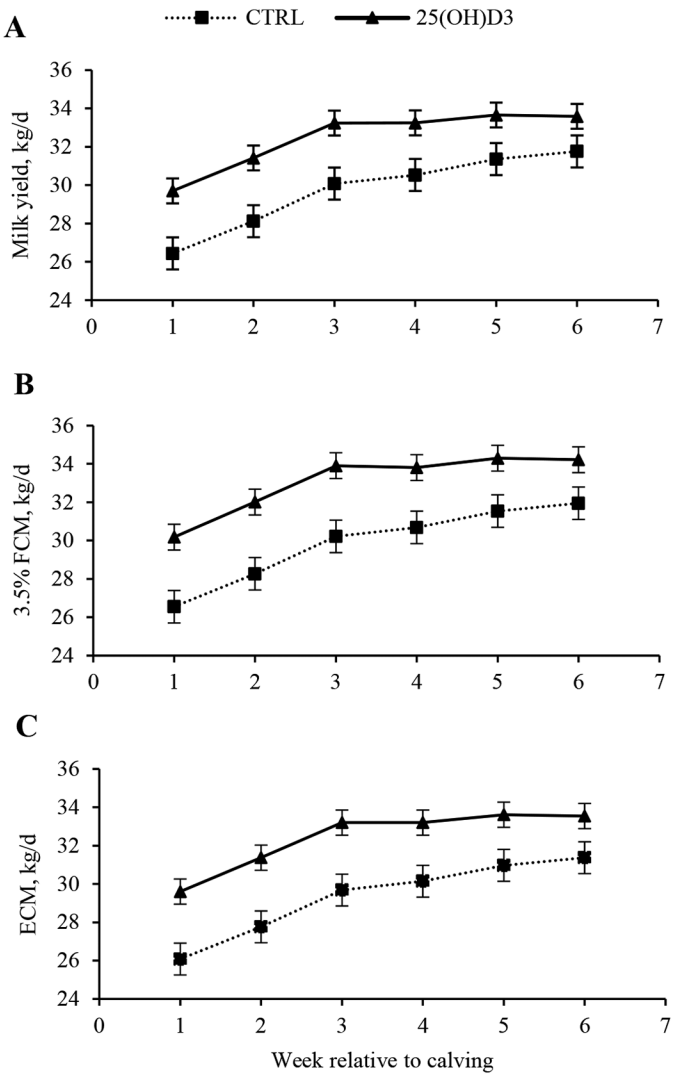

Figure 3. Milk yield (A), 3.5% FCM (B), and ECM (C) of multiparous pregnant Holstein dairy cows fed a prepartum acidogenic diet supplemented with CTRL or 25(OH)D_3. Acidogenic prepartum diets containing the treatments were fed 30 d before the expected calving date. CTRL: vitamin D_3 at 0.625 mg/d, equivalent to 25,000 IU of vitamin D_3/d (Rovimix D_3 500, DSM Nutritional Products) or 25-hydroxyvitamin D_3 [25(OH)D_3] at 3 mg/d (Rovimix Hy-D 1.25%, DSM Nutritional Products). Panel A: cows averaged (kg/d): CTRL = 29.7 ± 0.86; 25(OH)D_3 = 32.5 ± 0.88. Panel B: cows averaged (kg/d): CTRL = 29.9 ± 0.99; 25(OH)D_3 = 33.1 ± 1.01. Panel C: cows averaged (kg/d): CTRL = 29.3 ± 0.94; 25(OH)D_3 = 32.4 ± 0.92. Effect of treatment ($P = 0.03$), effect of time ($P < 0.0001$), and interaction between treatment and time ($P = 0.56$). Error bars denote SEM.

[25(OH)D_3] can be important in farms in the tropics to decrease the incidence of hypocalcemia and to improve the performance of Holstein dairy cows. To our knowledge, this is the first study reporting a decrease in plasma NEFA concentration and an increase in plasma glucose concentration, suggesting an improvement in energy metabolism of Holstein dairy cows in the transition period that were fed 25(OH)D_3.

