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# Reduced serum vitamin D concentrations in healthy early-lactation dairy cattle

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## ABSTRACT

Cattle obtain vitamin D by ingestion or cutaneous exposure to UV light. Dairy cattle diets are frequently supplemented with vitamin D to compensate for limited sun exposure or during times of increased metabolic demands, such as the periparturient period, to maintain calcium homeostasis. Whether housing and supplemental vitamin D practices supply adequate amounts of vitamin D to optimally support the transition from gestation to lactation in dairy cattle is unknown. Our objective was to determine how serum vitamin D concentrations of dairy cows change with season, age, parity, and stage of lactation. Clinically healthy cows (n = 183) from 5 commercial dairies were enrolled in the study. Serum samples were collected at dry off, within 7 d of entering the close-up group, and within 7 d after calving (calving+7). Vitamin D status was determined by measuring serum 25-hydroxyvitamin D [25(OH)D] by radioimmunoassay. We performed repeated-measures mixed-effects linear regression to determine the effects of season, age, parity, and lactation stage (dry off, close-up, and calving+7) on 25(OH)D concentrations in serum. Bivariable analysis indicated that parity, age, and season were not associated with serum 25(OH)D concentrations. Sample period affected 25(OH)D concentrations, with the highest 25(OH)D levels at dry off (99.7 ± 1.9 ng/mL) followed by close up (93.8 ± 2.1 ng/mL), with the lowest levels at calving+7 (82.6 ± 1.7 ng/mL). These data showed a large depletion of 25(OH)D in dairy cattle postpartum compared with late prepartum, although the biological significance of this change in these healthy cattle is unclear. Consumption of serum 25(OH)D by immune system functions and calcium homeostasis in early lactation likely caused the reduction in serum 25(OH)D concentrations after calving. These results suggest that determining whether serum 25(OH)D concentrations are associated with the incidence of transition period disease is an appropriate next step. Assessing the effects of enhanced vitamin D supplementation of cows in early lactation on postpartum diseases may be warranted.

**Key words:** 25-hydroxyvitamin D, dairy cattle, transition period

## INTRODUCTION

Dietary micronutrients are crucial for enhanced immune cell functions and improved production efficiency of transition dairy cows (Spears and Weiss, 2008; Sordillo, 2016). Vitamin D is essential for skeletal development and calcium homeostasis, but current evidence suggests that vitamin D also plays an important role in optimizing immune cell functions (Adams and Hewison, 2008). Vitamin D signaling in immune cells improved some indices of innate immunity and suppressed some proinflammatory measures of adaptive immunity in cattle (Nelson et al., 2010, 2012). Moreover, vitamin D improved disease resistance in models of chronic inflammatory-based diseases and reproductive disorders in cattle (Téllez-Pérez et al., 2012; Girard et al., 2015). Infusion of vitamin D into infected mammary gland quarters enhanced local immune function, decreased colonization by mastitis-causing bacteria, and reduced SCC in milk compared with untreated cows (Lippolis et al., 2011). These data collectively support the premise that vitamin D improves dairy cattle immunity and may be an important micronutrient essential to preventing health disorders in dairy cows.

Dairy cattle obtain vitamin D from ingestion of vitamin $D_3$ supplements, or following UV light exposure from sunlight, or by consuming vitamin $D_2$ from plant fungi in forages (Horst et al., 1994; Hymøller and Jensen, 2012). Cattle metabolize both vitamin $D_2$ and vitamin $D_3$ but preferentially utilize vitamin $D_3$ (Horst et al., 1994). Vitamin $D_3$ is readily metabolized to 25-hydroxyvitamin D [**25(OH)$D_3$**] by 25-hydroxylases in the liver and is the major circulating form of vitamin D in cattle. The active metabolite, 1,25-dihydroxyvita-

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min $D_3$ [**$1,25(OH)_2D_3$**] is formed by 1α-hydroxylation of 25(OH)$D_3$ (Horst et al., 1994). The conversion of 25(OH)$D_3$ to 1,25$(OH)_2D_3$ is tightly regulated in the kidney by parathyroid hormone (PTH) and locally by immune cells responding to immune signals (Horst et al., 1994; Adams and Hewison, 2008).

