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Effect of dietary vitamin D_3 and 25-hydroxyvitamin D_3 supplementation on plasma and milk 25-hydroxyvitamin D_3 concentration in dairy cows

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ABSTRACT

Milk enriched with vitamin D by supplementing dairy cow diets could provide a valuable dietary source of vitamin D, but information on the feasibility of this approach is limited. In the current study, the effects of supplementing dairy cows with either vitamin D_3 or $25(OH)D_3$ over the transition/early lactation period on plasma and milk vitamin D concentrations were compared. Sixty dairy cows were randomly allocated to 1 of 4 dietary treatments from 14 d precalving to 56 d postcalving. Treatments were a control diet (control) for both precalving and postcalving periods containing 0.625 mg/d of vitamin D_3; a precalving diet supplemented with 6 mg of $25(OH)D_3$/d, but with a postcalving diet matching that of the control diet [$25(OH)D_3$ precalving]; the control diet precalving but with the postcalving diet supplemented with 2 mg of vitamin D_3/d (D_3max), and the control diet precalving but with the postcalving diet supplemented with 1.5 mg of $25(OH)D_3$/d [$25(OH)D_3$ postcalving]. No treatment effect on milk yield, composition or $25(OH)D_3$ concentration was observed. However, an interaction was observed of treatment and time for plasma $25(OH)D_3$ concentration; this increased within 2 wk of supplementation for the $25(OH)D_3$ precalving treatment (peaking just after calving, 202 ng/mL), whereas that of the $25(OH)D_3$ postcalving group had a slower response following supplementation, continuing to increase at 56 d. Correlations were observed between plasma and milk $25(OH)D_3$ concentrations at d 4 and 14 of lactation, but not at later sampling times. The D_3max treatment did not increase $25(OH)D_3$ concentration in plasma or milk. Overall, results from this study indicate that supplemental $25(OH)D_3$ is an effective means of enhancing dairy cow plasma $25(OH)D_3$ concentrations compared with vitamin D_3 supplementation, but not necessarily milk concentrations.

Key words: vitamin D_3, $25(OH)D_3$, milk, enrichment

INTRODUCTION

Vitamin D is important for bone health, and mounting evidence demonstrates that vitamin D status is inversely associated with risk of some chronic diseases, such as cardiovascular diseases, diabetes, and cancers (Holick and Chen, 2008; Borradale and Kimlin, 2009). There is increasing evidence that vitamin D deficiency in humans is prevalent throughout the world, including the United Kingdom (Hilger et al., 2014; Cashman and Kiely, 2016). Several factors have contributed to low vitamin D status such as geographic variation (latitude, season, and time of day), human characteristics (skin pigmentation, aging, and clothing) and lifestyle choices (use of sun screen, increased indoor lifestyle; Holick, 1995; Tsiaras and Weinstock, 2011). Dietary sources have therefore become more important in sustaining adequate vitamin D status (Spiro and Buttriss, 2014). However, few types of foods are naturally high in vitamin D (Schmid and Walther, 2013), and therefore, vitamin D food fortification has been recommended as a strategy to increase vitamin D intake across the population (Cashman, 2015).

Vitamin D concentrations of milk and dairy products are naturally low (McDermott et al., 1985). However, because milk and dairy products are widely consumed, they have been identified as an ideal vehicle to increase vitamin D consumption. One way of achieving this is through fortification, which does occur in some countries, such as Canada, United States, and Finland (Cashman and Kiely, 2016), but not all (de Lourdes Samaniego-Vaesken et al., 2012; Cashman and Kiely, 2016). Thus, additional strategies of enrichment of animal food sources by adding vitamin D into the livestock feeds could have potential to increase the vitamin D status of humans (Cashman and Kiely, 2016). In

practice, vitamin D_3 is the form of vitamin D commonly used for food fortification although there is now evidence that the metabolically active form, $25(OH)D_3$, is more effective in raising human blood $25(OH)D_3$ concentrations than vitamin D_3, and may be absorbed faster than vitamin D_3 from the human digestive tract (Barger-Lux et al., 1998; Cashman et al., 2012; Jetter et al., 2013). Previous studies investigating the effect of supplementing dairy cow diets with vitamin D_3 (Hollis et al., 1981; Thompson and Hidiroglou, 1983; McDermott et al., 1985) suggest concentrations in milk following supplementation remain relatively low compared with that needed to meet the UK Reference Nutrient Intake of 10 µg/d of vitamin D (SACN, 2015). To date, only a few studies (Wilkens et al., 2012; Weiss et al., 2015) have examined the effect of supplementing cow diets with $25(OH)D_3$ on serum/plasma or milk vitamin D concentration. However, the main objective of these studies (Wilkens et al., 2012; Weiss et al., 2015) was to reduce the prevalence of postpartum hypocalcemia in the cow, rather than increasing milk vitamin D concentration.