One of the most common effects of acidogenic diets is the reduced DMI when compared with alkalogenic diets in the prepartum period (Lean et al., 2019; Santos et al., 2019). The exact mechanism involved in this reduction in the number of cows fed acidogenic diets is not completely understood, but the metabolic acidosis caused by these diets can be one of the reasons for the reduction of DMI in the prepartum period (Zimpel et al., 2018). Average DMI in the current study was similar to the report by Santos et al. (2019), which observed 11.2 kg/d of DMI for multiparous Holstein cows with a weight, BCS, and milk yield of 715 kg, 3.57, and 36.7 kg/d, respectively, after being fed an acidogenic diet. However, in contrast to Martinez et al. (2018a), in the present study cows fed 25(OH)D_3 tended to increase the prepartum DMI (+1.0 kg/d).

The lack of treatment effect on prepartum energy balance can be explained by the similar NE of the diets, although the caloric intake was higher for cows fed 25(OH)D_3 supported by the higher DMI. Martinez et al. (2018a) did not observe differences in prepartum energy balance or BW change for cows fed vitamin D_3 or 25(OH)D_3 21 d before calving. Cows fed 25(OH)D_3 had a higher BW change in the present study, possibly related to higher weight of the calves at birth (data not shown).

Around calving days, energy requirements increase due to colostrum production and increased mammary gland metabolism. The increase in colostrum yield (+2.06 kg/d) observed in the current study for cows fed 25(OH)D_3 is probably due to the higher glucose concentration in the plasma for 25(OH)D_3 group at calving day. Glucose is an important substrate to produce lactose (Cant et al., 2002) and, consequently, one of the main substrates for milk production (Danes et al., 2020). Cows fed 25(OH)D_3 tended to increase the DMI (+1.0 kg/d) and this could explain the higher glucose concentration on calving day that resulted in higher colostrum yield; however, the extra amount of colostrum did not result in more Ca secreted. In contrast to the current study, Rodney et al. (2018a) observed a trend for higher colostrum yield and Ca secretion in colostrum from cows fed 25(OH)D_3.

In the present study, the iCa and tCa concentrations were 1.3 and 2.5 mM, respectively. This normocalcemic state is likely due to an effective acidogenic diet, given that the urine pH was below 6.0 for both groups in the prepartum period. The value to diagnose hypocalcemia is iCa <1.0 mM (Oetzel et al., 1988), tCa <2.0 mM (Reinhardt et al., 2011), or tCa <2.15 mM (Martinez et al., 2012) and even though the cows remained with a mean value above the threshold, a decrease in the serum Ca at calving was present when compared with prepartum and 21 d postpartum. The lack of effect of treatment

Table 7. Postpartum DMI, net energy (NE) secreted as milk, caloric intake, net energy balance, BW, BW change, BCS, and rumination of multiparous pregnant Holstein dairy cows fed a prepartum acidogenic diet supplemented with CTRL or 25(OH)D_3

	Treatment[1]			*P*-value		
Item	CTRL	25(OH)D_3	SEM	Treatment	Week[2]	Treatment × week
DMI, kg/d	20.2	19.4	0.51	0.31	<0.0001	0.34
Milk NE, Mcal/d	19.9	21.7	0.68	0.08	<0.0001	0.50
NE intake, Mcal/d	29.4	29.1	0.71	0.76	<0.0001	0.09
NE balance, Mcal/d	−0.27	−2.30	0.78	0.08	<0.0001	0.09
BW, kg	597.1	600.1	12.92	0.87	<0.0001	0.41
BW change, kg/d	−1.00	−1.30	0.25	0.15	<0.0001	0.26
BCS, 1 to 5 scale	3.10	2.90	0.102	0.38	0.003	0.26
Rumination,[3] min/d	532	524	11.2	0.66	<0.0001	0.03

[1]Acidogenic prepartum diets containing the treatments were fed 30 d before the expected calving date. CTRL: vitamin D_3 at 0.625 mg/d, equivalent to 25,000 IU of vitamin D_3/d (Rovimix D_3 500, DSM Nutritional Products) or 25-hydroxyvitamin D_3 [25(OH)D_3] at 3 mg/d (Rovimix Hy-D 1.25%, DSM Nutritional Products).

[2]Week relative to calving (1, 2, 3, 4, 5, and 6) was used as a repeated measure for the postpartum DMI and energy balance.

[3]Time spent ruminating was evaluated at wk −1, 1, 2, 3, 4, 5, and 6 relative to calving.

on plasmatic PTH is probably due to very minimal differences in serum Ca at calving. Additionally, PTH is mainly a calcicotrophic hormone when Ca in the blood is under normal levels (Hernández-Castellano et al., 2020). Calcium is so tightly regulated that very low Ca concentration in the serum could promote changes in plasma PTH (DeGaris and Lean, 2008). Even the PTH challenge model by Vieira-Neto et al. (2021) could barely detect changes in 1,25(OH)$_2D_3$; therefore, in the present study, it is unlikely a change in PTH would be observed from dietary vitamin D.