Dairy cattle typically receive dietary vitamin $D_3$ supplementation to maintain consistent concentrations of 25(OH)D (the lack of subscript indicates $D_2$ and $D_3$) in the blood. The National Research Council recommends 21,000 IU/d of vitamin D for dairy cows to maintain serum 25(OH)D concentrations between 20 and 50 ng/mL and support calcium homeostasis (NRC, 2001). Supplementation is necessary because the vitamin $D_2$ content of forages is inconsistent and vitamin $D_2$ is less potent and effective at maintaining serum 25(OH)D concentrations compared with vitamin $D_3$ (Horst et al., 1994). Exposure to UV light from sunlight is affected by season (Hymøller and Jensen, 2010, 2012) and husbandry. Many lactating cattle are housed indoors, limiting endogenous vitamin $D_3$ production and making diet the major source of vitamin D in these cows.

The concentrations of 25(OH)D in blood that correspond to optimal health and performance of animals and humans remain unknown. A minimum threshold of 30 ng/mL to support immune function was proposed based on epidemiological data from human populations but remains hypothetical (Gunville et al., 2013). A recent survey of vitamin D status of dairy cows showed that the majority of lactating cows received 1.5 to 2.5 times the NRC recommendation for supplemental vitamin $D_3$ and had average serum 25(OH)D concentrations of 60 to 70 ng/mL, ranging between 40 to 100 ng/mL (Nelson et al., 2016). The broad range in serum 25(OH)D concentrations in these dairy cattle might be due to metabolic demands that occur during the production cycle and dramatic shifts in DMI (Horst et al., 1994; Ingvartsen and Andersen, 2000; Hayirli et al., 2002). However, limited data exist describing the effects of external influences on serum 25(OH)D concentrations in dairy cows. The objective of this study was to determine how serum 25(OH)D concentrations of healthy dairy cattle are affected by season, age, parity, and stage of lactation as dairy cattle transition from dry off to the close-up and early postpartum periods. We hypothesized that serum 25(OH)D concentrations would be significantly lower in dairy cattle during early lactation compared with dry off and the close-up period. We performed a longitudinal, herd-based epidemiologic investigation of serum 25(OH)D concentrations in healthy dairy cattle on 5 commercial farms by sampling dairy cattle of varying parities and ages during different seasons and at 3 specific times during the lactation cycle.

## MATERIALS AND METHODS

### Animals

The Animal Use and Care Committee at Michigan State University (East Lansing) approved this study and all animal protocols. A total of 300 cows from 5 commercial dairy herds in Michigan were enrolled. Cohorts containing 15 cows/cohort from each of the 5 farms were included in the study. Cows in the cohorts were selected randomly from the group of animals to be dried off each week and were enrolled based on date of dry off and stage of lactation. Each cohort of 15 cows contained 3 groups of 5 cows per group that included 5 heifers <25 mo old, 5 second-lactation cows, and 5 cows that were third or greater lactation. Cows were approximately 200 to 230 d pregnant and <380 DIM at the time of dry-off. All cows were bred by AI to ensure more accurate calving dates. The health status of each cow was monitored from the nonlactating period through the lactation cycle using Dairy COMP305 (Alta Genetics Inc., Watertown, WI) or PCDART (NorthStar Michigan Lab, Grand Ledge, MI) on-farm software. All animal health records were maintained in Dairy COMP305 or PCDART using established treatment protocols so that disease incidence was recorded consistently. Cows diagnosed with mastitis, metritis, ketosis, lameness, displaced abomasum, pneumonia, milk fever, or retained placenta during the first 30 d of lactation, and cows with other negative health outcomes including abortion and death were excluded from the analysis. Each farm fed a TMR supplemented with vitamin $D_3$ (Table 1).