The main objective of the present study was to investigate the effect of supplemental vitamin D_3 or $25(OH)D_3$ in dairy cow diets on the $25(OH)D_3$ concentration of both plasma and milk over the transition period from late pregnancy to early lactation. Vitamin D_3 was included as a measurement in the milk as it is the precursor form of $25(OH)D_3$ (McDermott et al., 1985). We hypothesized that supplementing cows with $25(OH)D_3$ would be more efficient at increasing $25(OH)D_3$ concentrations of plasma and milk than vitamin D_3 supplementation. As hypocalcemia is common in dairy cows during the transition period (DeGaris and Lean, 2008), the secondary objective of the study was to investigate the effect of supplemental $25(OH)D_3$ on plasma calcium concentration during the precalving period. Furthermore, as hypocalcemia is usually accompanied by changes in plasma magnesium and phosphorus concentrations (Klimiene et al., 2005), the balance of which is crucial during the calving period (Reinhardt et al., 1988), the effects of treatments on plasma and milk magnesium and phosphorus concentrations were also studied.

MATERIALS AND METHODS

Animals and Management

All licensed procedures were conducted according to Scientific Procedures Act 1986 under the authority of Home Office Project License 70/7727. Sixty nonlactating (parity 2 or greater) Holstein-Friesian dairy cows with previous lactation yield (305 d) of 10,141 kg (SE = 177) and initial live weight of 725 kg (SE = 7.5) were randomly allocated to 1 of 4 experimental diets using a continuous design, at 14 d before calving [average duration of the precalving period was 14 d (SE = 0.5)]. When not restrained for measurements, cows were loose-housed in a straw-covered yard in the late gestation phase and in a cubicle yard with washed sand bedding and automatic alley scrapers during the lactation phase. For the period immediately around parturition, cows were housed in straw-bedded maternity pens.

Cows were group fed during the precalving period and for the first week postcalving. From d 7 of lactation onward, all cows were fed individually using Calan gates (Calan Broadbent Feeding System, American Calan Inc., Northwood, NH) for the remainder of the study. Cows were milked twice daily in the morning and afternoon at unequal intervals (0500 and 1500 h) through a 50-point rotary parlor (Dairymaster, Tralee, Co. Kerry, Ireland). All cows were housed at the University of Reading's Centre for Dairy Research during the winter period of October 2013 to March 2014 to avoid the confounding factor of in vivo vitamin D synthesis due to UV radiation from sunlight exposure.

Treatment Diets, Experimental Design, and Blocking

The 4 treatment diets were as follows: The control group was fed a basal transition cow diet from 14 d precalving, and a basal early lactation diet until 56 d postcalving (Table 1), both supplemented with 0.625 mg (25,000 IU) of vitamin D_3 (NRC, 2001) per cow per day (Table 2). The $25(OH)D_3$ precalving treatment received an additional 6 mg (240,000 IU) of $25(OH)D_3$ per cow per day (Rovimix HyD, DSM Nutritional Products, Basel, Switzerland) to the precalving diet, and the postcalving was the same as control. The $25(OH)D_3$ postcalving and D_3max treatments received the precalving control diet up to calving. The $25(OH)D_3$ postcalving treatment then received an additional 1.5 mg (60,000 IU) of $25(OH)D_3$ (Rovimix HyD 1.25%, DSM Nutritional Products) per cow per day, and D_3max received 2 mg of vitamin D_3 (maximum permitted EU level; EC, 2004) postcalving until 56 d (Table 2).

All supplements were formulated to provide the daily required dose of vitamin D_3 or $25(OH)D_3$ (or both) within 250 g of a ground wheat carrier. All diets were formulated to meet the animals' nutritional requirements according to the UK Feed Into Milk model (Thomas, 2004). The composition and estimated nutritive value of pre- and postcalving diets are described in Table 1. All diets were fed as TMR and were offered ad libitum to achieve 5% refusals. Oven DM (80°C for 24 h) of the TMR, silages, and concentrate blend were measured 3 times and once (for concentrate) per week.

Table 1. Composition and estimated nutritive value of pre- and postcalving TMR

Item	Precalving diet	Postcalving diet
Ingredient[1] (g/kg of DM)		
Grass silage[2]	244	224
Maize silage[3]	344	242
Wheat straw	160	18
Grass hay	—	39
Calcium salts of palm oil distillate	—	12
Minerals[4]	14	10
Sodium chloride	—	4
Ammonium chloride	14	—
Magnesium chloride	14	—
Concentrate blend[5]	210	458
Calculated nutritive value, DM		
CP (g/kg)	144	173
ME (MJ/kg)	10.2	11.8
Starch (g/kg)	169	203
NDF (g/kg)	450	351
Oil[6] (g/kg)	44	58
Ash (g/kg)	109	83
Water-soluble carbohydrates (g/kg)	25	48

[1]Containing dietary cation-anion difference −98 mEq/kg of DM precalving.

[2]Grass silage: 508 g/kg of DM NDF; 118 g/kg of DM CP; DM 294 g/kg.

[3]Maize silage: 357 g/kg of DM NDF; 73 g/kg of DM CP; DM 395 g/kg.