When tCa is <2.5 m*M*, the parathyroid gland produces PTH (Goff et al., 2014). As mentioned above, the cows in the present study had normal levels of blood Ca. Another reason all cows remained normocalcemic was that the serum Mg concentration was above 2.2 mg/dL. The Mg concentration above 1.9 mg/dL suggests no suppression of PTH responsiveness (Rude and Gruber, 2004).

In the current study, cows fed 25(OH)D_3 tended to increase (+0.07 m*M*) the serum iCa concentration in the postpartum period; however, Rodney et al. (2018a) observed an increase in serum iCa concentration in the prepartum period, but not during the postpartum period for cows fed 25(OH)D_3 at 3 mg/d associated with a negative DCAD diet (−126.6 mEq/kg of DM). The increase in serum iCa observed in the present study (+0.07 m*M*) is consistent with Poindexter et al. (2020) and Rodney et al. (2018a), which also evaluated the effects of feeding an acidogenic diet supplemented with 25(OH)D_3 during the transition period.

The intestinal absorption of Ca is mediated by the transcellular pathway through the Ca entrance in the specific sites present in the brush border and, finally, an intracellular transport mediated by Ca-binding protein and calbindin-D_{9k} (Lieben et al., 2011). The activation of intestinal absorption of Ca is mediated mainly by the active form of vitamin D. In the current study, the cows fed 25(OH)D_3 had 268% higher 25(OH)D_3 in the plasma. This increase of plasma 25(OH)D_3 suggests an increased Ca intestinal absorption; however, this is not conclusive in our study because we do not use markers for the determination of Ca absorption, although previous data showed increased Ca absorption in the intestine of Holstein dairy cows fed 25(OH)D_3 (Oehlschlaeger et al., 2014). In addition, the involvement of vitamin D on intestinal absorption of Ca is largely acceptable in the literature (McGrath et al., 2013).

Moreover, cows fed vitamin D_3 had a mean 25(OH)D_3 of 66.6 ng/mL during the experiment in the present study. Dairy cows fed 25,000 IU/d of vitamin D_3 normally present this range of plasma 25(OH)D_3, although the data of the present study suggest that it is not enough to improve Ca metabolism. In a previous study, Rodney et al. (2018a) found a possible limitation of the conversion of vitamin D_3 to 25(OH)D_3 on the vitamin D_3 supplemented group with Holstein dairy cows fed 21 d before calving with 120,000 IU/d vitamin D_3. This lower blood plasma 25(OH)D_3 for the vitamin D_3 treatment possibly decreased the intestinal absorption of Ca compared with cows fed 25(OH)D_3 in the present study. Additionally, at the onset of lactation, the Ca supplied from the diet is the main source of Ca for the transition dairy cows because the supply is mainly mediated by the 1,25(OH)$_2D_3$ (Horst et al., 1994) that increases the Ca efflux and decreases the Ca influx in bone (Felsenfeld et al., 2013).

When the blood concentration of Ca and Mg increases above normal levels, the urinary excretion increases to maintain normal physiological levels. Therefore, the lack of effect of 25(OH)D_3 supplementation in urinary Mg excretion in the pre- and postpartum periods re-