### Measurement of 25-Hydroxyvitamin D

Blood samples were collected from the tail vein at dry off, within 7 d of entering the close-up group, and within 7 d of calving (calving+7). Sampling interval between close-up and calving+7 was 22.3 ± 7.5 d (mean ± SD). Serum was harvested and stored at −20°C for batch analysis of 25(OH)D for 1 to 6 mo. The metabolite 25(OH)D is robust and stable when stored in sealed glass or plastic vials at −20°C for up to 48 mo (Ockè et al., 1995). The total concentration of 25(OH)D (the sum of 25-hydroxyvitamin $D_2$ and 25-hydrovitamin $D_3$) in serum samples was measured by RIA by Heartland Assays (Iowa State University Research Park, Ames; Farrell et al., 2012) in singlicates. The detection range of the assay was 2.5 to 100 ng/mL, with analytical sensitivity of 1.5 ng/mL. Analytical specificity based on cross reactivity of other metabolites was 100% for 25(OH)$D_3$, 25(OH)$D_2$, 24,25$(OH)_2D_2$, 24,25$(OH)_2D_3$, 25,26$(OH)_2D_2$, 25,26$(OH)_2D_3$, and 11% for 1,25(OH)$D_2$

**Table 1**. Vitamin D (kIU/kg of DM) supplemented in feed by farms for heifers and cows during 2 to 10 DIM, close up, and dry off

| | 2–10 DIM | | Close up[1] | | Dry off[2] | |
|---|---|---|---|---|---|---|
| Farm | Cow | Heifer | Cow | Heifer | Cow | Heifer |
| 1 | 1.67 | 1.67 | 4.40 | 2.73 | 1.78 | 2.2 |
| 2 | 1.21 | 1.21 | 9.30 | 9.30 | 4.47 | 4.47 |
| 3 | 2.42 | 2.42 | 4.38 | 2.87 | 3.96 | 4.38 |
| 4 | 1.45 | 1.45 | 1.45 | 1.45 | 1.45 | 1.45 |
| 5 | 1.32 | 1.32 | 1.32 | 1.32 | 1.32 | 1.32 |
| Mean ± SD | 1.60 ± 0.48 | 1.60 ± 0.48 | 4.18 ± 3.20 | 3.54 ± 3.3 | 2.60 ± 1.50 | 2.77 ± 1.56 |

[1]Close up = approximately 21 d prepartum.
[2]Dry off = approximately 60 d prepartum.

and 1,25(OH)$D_3$. The assay has <0.8% cross reactivity with vitamin $D_2$ and vitamin $D_3$. The interassay and intraassay coefficients of variation were 13.3 and 6.5%, respectively. The assay trueness was checked by serial dilution and recovery test for linearity (parallelism).