[4]Minerals consisted of (g/kg, as fed) calcium (210), phosphorus (40), magnesium (80), sodium (60), sodium selenite (0.03), cobalt carbonate (0.085), calcium iodate (0.4), manganese oxide (5), zinc oxide (5), cupric sulfate (2), vitamin A (400,000 IU/kg), vitamin E (2,500 IU/kg), and biotin (0.085).

[5]Concentrate blend consisted of (g/kg of DM) rolled wheat (313), soybean meal (159), soy hulls (60), palm kernel meal (120), rapeseed meal (170), wheat feed (129), calcium salts of palm oil distillate (16; Megalac, Volac International Ltd., Royston, UK), and molasses (33).

[6]Oil refers to oil B extraction.

Diets offered were adjusted once per week according to the mean of the last 3 forage DM results. Diets were prepared daily and dispensed between 0730 and 0900 h. Milk yield were recorded daily throughout the whole study.

Experimental Sampling

Blood samples were collected from the tail vein of each cow on d 14 and 7 before expected calving date, on the day of calving (within the first 24 h after parturition), and d 4, 7, 14, 21, 28, 35, and 42 of lactation for plasma vitamin D$_3$, 25(OH)D$_3$, calcium, phosphorus, and magnesium analyses. Two samples were collected in 10-mL Vacutainers containing EDTA (Becton Dickinson) from each cow at each sampling time. Each collected sample was immediately centrifuged at 1,713.6 × g for 15 min at 15°C to separate plasma, and plasma then was stored at −80°C.

Milk samples were collected on d 4, 14, 28, 35, and 42 of lactation and analyzed for vitamin D concentrations [vitamin D$_3$ and 25(OH)D$_3$]. Two milk samples (am and pm) were pooled (100 mL) according to yield and then were split into 2 × 50 mL samples and immediately frozen at −80°C. In addition, 2 milk samples (am and pm) were collected at each day of 4, 28, and 42 d and were pooled for mineral analysis according to yield.

Chemical Analysis

Analysis of plasma for vitamin D$_3$ and 25(OH)D$_3$ analyses were conducted by DSM Nutritional Products Ltd. (Kaiseraugst, Switzerland). Analysis of milk for

Table 2. Details of experimental treatments

Treatment	Cows (n)	Daily feeding for each cow	
		14 d of precalving until calving	Feeding from calving to early lactation of 56 d
Control	15	Basal transition cow diet[1] plus vitamin D supplementation: 0.625 mg of vitamin D$_3$ No 25(OH)D$_3$	Basal early lactation diet[1] plus vitamin D supplementation: 0.625 mg of vitamin D$_3$ No 25(OH)D$_3$
25(OH)D$_3$ precalving	15	Basal transition cow diet plus vitamin D supplementation: 0.625 mg of vitamin D$_3$ 6 mg of 25(OH)D$_3$	Basal early lactation diet plus vitamin D supplementation: 0.625 mg of vitamin D$_3$ No 25(OH)D$_3$
D$_3$max	15	Basal transition cow diet plus vitamin D supplementation: 0.625 mg of vitamin D$_3$ No 25(OH)D$_3$	Basal early lactation diet plus vitamin D supplementation: 2 mg of vitamin D$_3$ No 25(OH)D$_3$
25(OH)D$_3$ postcalving	15	Basal transition cow diet plus vitamin D supplementation: 0.625 mg of vitamin D$_3$ No 25(OH)D$_3$	Basal early lactation diet plus vitamin D supplementation: 0.625 mg of vitamin D$_3$ 1.5 mg of 25(OH)D$_3$

[1]Composition and estimated nutritive value of basal transition and early lactation cow diet described in Table 1.

vitamin D_3 and 25(OH)D_3 analyses were conducted by RTC (Pomezia, Italy). The methods used were checked according to Food and Drug Administration (FDA, 2013) and European Medicines Agency (EMEA, 2011) bioanalytical guidelines.

In brief, for plasma vitamin D_3 and 25(OH)D_3, 100 µL of plasma was pipetted into a protein precipitation plate, with addition of 10 µL of a deuterated internal standard solution (0.500 µg/mL D6-25(OH)D_3 and 0.250 µg/mL D3-vitamin D_3), 250 µL of extraction solution of tetrahydrofuran, acetonitrile, and methanol (50:40:10, vol/vol/vol) were added and the plate was vortexed for 10 min. The plate was then centrifuged (2–3 min, 1,713.6 × g, 10°C) and evaporated to dryness (40–60 min, 45°C). The residue was reconstituted with 50 µL of injection solvent of methanol and acetonitrile (80:20, vol/vol) solution for 10 min. An aliquot of plasma sample extract was injected into the liquid chromatography (**LC**)-MS/MS system for analysis.

For milk vitamin D_3 quantification, 3 g of raw milk was added to 10 µL of internal standard solution (400 ng/mL D3-vitamin D_3), saponified with 3 mL of methanol, 3 mL of ethanol, and 3 mL of 47% potassium hydroxide for 20 min at 80°C. Then, 3 mL of water was added and vitamin D_3 was extracted by liquid/liquid extraction twice successively with 7 mL of cyclohexane. The cyclohexane phase was evaporated to dryness under a stream of nitrogen. The residue was reconstituted in methanol and acetonitrile solution (80/20, vol/vol) for 10 min. After the final extraction was filtered, an aliquot of supernatant was injected into the LC-MS/MS system.