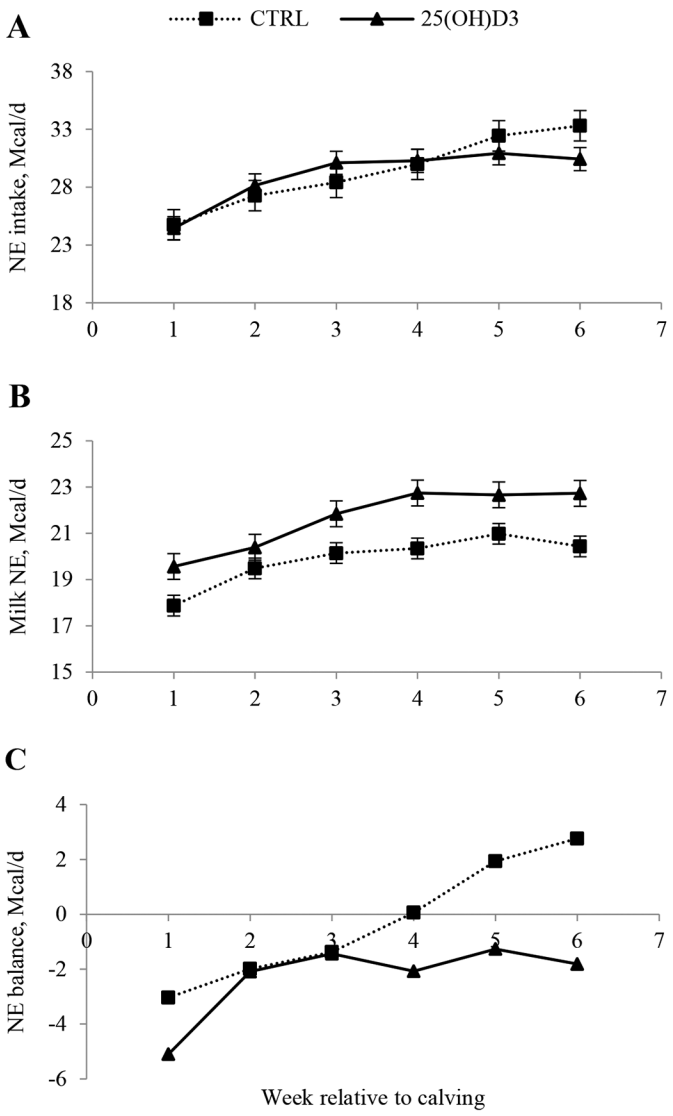

Figure 4. Postpartum caloric intake (A), milk net energy (NE; B), and NE balance (C) of multiparous pregnant Holstein dairy cows fed a prepartum acidogenic diet supplemented with CTRL or 25(OH)D_3. Acidogenic prepartum diets containing the treatments were fed 30 d before the expected calving date. CTRL: vitamin D_3 at 0.625 mg/d, equivalent to 25,000 IU of vitamin D_3/d (Rovimix D_3 500, DSM Nutritional Products) or 25-hydroxyvitamin D_3 [25(OH)D_3] at 3 mg/d (Rovimix Hy-D 1.25%, DSM Nutritional Products). Panel A: effect of treatment ($P = 0.76$), effect of the day ($P < 0.0001$), interaction between treatment and day ($P = 0.08$). Panel B: effect of treatment ($P = 0.07$), effect of day ($P < 0.0001$), interaction between treatment and the day ($P = 0.49$). Panel C: treatment effect ($P = 0.07$), effect of day ($P < 0.0001$), interaction between treatment and day of collection ($P = 0.09$). Error bars denote SEM.

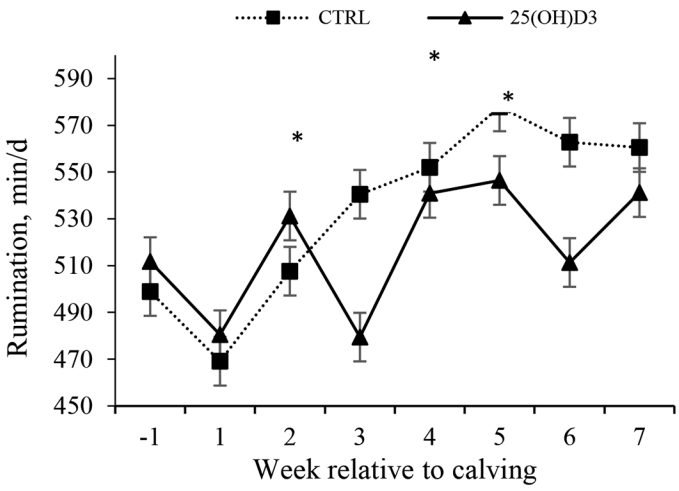

Figure 5. Rumination time of multiparous pregnant Holstein dairy cows fed a prepartum acidogenic diet supplemented with control (CTRL) or 25(OH)D_3. Acidogenic prepartum diets containing the treatments were fed 30 d before the expected calving date. CTRL: vitamin D_3 at 0.625 mg/d, equivalent to 25,000 IU of vitamin D_3/d (Rovimix D_3 500, DSM Nutritional Products) or 25-hydroxyvitamin D_3 [25(OH)D_3] at 3 mg/d (Rovimix Hy-D 1.25%, DSM Nutritional Products). Error bars denote SEM; asterisks denote interaction between vitamin D source and week of lactation.