### *Data Analysis*

Repeated-measures linear mixed-effects models were constructed using the Proc Mixed command in SAS 9.4 (SAS Institute Inc., Cary, NC) to analyze the effects of sample period, age, parity, and season on serum 25(OH)D concentrations. Age, parity, and season were treated as categorical fixed effects. Categories for age and parity were created based on the distribution of the data (Table 2). Season was based on what month the samples were collected (March, April, May = spring; June, July, August = summer; September, October, November = fall; December, January, February = winter). Sample period was coded as the average number of days from dry off for each of the 3 sample points, 0 d (dry off), 32 d (close up), and 55 d (calving+7). The final multivariable linear mixed-effects model was constructed using a backward elimination method, which entailed starting with a loaded mean structure model, selecting the covariance structure for random effects, selecting a residual covariance structure, and then testing the significance of interaction terms and fixed effects (West et al., 2007). Loaded mean structure models include all possible fixed effects and biologically plausible interaction terms. In addition, the loaded model included random intercepts for farm, cohort nested within farm, and cow nested within cohort and farm. To account for unequally distanced sampling points, the best fitting spatial covariance structure was selected based on Akaike's information criterion (smallest) or convergence of the model. Degrees of freedom were estimated using the Kenward-Roger approximation. Random effects of intercepts for farm, cohort, and cow were tested for retention in the model. Random effects and residual covariance structures were tested for significance using likelihood-ratio tests based on REML estimation. The test statistic for testing random effects was a mixture of chi-squared distributions with degrees of freedom of 0 to 1, and equal weights of 0.5. Multicollinearity of fixed effects was tested by estimating Spearman correlation coefficients before inclusion in the multivariable model building. Fixed effects and all plausible 2-way interaction terms were selected for inclusion in the model by comparing nested models using likelihood-ratio tests based on maximum likelihood estimation (MLE). Normal distribution of residuals and heteroskedasticity was assessed visually using histograms and Q-Q (quantile-quantile) plots of standardized residuals, and predicted-residual plots. Potential heteroskedasticity of sample period was also tested using a likelihood ratio tests based on REML estimation using the GROUP option in the REPEATED statement. Statistical significance was set at $P < 0.05$.

## RESULTS

Three hundred cows were enrolled and blood samples were collected from 283 cows from September 24, 2014, to December 15, 2015. One hundred cows were excluded from the study due to disease at some point during the study. The final study population included data from the remaining 183 cows, 11 of which had incomplete 25(OH)D data. Ten cows were missing vitamin D data for the close-up period because these 10 cows calved early. One of 11 cows was missing a 25(OH)D data point for calving+7 for an unknown reason. Descriptive statistics for 25(OH)D by parity, age, season, and sample period are shown in Table 2.

The bivariable analyses that evaluated the effects of parity, season, and age on serum 25(OH)D concentrations are shown in Table 3. Random intercepts for farm and cow significantly improved model fit ($P < 0.01$) in all 3 bivariable analyses. Cohort did not significantly improve model fit and was not included for parity ($P$

**Table 2**. Descriptive statistics for serum 25-hydroxyvitamin D [25(OH)D] concentrations (ng/mL) by parity, age, season, and sample period (n = 183 cows)

| Variable | No. of cows | No. of samples | Mean | SEM | Minimum | 25th percentile | Median | 75th wpercentile | Maximum |
|---|---|---|---|---|---|---|---|---|---|
| Sample period | | | | | | | | | |
| Dry off | — | 183 | 99.7 | 1.9 | 47.6 | 81.5 | 97.7 | 112.8 | 198.6 |
| Close up | — | 173 | 93.8 | 2.1 | 24.9 | 76.7 | 94.1 | 109.7 | 210.4 |
| Calving+7[1] | — | 182 | 82.6 | 1.7 | 40.1 | 64.2 | 82.6 | 96.5 | 166.4 |
| Lactation number (parity) | | | | | | | | | |
| First | 68 | 198 | 89.1 | 2.2 | 24.9 | 68.0 | 84.2 | 106.4 | 210.4 |
| Second | 66 | 195 | 94.6 | 1.7 | 47.7 | 77.6 | 94.1 | 110.4 | 185.6 |
| Third | 27 | 81 | 93.4 | 2.7 | 36.2 | 76.7 | 94.7 | 105.9 | 176.1 |
| Fourth or more | 22 | 64 | 91.2 | 3.1 | 32.4 | 79.2 | 95.7 | 107.0 | 132.5 |
| Age (yr) | | | | | | | | | |
| ≤2 | 68 | 198 | 89.2 | 2.2 | 24.9 | 68.0 | 84.2 | 106.4 | 210.4 |
| 3 | 65 | 192 | 94.5 | 1.7 | 47.7 | 77.7 | 93.9 | 109.7 | 185.6 |
| 4 | 26 | 78 | 93.9 | 2.8 | 36.2 | 76.7 | 94.8 | 106.4 | 176.1 |
| ≥5 | 23 | 67 | 90.7 | 3.0 | 32.4 | 78.2 | 95.6 | 106.9 | 132.5 |
| Season | | | | | | | | | |
| Spring | — | 164 | 93.2 | 2.5 | 32.4 | 70.6 | 89.9 | 112.9 | 210.4 |
| Summer | — | 73 | 84.4 | 2.2 | 43.6 | 72.9 | 85.0 | 95.8 | 129.1 |
| Fall | — | 248 | 94.5 | 1.5 | 24.9 | 79.5 | 96.1 | 109.8 | 176.4 |
| Winter | — | 53 | 86.9 | 4.2 | 35.8 | 67.1 | 81.5 | 96.7 | 198.6 |
| Total | 183 | 538 | 92.0 | 1.2 | 24.9 | 73.7 | 90.5 | 108.1 | 210.4 |