For milk 25(OH)D_3 quantification, 8 g of raw milk was added to 25 µL of internal standard solution [250 ng/mL D6-25(OH)D_3], and saponification was performed by adding 3 mL of methanol and 1.5 mL of potassium hydroxide (47%, vol/vol) for 30 min at 80°C, a liquid/liquid extraction with tert-butyl methyl ether was then used to extract 25(OH)D_3. After evaporation of the extract, the sample was cleaned by solid-phase extraction. With elution with a methanol and acetonitrile solution (80:20, vol/vol), the eluate was evaporated until dryness under nitrogen. The final residue was reconstituted in methanol/acetonitrile solvent and filtrated before injection into the LC-MS/MS system.

Concentrations of vitamin D_3 and 25(OH)D_3 in all samples were determined by a LC-MS/MS system (Agilent 1290, Agilent Technologies, Santa Clara, CA) using a reverse-phase column, coupled with an atmospheric pressure photospray ionization source (ABSciex 4000, Darmstadt, Germany) using an atmospheric pressure photospray ionization source in positive mode. The detection of the specific fragment ions was performed by using multiple reactions monitoring mode. Data acquisition of extracted ion chromatograms, integration, and quantification was performed by Analyst software from ABSciex.

Analysis of milk for calcium, phosphorus, and magnesium was performed by Sciantec Analytical, Yorkshire, United Kingdom (Walk et al., 2015). Briefly, milk samples were homogenized by vigorous shaking and 10 g of the milk sample was transferred into a digestion vessel. After addition of 30 mL of concentrated nitric acid (Romilk High Purity SpA), the samples were heated at 110°C for 4 h. After cooling to room temperature, each sample was mixed with deionized water followed by a 10-fold dilution with deionized water before ICP-MS analysis.

Plasma concentrations of calcium, phosphorus, and magnesium were determined via established methods (Veterinary Laboratories Agency, Shrewsbury, UK) by using an Olympus AU 400 chemistry analyzer and standard kits with appropriate quality control as defined by the Animal and Plant Health Agency in the United Kingdom, according to the manufacturer's instructions (Guerra-Ordaz et al., 2013).

Milk composition measurements including fat, protein, lactose, casein., urea and SCC were analyzed by Fourier transform-infrared spectrophotometry (National Milk Laboratories, Wolverhampton, UK) using the method described elsewhere (Reynolds et al., 2014) and the analyses were conducted by National Milk Laboratories, United Kingdom.

Statistical Analysis

Results were averaged for each cow and sampling period, and were analyzed using the Mixed procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC), including fixed effects of dietary treatment and time, and random effect of cow, with time as a repeated effect within cow. Milk yield and composition was only analyzed for the postcalving period, whereas other data were analyzed for the whole study. Compound symmetry, heterogeneous compound symmetry, first-order autoregressive, or a heterogeneous first-order regressive covariance structure were used for repeated measures analysis based on goodness of the fit criteria for each analyzed variable. Orthogonal contrasts were applied to investigate the difference between treatments: control versus all other diets, 25(OH)D_3 precalving versus 25(OH)D_3 postcalving to assess whether pre- or postcalving supplementation is most suitable to achieve optimal results, and D_3max versus 25(OH)D_3 postcalving to assess which of the postcalving treatments was more successful at enhancing milk concentrations of vitamin

D. Least squares means (±SEM) were reported, and treatment effects were considered significant at $P < 0.05$.

Area under the curve (**AUC**) for plasma and milk vitamin D concentrations over time were calculated according to the trapezium rule as the summation measure for each treatment, which was analyzed by 1-way ANOVA in Stata (version 13.0, 2014, StataCorp, College Station, TX), Bonferroni correction was used subsequently to compute the multiple pairwise comparisons if there was a significant effect of the investigated variables between treatments. Furthermore, to investigate the relationship of 25(OH)D$_3$, calcium, phosphorus, or magnesium between plasma and milk at each time point, the correlation of 25(OH)D$_3$, calcium, phosphorus, and magnesium in plasma and milk were conducted (across all treatments) by using the general linear regression model in Stata.

RESULTS

There were no overall significant effects of treatment or treatment by time interactions for milk yield, milk composition, and DMI (Table 3). Milk yield ($P < 0.001$), FCM yield ($P = 0.024$), and lactose yield ($P < 0.001$) increased over time, whereas daily milk fat yield decreased ($P = 0.001$) over time (Table 3). Content of fat, protein, and casein concentrations decreased in milk ($P < 0.001$), whereas lactose concentration ($P = 0.001$) increased over time (Table 3). Somatic cell count and DM increased ($P < 0.001$) over time (Table 3).