flected the lack of treatment effect on serum blood Mg. In contrast, the decrease of blood Mg resulted in lower urinary Mg excretion in cows fed 25(OH)D_3 21 d before calving associated with an acidogenic diet (DCAD = −126.6 mEq/kg of DM; Rodney et al., 2018a). Treatment did not affect tP in the current study (mean = 2.22 m*M*). This is important to note because P concentration in blood is directly related to P intake (Horst, 1986). In the current study, the P concentration of the prepartum diets was 0.27%; therefore, high P diets can decrease the conversion of 25(OH)D_3 to 1,25(OH)$_2D_3$ by impairment of the hydroxylation mediated by 1-α hydroxylase (Anderson et al., 2017).

A large amount of glucose is needed for a variety of functions and physiological mechanisms such as the immune system, energy metabolism, milk production, and reproduction. The glucose requirement is usually 2.7 times greater for cows in early lactation (Sundrum, 2015). In the present study, supplementation with 25(OH)D_3 increased plasma glucose concentration by 0.45 m*M*, which means more glucose was available to be used for a variety of functions. Cows in early lactation can experience some degree of insulin resistance in response to physiological changes (De Koster and Opsomer, 2013; Mair et al., 2016) and low insulin concentration (Gärtner et al., 2019). The present study, in agreement with Martinez et al. (2018a), found insulin concentration was not affected by treatments.

Lipolysis also occurs frequently in early lactation due to great energetic demand during this period (Contreras and Sordillo, 2011). Lipid mobilization is related to decreased postpartum DMI, lower percentage of neutrophils with oxidative burst activity, and lower production of reactive oxygen species per neutrophil (Martinez et al., 2014). Plasma concentration of NEFA

Table 8. Urinary volume, creatinine, Ca, and Mg of multiparous pregnant Holstein dairy cows fed a prepartum acidogenic diet supplemented with CTRL or 25(OH)D_3

	Treatment[1]			*P*-value		
Item	CTRL	25(OH)D_3	SEM	Treatment	Day[2]	Treatment × day
Urine prepartum						
Urine, L/d	26.9	28.8	2.64	0.54	—	—
Creatinine, g/L	0.66	0.69	0.05	0.67	—	—
Ca, mg/L	345.0	448.3	38.16	0.03	—	—
Ca, g/d	8.01	12.83	0.874	0.0002	—	—
Mg, mg/L	66.3	63.9	3.62	0.51	—	—
Mg, g/d	1.75	1.88	0.182	0.52	—	—
Urine pH	5.69	5.79	0.06	0.25		
Urine postpartum						
Urine, L/d	30.5	28.1	2.409	0.41	0.23	0.34
Creatinine, g/L	0.96	0.61	0.289	0.38	0.25	0.33
Ca, mg/L	23.8	20.2	4.16	0.50	0.03	0.69
Ca, g/d	0.82	0.58	0.204	0.41	0.06	0.52
Mg, mg/L	63.2	64.2	1.462	0.61	0.84	0.02
Mg, g/d	1.94	1.85	0.189	0.69	0.77	0.96

[1]Acidogenic prepartum diets containing the treatments were fed 30 d before the expected calving date. CTRL: vitamin D_3 at 0.625 mg/d, equivalent to 25,000 IU of vitamin D_3/d (Rovimix D_3 500, DSM Nutritional Products) or 25-hydroxyvitamin D_3 [25(OH)D_3] at 3 mg/d (Rovimix Hy-D 1.25%, DSM Nutritional Products).

[2]Urinary production and mineral excretion were performed at d 21 and 42 relative to calving.

is exacerbated by the increased demand for energy during the postpartum period followed by low DMI. In the present study, cows fed 25(OH)D_3 had lower plasma NEFA concentrations compared with the CTRL probably due to the higher glucose concentration on the day of calving. In the present study, the average plasma NEFA concentration on the cows fed 25(OH)D_3 during the experimental period was 0.32 m*M*, with the higher values on calving day (0.68 m*M*) and 7 d postpartum (0.58 m*M*). Similar to diabetes type 2, insulin secretion is not compromised by β cells in the pancreas, but the glucose uptake on target tissues is affected by low insulin sensitivity. As mentioned earlier, no effect of the treatment on insulin concentration was observed in the present study; however, insulin resistance can occur in specific target tissues (De Koster and Opsomer, 2013), and the higher lipid mobilization of the cows fed vitamin D_3 supports this hypothesis in the present study. Furthermore, Weber et al. (2013) showed that cows classified with low, medium, and high fat liver concentrations had higher glucose and lower plasma NEFA concentrations.