[1]Within 7 d after calving.

= 0.33), season ($P = 0.30$), or age ($P = 0.33$) analyses. Parity, age, and season were not significantly associated with serum 25(OH)D concentrations ($P = 0.26$, 0.37, and 0.40, respectively).

In the multivariable analysis, age was not included due to its high correlation with parity (r = 0.99). The spatial power residual covariance structure was used to account for unequal spacing between sample

**Table 3**. Summary of bivariable analyses estimating associations between serum 25-hydroxyvitamin D [25(OH)D] concentrations (ng/mL) and parity, age, and season

| | | | 95% CI | | | |
|---|---|---|---|---|---|---|
| Parameter | Estimate | SE | Lower | Upper | *P*-value | ICC[1] |
| Sample period[2] | −0.30 | 0.03 | −0.36 | −0.24 | <0.01 | 0.61 |
| Intercept | 100.49 | 6.44 | 82.91 | 118.07 | <0.01 | |
| Lactation number (parity)[3] | | | | | | |
| Intercept | 88.89 | 6.00 | 77.14 | 100.65 | 0.26 | 0.53 |
| First (referent) | — | — | — | — | | |
| Second | 5.42 | 3.21 | −0.87 | 11.72 | | |
| Third | 5.65 | 4.26 | −2.70 | 14.00 | | |
| Fourth or more | −0.12 | 4.57 | −9.08 | 8.84 | | |
| Age (yr)[4] | | | | | | |
| Intercept | 88.89 | 6.00 | 77.15 | 100.64 | 0.37 | 0.54 |
| ≤2 (referent) | — | — | — | — | | |
| 3 | 5.43 | 3.23 | −0.91 | 11.77 | | |
| 4 | 5.72 | 4.32 | −2.75 | 14.20 | | |
| ≥5 | 0.02 | 4.52 | −8.84 | 8.87 | | |
| Season[5] | | | | | | |
| Intercept | 92.15 | 5.74 | 82.86 | 105.37 | 0.40 | 0.54 |
| Spring (referent) | — | — | — | — | | |
| Summer | −2.92 | 2.95 | −8.70 | 2.85 | | |
| Fall | 0.61 | 2.84 | −4.96 | 6.19 | | |
| Winter | −3.41 | 3.50 | −10.27 | 3.44 | | |

[1]ICC = intraclass correlation coefficient at the cow-within-farm level.

[2]Final model. Random effect estimates, farm: 193.34 (95% CI: 66.93, 1,867.09), cow: 256.68 (95% CI: 197.05, 348.32), spatial power 0.53 (95% CI: −11.27, 12.34).

[3]Random intercept estimates, farm: 154.01 (95% CI: 41.38, 573.19), cow: 224.77 (95% CI: 163.23, 309.52).

[4]Random intercept estimates, farm: 153.36 (95% CI: 41.17, 571.26), cow: 227.98 (95% CI: 165.90, 313.30).

