



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 D₃ supplements, or following UV light exposure from sunlight, or by consuming vitamin D₂ from plant fungi in forages (Horst et al., 1994; Hymøller and Jensen, 2012). Cattle metabolize both vitamin D₂ and vitamin D₃ but preferentially utilize vitamin D₃ (Horst et al., 1994). Vitamin D₃ is readily metabolized to 25-hydroxyvitamin D [25(OH)D₃] by 25-hydroxylases in the liver and is the major circulating form of vitamin D in cattle. The active metabolite, 1,25-dihydroxyvita-

Received July 21, 2017.

Accepted October 8, 2017.

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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; Ingvarsen 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 $\pm$ 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-hydroxyvitamin 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 $1,25(OH)_2D_2$.

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