The plasma BHB concentration was not affected by treatments in the present study. Martinez et al. (2018a) did not observe an effect of the source of vitamin D on plasma BHB concentration. One possible explanation is that the liver's oxidative capacity was not exceeded in the present study. Plasma BHB levels are usually lower than 1.2 m*M* and when they increase to levels above 1.2 to 1.5 m*M*, cows can exhibit subclinical or clinical ketosis, respectively (Iwersen et al., 2013). In the present study, the cows fed 25(OH)D_3 or the CTRL had 0.72 and 0.70 m*M* plasma BHB concentration, respectively.

Cows fed 25(OH)D_3 produced 2.8 kg/d more milk than those fed CTRL. During lactation, most of the glucose available (50 to 80%) is directed to the mam-

Table 9. Mineral balance during prepartum and at the day of calving of multiparous pregnant Holstein dairy cows fed a prepartum acidogenic diet supplemented with CTRL or 25(OH)D_3

	Treatment[1]			
Item	CTRL	25(OH)D_3	SEM	*P*-value
Prepartum mineral balance				
Ca, g/d	24.0	11.9	3.67	<0.0001
Mg, g/d	18.7	19.1	1.31	0.64
Postpartum mineral balance				
Ca, g/d	13.2	–16.3	7.81	0.001
Mg, g/d	19.5	14.4	2.18	0.11

[1]Acidogenic prepartum diets containing the treatments were fed 30 d before the expected calving date. CTRL: vitamin D_3 at 0.625 mg/d, equivalent to 25,000 IU of vitamin D_3/d (Rovimix D_3 500, DSM Nutritional Products) or 25-hydroxyvitamin D_3 [25(OH)D_3] at 3 mg/d (Rovimix Hy-D 1.25%, DSM Nutritional Products).

mary gland (Duhlmeier et al., 2005; Zhao and Keating, 2007). In this context, Danes et al. (2020) observed an increase of 6.4 kg/d of milk yield for cows receiving abomasal infusion of 3.1 Mcal/d of glucose. In the current study, cows fed 25(OH)D_3 increased plasma glucose concentration and milk yield.

Glucose in ruminants is mostly from the hepatic gluconeogenesis, and its transport is mediated by glucose transporter (GLUT4) stimulated by insulin secretion and GLUT1 to the mammary gland that does not depend on insulin signals (De Koster and Opsomer, 2013). Although not expected, the plasma insulin concentration was not influenced by treatments in the present study.

Rodney et al. (2018b) observed a positive correlation between 25(OH)D_3 and the IGF-1, and also between the IGF-1 and glucose; therefore, this could explain, in part, the higher milk yield for the cows fed 25(OH)D_3 in the current study. Unfortunately, the IGF-1 was not measured in the present study. Additionally, Lee et al. (2007) demonstrated in mice models an endocrine regulation of energy metabolism. In mouse models, IGF-1 regulates the levels of nutrients in the blood and drives cell growth and division (Feng and Levine, 2010).

The supplementation with 25(OH)D_3 increased the energy secreted as milk (+1.8 Mcal/d), although the milk composition was not affected by treatments. Due to higher energy secreted as milk, the cows fed 25(OH)D_3 tended to have a higher NEB in the present study. Likewise, Martinez et al. (2018a) reported an increase of energy secreted in milk produced by cows fed an acidogenic diet with 25(OH)D_3 21 d before calving; therefore, such authors also reported a lower NEB (−7.5 Mcal/d), but higher than the results of the present study (−2.3 Mcal/d). The lower energy balance in the present study compared with Martinez et al. (2018a) was probably due to the higher DMI (19.4 vs. 18.4 kg/d) and lower milk fat concentration (3.59 vs. 4.77%).