[5]Random intercept estimates, farm: 144.76 (95% CI: 38.57, 543.30), cow: 234.32 (95% CI: 171.05, 320.99).

periods. Inspection of the histograms and Q-Q plots of standardized residuals indicated normality. Similar to the bivariable analyses, random intercepts for farm and cow significantly improved model fit ($P < 0.001$), whereas cohort was excluded because it did not improve the model fit ($P = 0.32$). Parity and season were removed from the model during backward elimination due to a lack of association with serum 25(OH)D concentrations ($P = 0.25$ and $P = 0.75$, respectively). Only sample period remained in the final model and it was significant ($P < 0.01$), with the highest serum 25(OH)D concentrations at dry off followed by close up, and the lowest levels being detected at calving+7 (Table 3).

## DISCUSSION

The incidence and severity of dairy cattle disease are greatest during the physiologic transition from late gestation to early lactation (Castillo et al., 2005; Sordillo and Raphael, 2013). Disease risk in these cows is associated with increased metabolic pressures and dysfunctional immunity that occur late in pregnancy and during early lactation (Sordillo, 2016). Vitamin D supplementation enhances innate immunity by stimulating the production of pattern recognition receptors, antimicrobial peptides and cytokines by immunologically responsive cells (Dimitrov and White, 2017) and improves metabolic and reproductive profiles in transition dairy cattle (Omur et al., 2016). Vitamin D administration is also associated with diminished oxidative stress by increasing total oxidative capacity in transition water buffaloes with subclinical mastitis (Dimri et al., 2013). Our results showed that serum 25(OH)D concentrations were significantly lower in the transition period during early lactation compared with the close-up and dry-off periods, at a time when dairy cattle are most vulnerable to disease and metabolic stresses. The biological significance of a reduction in serum 25(OH)D concentrations in these healthy dairy cows is unclear and likely negligible. However, diminished serum 25(OH)D concentrations in transition dairy cattle may enhance oxidative stress and play a role in disease susceptibility. Future studies determining associations between changes in serum 25(OH)D concentrations and occurrence of transition period diseases and establishing the optimum serum concentrations of 25(OH)D to support immune function in dairy cattle may be warranted.

Determining the cause of the decrease in serum 25(OH)D during the transition period was beyond the scope of this study. Likely, the decrement in serum 25(OH)D concentration was due to increased consumption of vitamin D metabolites by immunological and calcium homeostatic systems during the early postpartum period. Nelson and colleagues surveyed serum 25(OH)D in dairy cattle at different stages of lactation and noted that serum 25(OH)D was lower in early-lactation cows compared with mid- and late-lactation dairy cattle (Nelson et al., 2016). Olsen and colleagues reported that stage of lactation had a significant effect on the serum concentrations of vitamin D binding protein (**DBP**) and 25(OH)D (Olsen et al., 2016); DBP is bound to 25(OH)D in circulation. Both DBP and 25(OH)D were lowest at the beginning of lactation and increased as lactation progressed (Olsen et al., 2016), suggesting that consumption of these nutrients at parturition led to their depletion.

The total antioxidant potential and many specific antioxidants, including vitamin E, selenium, and ascorbic acid, are depleted during the transition period in dairy cattle (Weiss et al., 1990; Bernabucci et al., 2005; Castillo et al., 2005; Sordillo et al., 2007) supporting the premise that consumption of 25(OH)D metabolites during early lactation may account for the decrement in serum concentrations. Oxidative stress, an imbalance between the production of reactive oxygen metabolites and antioxidants, occurs during the transition period, leading to antioxidant depletion in an attempt to balance the pro-oxidative status of the cow (Bernabucci et al., 2005; Sordillo et al., 2007). Parturition is an inflammatory event (Bradford et al., 2015). The highest serum concentrations of the acute phase proteins haptoglobin and C-reactive protein were measured in Holstein-Friesian dairy cows within the first postpartum month compared with prepartum and late-lactation samples (Dębski et al., 2016). An inverse correlation was reported between C-reactive protein and 25(OH)D in humans, with a 40% decrement in 25(OH)D when C-reactive protein increased from <5 to >80 mg/L (Duncan et al., 2012). Systemic inflammatory processes, such as parturition and oxidative stress, reduce serum 25(OH)D concentrations due to intracellular hydroxylation of 25(OH)D to $1,25(OH)_2D_3$, enhancing consumption of vitamin D metabolites (Heaney and Armas, 2015).