In addition, there was no significant effect of treatment or interaction between treatment and time on plasma and milk calcium, phosphorus, and magnesium concentrations (Table 4). However, time affected ($P < 0.001$) concentrations of all minerals in both plasma and milk. Plasma calcium concentrations experienced a sharp decrease around the immediate calving period and then increased again and remained relatively consistent after d 7 postcalving ($P < 0.001$, Table 4). In contrast, milk calcium concentration gradually decreased after d 7 postcalving ($P < 0.001$, Table 4). Similarly, plasma and milk phosphorus concentration had a similar pattern to plasma and milk calcium concentration, respectively ($P < 0.001$, Table 4). Plasma magnesium concentrations increased around the calving period and followed by a sharp decrease immediately postcalving, and then increased again and remained constant ($P < 0.001$), whereas milk magnesium concentration gradually decreased after calving ($P < 0.001$, Table 4).

Effects ($P < 0.001$) were observed of treatment, time, and an interaction between the 2 for plasma 25(OH)D$_3$ concentration. The 25(OH)D$_3$ precalving treatment resulted in a greater mean plasma concentration

Table 3. Effect of supplements on milk yield, milk composition, and DMI of cows (LSM)

Characteristic	Treatment				SEM[1]	P-value[2]			Contrast P-value[3]		
	Control	25(OH)D$_3$ precalving	D$_3$max	25(OH)D$_3$ postcalving		Treatment	Time	Treatment × time	1	2	3
Milk yield											
Milk yield (kg/d)	43.0	42.4	42.3	44.2	1.39	0.735	<0.001	0.320	0.993	0.355	0.311
FCM (kg/d)	46.5	44.3	45.7	46.6	1.71	0.718	0.024	0.183	0.604	0.313	0.680
ECM (kg/d)	45.2	43.2	44.4	45.4	1.60	0.745	0.071	0.210	0.629	0.328	0.634
Fat (g/d)	1,722	1,587	1,689	1,697	74.6	0.625	0.001	0.182	0.447	0.332	0.937
Protein (g/d)	1,292	1,267	1,266	1,311	42.2	0.841	0.601	0.645	0.819	0.457	0.441
Lactose (g/d)	1,926	1,895	1,901	1,979	62.2	0.747	<0.001	0.313	0.993	0.324	0.358
Milk composition											
Fat (g/kg)	40.3	37.3	40.1	38.5	1.16	0.187	<0.001	0.055	0.198	0.479	0.290
Protein (g/kg)	30.2	30.1	29.9	29.7	0.42	0.842	<0.001	0.439	0.582	0.476	0.814
Lactose (g/kg)	44.8	44.7	44.9	44.8	0.25	0.946	0.001	0.779	0.916	0.784	0.755
Casein (g/kg)	22.9	22.9	23.0	22.7	0.45	0.946	<0.001	0.613	0.844	0.569	0.764
Urea N (mg/L)	244	239	231	244	13.1	0.762	0.389	0.618	0.620	0.728	0.352
SCC (× 10,000/mL)	4.2	7.5	5.5	8.8	0.1	0.293	<0.001	0.257	0.120	0.679	0.249
DMI (kg/d)	25.0	24.5	24.9	25.5	0.93	0.803	<0.001	0.734	0.975	0.332	0.542

[1]Standard error of the mean for n = 15 measurements.
[2]Probability corresponding to the effect of treatment, time, or treatment by time interaction.
[3]1 = control vs. all other diets; 2 = 25(OH)D$_3$ precalving vs. 25(OH)D$_3$ postcalving; 3 = D$_3$max vs. postcalving.

Table 4. Effect of supplements on milk and plasma 25-hydroxyvitamin D_3 and mineral concentrations (LSM)

Measurement	Treatment				SEM[1]	P-value[2]			Contrast P-value[3]		
	Control	25(OH)D_3 precalving	D_3max	25(OH)D_3 postcalving		Treatment	Time	Treatment × time	1	2	3
Plasma (whole study)											
25(OH)D_3 (ng/mL)	43.0	122.7	39.5	87.1	5.11	<0.0001	<0.001	<0.001	<0.001	<0.001	<0.001
Calcium (mmol/L)	2.38	2.34	2.35	2.33	0.028	0.399	<0.001	0.715	0.118	0.593	0.515
Phosphorus (mmol/L)	1.64	1.73	1.72	1.77	0.053	0.130	<0.001	0.717	0.030	0.482	0.344
Magnesium (mmol/L)	0.98	0.97	0.98	0.97	0.021	0.933	<0.001	0.379	0.733	0.875	0.875
Milk											
25(OH)D_3 (ng/kg)	869	1,132	889	1,001	130.7	0.193	0.004	<0.001	0.214	0.339	0.412
Calcium (mg/kg)	1,105	1,064	1,085	1,133	26.9	0.111	<0.001	0.782	0.644	0.020	0.111
Phosphorus (mg/kg)	896	923	880	930	24.4	0.215	<0.001	0.240	0.484	0.789	0.065
Magnesium (mg/kg)	95.8	96.3	93.9	98.2	2.95	0.591	<0.001	0.906	0.901	0.554	0.175
Area under curve											
Plasma 25(OH)D_3 (ng/mL × 70 d)	2,964[a]	8,274[b]	2,619[a]	6,806[b]	384	<0.0001	—	—	—	—	—
Milk 25(OH)D_3 (ng/kg × 39 d)	33,659	42,799	31,309	34,823	1,874	0.142	—	—	—	—	—

[a,b]Means within a row with different superscripts differ ($P < 0.05$).