The BW change and BCS were not affected by treatments. Due to the higher milk yield for the cows fed 25(OH)D_3, a greater fat mobilization was expected to compensate for the higher energy demand for production; however, 25(OH)D_3 supplementation decreased fat mobilization and showed an improvement of homeorhetic mechanisms, and cows fed 25(OH)D_3 had a decreased NEB when compared with cows fed the CTRL. The β-cell proliferation, normal insulin secretion, and decrease of insulin resistance through the uncarboxylated osteocalcin promote the suppression mechanisms of reserved mobilization (Wolf, 2008). Furthermore, the active metabolite of vitamin D (calcitriol) induces the osteocalcin by the osteoblasts (Wolf and Phil, 1996).

Several studies have shown the influence of the near parturition on rumination time (Calamari et al., 2014; Goff et al., 2014; Kaufman et al., 2016) and the reaction to physiological and metabolic changes in relation to the low blood Ca concentration (Goff et al., 2020) and strong reduction of the DMI (Liboreiro et al., 2015). In the present study, both treatments had an abrupt decrease in DMI at parturition. Nevertheless, rumination time of cows fed 25(OH)D_3 decreased on calving day, but the cows fed vitamin D_3 had lower rumination time from parturition until the third week postpartum. Rumination time is closely linked with ruminal motility, which is also controlled by the Ca availability and can affect DMI (Jørgensen et al., 1998). The ruminal contraction usually ceases when the iCa is lower than 0.6 mM (Jørgensen et al., 1998). Additionally, a direct relationship exists between plasma Ca concentration and rumination rate ($r = 0.75$; $P = 0.01$) on the first day of lactation (Goff et al., 2020). Our data suggest that the trend for higher serum iCa postpartum on cows feeding 25(OH)D_3 was enough for increased rumination time after parturition.

The increased prepartum Ca excretion (+4.82 g/d) in the current study suggests a compensatory mechanism (Kovács et al., 2015) on cows fed 25(OH)D_3. The normal levels of blood Ca are tightly regulated, and the higher concentration of blood Ca results in a hypercalciuria status (Constable et al., 2019) that can lead to a higher urinary Ca excretion; however, when blood Ca decreases, the renal reabsorption increases to maintain the normal levels of blood Ca (Blaine et al., 2015). The cows fed 25(OH)D_3 in the current study possibly have increased Ca transport by the increase of the expression of luminal Ca channels mainly by the transient receptor potential vanilloid type 5 and 6 (Hoenderop et al., 2005). This increase in the Ca flux exchange promotes a decrease in renal Ca reabsorption (Fredeen et al., 1988) and, therefore, more Ca is excreted, as observed in the current study. In the same way, the urinary Ca excretion increased (+4.10 g/d) for cows fed an acidogenic diet (−68 mEq/kg of DM) supplemented with 25(OH)D_3 (Oehlschlaeger et al., 2014).

Due to the higher urinary excretion of Ca in cows fed 25(OH)D_3 (8.01 vs. 12.83 g/d for the cows fed the CTRL treatment and cows fed 25(OH)D_3, respectively), the Ca balance (even positive) was lower during the prepartum period and negative during the postpartum period. Nevertheless, the estimation of Ca balance did not account for a likely effect of treatment on Ca absorption; therefore, Ca balance may be underestimated in the current study. In addition, cows fed 25(OH)D_3 produced 2.06 kg/d more colostrum, which resulted in 7.0 g/d more Ca secreted in the colostrum. Although

this increase was not statistically significant, it is possible the Ca secreted in colostrum affected the Ca balance. In agreement with the present study, Rodney et al. (2018a) observed similar values for the Ca balance, but the negative balance in that study was smaller than the observed in the current trial, probably due to the lack of the effect of treatment on colostrum yield observed by Rodney et al. (2018a). In the present study, the Mg balance was not influenced by treatments, probably due to the lack of effect of treatment on urinary Mg excretion and Mg concentration in the colostrum. In contrast, according to Rodney et al., (2018a), feeding cows 25(OH)D_3 during the transition period increased the negative balance of Mg.

CONCLUSIONS

Cows fed 25(OH)D_3 at 3 mg/d tended to have an increased serum iCa concentration and increase urinary excretion of Ca. Also, feeding 25(OH)D_3 at 3 mg/d increases colostrum yield and plasma glucose concentration on the day of calving. Feeding 25(OH)D_3 increases milk yield, 3.5% FCM, and ECM, and improves milk yield components in early lactation. Overall, these findings suggest that 25(OH)D_3 at 3 mg/d can improve the energy metabolism and lactation performance compared with the current feeding practice of supplementing vitamin D_3.

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