Calcium demand by the mammary gland in early lactation overwhelms a cow's ability to maintain normal plasma calcium (Horst et al., 1994). Increased parathyroid hormone reduces urinary calcium losses, stimulates bone calcium resorption, and increases $1,25(OH)_2D_3$ synthesis to enhance the active transport of calcium in the intestine. The increase in $1,25(OH)_2D_3$ at parturition drives the vitamin D metabolite degradation pathways, leading to the depletion of serum 25(OH)D concentrations (Horst et al., 1994). Colostrogenesis also consumes 25(OH)D (Horst et al., 2005). Colostrum contains 2 to 3 times as much $25(OH)D_3$ as samples taken at the sixth milking and 28 DIM (Weiss et al., 2015). The combined effects of these lactation and im-

mune system events likely caused the decline in serum 25(OH)D concentrations measured at calving+7 in this study.

Transient renal dysfunction and disruption of renal tubular cells could lead to diminished serum 25(OH)D concentrations in early-lactation cows. Tubular cell dysfunction and alterations in tubular cell proteins can cause excretion of 25(OH)D/DBP in the urine, resulting in a lower serum concentration of 25(OH)D (Anderson et al., 2010). The cows in our study were clinically healthy, making even subclinical kidney dysfunction unlikely. However, subclinical injury might precede kidney dysfunction. Serum creatinine was increased in dairy cattle at parturition compared with 3 wk postpartum (Omur et al., 2016). Increased serum creatinine is indicative of parenchymal injury and kidney dysfunction. In that study, dairy cattle supplemented with antioxidant vitamins, including vitamin D and trace elements, showed no increase in serum creatinine in postpartum early lactation compared with prepartum measurements, and the creatinine values at parturition in the supplemented cattle were significantly lower than in controls (Omur et al., 2016).

Measurement of individual cow DMI was beyond the scope of this study. The diminished serum 25(OH)D concentrations measured at calving+7 might have been due in part to decreased vitamin D dietary consumption. Substantial decrements in DMI occur in late pregnancy and continue into early lactation in dairy cattle (Ingvartsen and Andersen, 2000). Dry matter intake decreased 32% during the final 3 wk of gestation, and 89% of that decline occurred during the final week of gestation (Hayirli et al., 2002). Plasma half-life of 25(OH)$D_3$ in dairy cows is 16 to 32 d but depends on amount supplemented, parity, and diet (Wilkens et al., 2013).

Dairy cattle in the current study were from 5 commercial Michigan farms located in a latitude range of 43 to 44°N and housed largely indoors, limiting their exposure to UV light. Indoor housing was likely responsible for the lack of a measurable season effect of serum 25(OH)D in the study population. Parity and age were not significantly associated with serum 25(OH)D concentrations in our study, supporting the results of a large survey of serum 25(OH)D concentrations in dairy cattle (Nelson et al., 2016).

## CONCLUSIONS

Serum 25(OH)D concentrations were lowest during early lactation, a time of increased disease susceptibility. The reason for the decrement in serum 25(OH)D concentrations at calving+7 is unclear but might be due to increased consumption of vitamin D by immune and calcium homeostatic systems. Establishing associations between serum 25(OH)D concentrations and transition period diseases is an important next step toward determining optimum 25(OH)D concentrations to support immune health in dairy cows.

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