[1]Standard error of the mean for n = 15 measurements.

[2]Provability corresponding to the effect of treatment, time, or treatment by time interaction.

[3]1 = control vs. all other diets; 2 = 25(OH)D_3 precalving vs. 25(OH)D_3 postcalving; 3 = D_3max vs. postcalving.
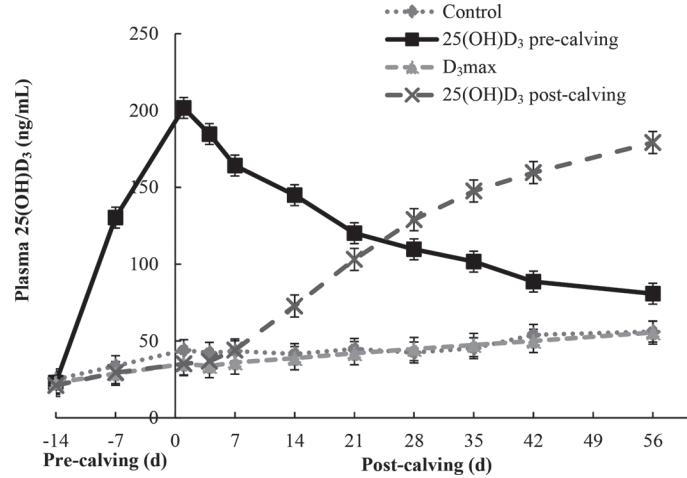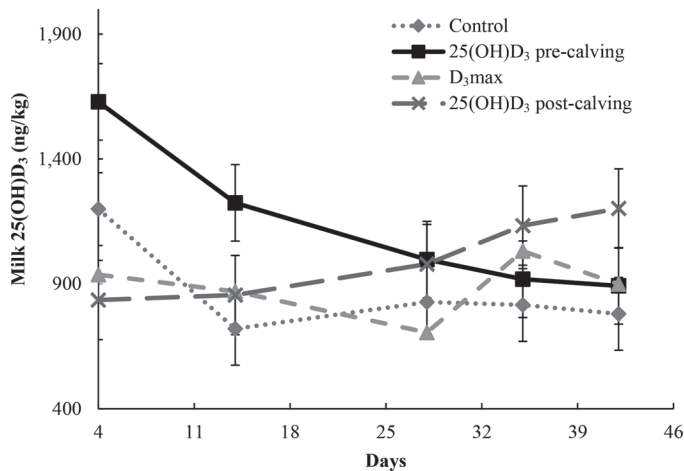

Figure 1. Effect of treatments on 25(OH)D_3 concentrations in plasma. Control for both transition and early lactation containing 0.625 mg of vitamin D_3; 25(OH)D_3 precalving had same diet as the control at early lactation, but the transition diet was supplemented with 6 mg of 25(OH)D_3 during precalving in addition to the control diet; 25(OH)D_3 postcalving had same transition diet as the control, but the early lactation diet included 1.5 mg of 25(OH)D_3 supplements in addition to the control; D_3max had same transition diet as the control, but with supplemented 2 mg of vitamin D_3 in addition to the control diet. Least squares means ± SEM for 15 measurements.

of 25(OH)D_3 across the whole study (Table 4; Figure 1), with a peak 25(OH)D_3 concentration of 202 ng/mL achieved by d 1 of lactation, before decreasing gradually. In comparison, the 25(OH)D_3 postcalving treatment resulted in an increase in plasma 25(OH)D_3 concentration following 14 d of supplementation, reaching 179 ng/mL at d 56. By calculating the AUC for plasma 25(OH)D_3 from 14 d precalving to 56 d after calving, a difference was found between treatments: plasma $AUC_{14-56\ d}$ of 25(OH)D_3 precalving and 25(OH)D_3 postcalving were significantly higher than control and D_3max postcalving treatments ($P < 0.0001$). No difference was present between 25(OH)D_3 precalving and 25(OH)D_3 postcalving, or between control and D_3max.

A treatment by time interaction ($P < 0.001$) and an effect of time ($P = 0.004$) were observed, but no treatment effect for 25(OH)D_3 concentration in milk was present (Table 4, Figure 2). Vitamin D_3 was measured in milk, but 87% of values were below the limit of detection of 60 ng/kg, so data analysis was not possible. No difference ($P = 0.142$) was present between treatments on $AUC_{4-42\ d}$ for milk 25(OH)D_3 concentrations.

Concentrations of 25(OH)D_3 in plasma and milk were correlated at d 4 ($R^2 = 0.25$; $P = 0.009$) and 14 ($R^2 = 0.24$; $P = 0.01$) of lactation, but not at d 28, 35, or 42 (Figure 3). No correlation was present between concentrations of calcium, phosphorus, or magnesium in plasma and milk ($P > 0.05$; data not shown).

Figure 2. Effect of treatments on 25(OH)D₃ concentrations in milk. Control for both transition and early lactation containing 0.625 mg of vitamin D₃; 25(OH)D₃ precalving had same diet as the control at early lactation, but the transition diet was supplemented with 6 mg of 25(OH)D₃ during precalving in addition to the control diet; 25(OH)D₃ postcalving had same transition diet as the control, but the early lactation diet included 1.5 mg of 25(OH)D₃ supplements in addition to the control; D₃max had same transition diet as the control, but with supplemented 2 mg of vitamin D₃ in addition to the control diet. Least squares means ± SEM for 15 measurements.

DISCUSSION

Vitamin D metabolite form of 25(OH)D₃ is more effective than vitamin D₃ in raising human serum 25(OH)D₃ concentrations (Barger-Lux et al., 1998; Cashman et al., 2012; Jetter et al., 2013). To our knowledge, the current study is the first to compare the effects of supplementing both precalving and postcalving cow diets with 25(OH)D₃ and vitamin D₃ on plasma and milk 25(OH)D₃ concentrations. The current study demonstrated that a daily oral supplementation of 6 mg of 25(OH)D₃ during 2 wk precalving or 1.5 mg of 25(OH)D₃ during 8 wk postcalving is more effective in raising plasma 25(OH)D₃ concentrations than vitamin D₃ supplementation. However, we did not detect significant treatment effects on 25(OH)D₃ and mineral concentrations of the milk.

Supplementing cows with 25(OH)D₃ for 2 wk precalving increased plasma 25(OH)D₃ concentration, which reached a peak just after calving (d 1; 202 ng/mL) when supplementation stopped. This result is consistent with previous studies (Wilkens et al., 2012; Weiss et al., 2015) who reported that precalving 25(OH)D₃ supplementation is effective at increasing plasma concentrations, peaking at the same time. The daily 25(OH)D₃ supplementation dose (6 mg) in the current study was the same as that used by Weiss et al. (2015), and yet the earlier study resulted in a higher peak concentration (274 ng/mL). Wilkens et al. (2012)

supplemented with less 25(OH)D₃ (3 mg/d) but the peak plasma concentration was similar to that of the current study (198 ng/mL). The reason for these differences is unclear, as supplementation time was similar for all studies and a comparable number of cows were included per treatment, but may be due to the differences in the absorbance of vitamin D form the digestive tract of individual animals.

Plasma 25(OH)D₃ concentration following supplementation with vitamin D₃ postcalving (D₃max) was not different to that of cows consuming the control diet. Our results were consistent with those of McDermott et al. (1985), who compared 3 daily doses of vitamin D₃ supplements (0.25, 1.25, or 6.25 mg), with results demonstrating that only the 6.25 mg dose significantly enhanced plasma 25(OH)D₃ concentration. Thus, it is perhaps not surprising the mean plasma 25(OH)D₃ concentration of the vitamin D₃ treatment (at 2 mg/cow per d) in the current study is followed at a similar level with the control. To our knowledge, the current study is the first to directly compare the effect of postcalving supplementation of vitamin D₃ with 25(OH)D₃, on plasma 25(OH)D₃ concentrations, and our results are consistent with similar studies involving humans (Cashman et al., 2012; Guo et al., 2017) where 25(OH)D₃ supplementation was more effective at raising plasma or serum 25(OH)D₃ than vitamin D₃ supplementation.

Milk concentration of 25(OH)D₃ was not affected by treatment in the current study, and the mean concentration of 25(OH)D₃ concentration in milk through whole study was 875 ng/kg, results that are consistent with previous studies (Hollis et al., 1981; McDermott et al., 1985). Hollis et al. (1981) fed cows with 0.1 or 10 mg of vitamin D₃ per day, but this 10-fold elevated supplementation level only resulted in a 2-fold increase in milk 25(OH)D₃ concentration (3.72×10^{-4} mg/L increased to 6.85×10^{-4} mg/L). McDermott et al. (1985) supplemented cow diets with a higher daily dose of vitamin D₃ (1.25 or 6.25 mg) for 14 wk from 2 wk precalving until 12 wk postcalving, and reported that milk 25(OH)D₃ concentration only slightly increased from 7.5×10^{-4} to 9.25×10^{-4} mg/L. The current study is also in agreement with Weiss et al. (2015), in that milk concentrations of 25(OH)D₃ were highest earlier in lactation compared with later. In addition, the current study found a correlation between plasma and milk 25(OH)D₃ concentrations up to 14 d postcalving but not after, which is also in agreement with Weiss et al. (2015), and may be due to a greater transfer of 25(OH)D₃ from plasma to milk via vitamin D binding protein so that colostrum contains a higher concentration for the neonate. Indeed, colostrum has a higher concentration of vitamin D binding protein than milk from later in lactation (Larson and Jorgensen, 1974).
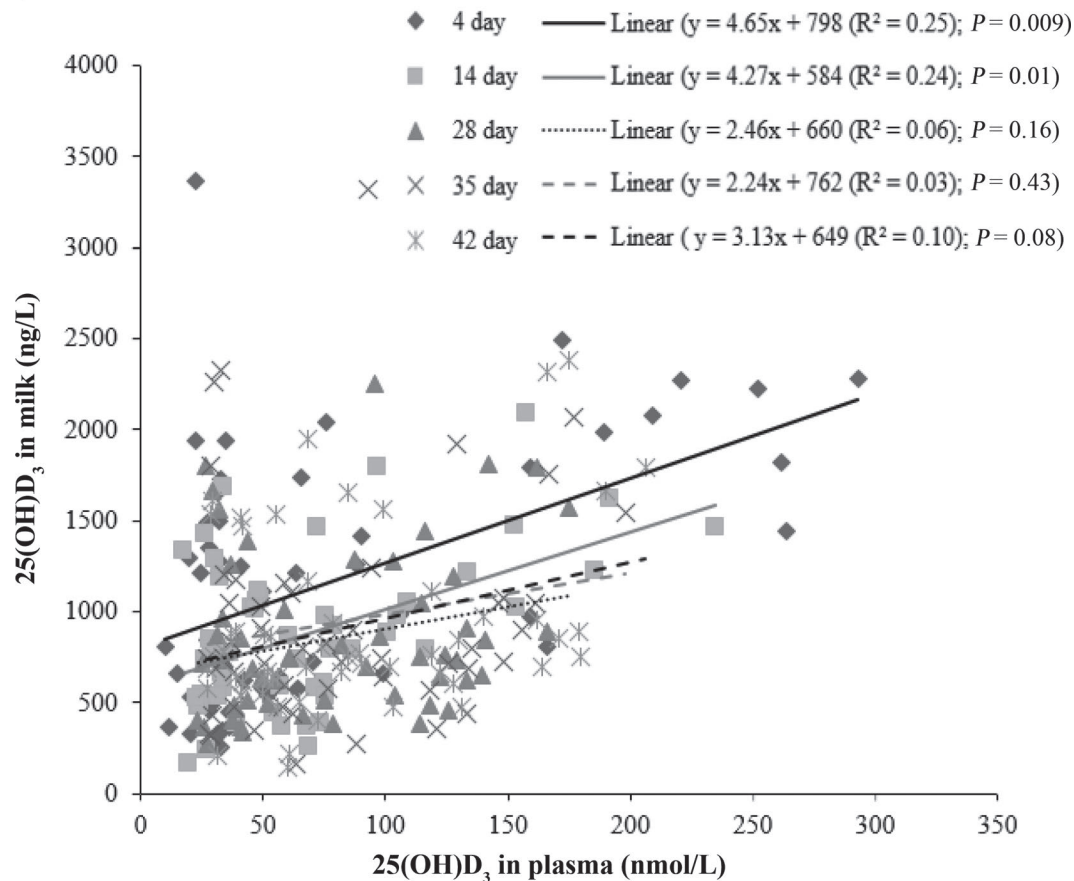
Figure 3. Corrections between 25-hydroxyvitamin D_3 in plasma and in milk.

Postcalving plasma and milk concentrations of calcium, phosphorus, and magnesium were not increased by vitamin D_3 or 25(OH)D_3 supplementation in the current study. This is consistent with Weiss et al. (2015) who also reported no effect on postpartum serum calcium, phosphorus, and magnesium after feeding the same dose of 6 mg/d of 25(OH)D_3 with a dietary cation-anion difference of −138 mEq/kg during the last 13 d of precalving. In contrast, Wilkens et al. (2012) reported a 24-h postpartum increased serum calcium concentration after feeding cows 3 mg of 25(OH)D_3 per day with dietary cation-anion difference of −168 mEq/kg during the last 10 d of precalving. Therefore, further research is required on the supplementation of vitamin D diet to reduce hypocalcemia in newly calved cows.

Milk concentration of 25(OH)D_3 in the current study ranged from 100 to 3,300 ng/kg, which, for a typical milk serving of 200 mL (FSA, 2005) would contribute 0.02 to 0.66 µg, well below the current UK vitamin D recommended intake of 10 µg/d (SACN, 2015). Nevertheless, supplementing diets with 25(OH)D_3 is more effective at raising plasma 25(OH)D_3 concentrations (which has a greater biological activity) than supplementing the same dose of vitamin D_3.

CONCLUSIONS

Supplementing dairy cows with 25(OH)D_3 was a successful strategy for increasing circulating concentrations of 25(OH)D_3 in the cow. However, transfer of this into milk appeared to be greater during early lactation (0–14 d). Therefore, supplementation of cow diets at this supplementation level is unlikely to be an effective dietary strategy for increasing 25(OH)D_3 content of milk further into lactation to address the current suboptimal vitamin D status within the general population.

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