Vitamin D status of dairy cattle: Outcomes of current practices in the dairy industry

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ABSTRACT

The need for vitamin D supplementation of dairy cattle has been known for the better part of the last century and is well appreciated by dairy producers and nutritionists. Whether current recommendations and practices for supplemental vitamin D are meeting the needs of dairy cattle, however, is not well known. The vitamin D status of animals is reliably indicated by the concentration of the 25-hydroxyvitamin D [25(OH)D] metabolite in serum or plasma, with a concentration of 30 ng/mL proposed as a lower threshold for sufficiency. The objective of this study was to determine the typical serum 25(OH)D concentrations of dairy cattle across various dairy operations. The serum 25(OH)D concentration of 702 samples collected from cows across various stages of lactation, housing systems, and locations in the United States was 68 ± 22 ng/mL (mean $\pm$ standard deviation), with the majority of samples between 40 and 100 ng/mL. Most of the 12 herds surveyed supplemented cows with 30,000 to 50,000 IU of vitamin D₃/d, and average serum 25(OH)D of cows at 100 to 300 DIM in each of those herds was near or above 70 ng/mL regardless of season or housing. In contrast, average serum 25(OH)D of a herd supplementing with 20,000 IU/d was 42 ± 15 ng/mL, with 22% below 30 ng/mL. Cows in early lactation (0 to 30 d in milk) also had lower serum 25(OH)D than did mid- to late-lactation cows (57 ± 17 vs. 71 ± 20 ng/mL, respectively). Serum 25(OH)D of yearling heifers receiving 11,000 to 12,000 IU of vitamin D₃/d was near that of cows at 76 ± 15 ng/mL. Serum 25(OH)D concentrations of calves, on the other hand, was 15 ± 11 ng/mL at birth and remained near or below 15 ng/mL through 1 mo of age if they were fed pasteurized

waste milk with little to no summer sun exposure. In contrast, serum 25(OH)D of calves fed milk replacer containing 6,600 and 11,000 IU of vitamin D₂/kg of dry matter were 59 ± 8 and 98 ± 33 ng/mL, respectively, at 1 mo of age. Experimental data from calves similarly indicated that serum 25(OH)D achieved at approximately 1 mo of age would increase 6 to 7 ng/mL for every 1,000 IU of vitamin D₃/kg of dry matter of milk replacer. In conclusion, vitamin D status of dairy cattle supplemented with vitamin D₃ according to typical practices, about 1.5 to 2.5 times the National Research Council recommendation, is sufficient as defined by serum 25(OH)D concentrations. Newborn calves and calves fed milk without supplemental vitamin D₃, however, are prone to deficiency.

Key words: vitamin D, dairy cattle

INTRODUCTION

The requirement of vitamin D for normal growth and development of cattle was established not long after the discovery of the fat-soluble factor that prevented rickets (Rupel et al., 1932; Bechdel et al., 1935; Wallis, 1937). The minimal requirement of 6.7 IU of vitamin D/kg of BW was originally established as the amount needed to prevent rickets in calves (Huffman and Duncan, 1935; Bechdel et al., 1937). Eventually, it was established that vitamin D was not simply a nutrient required for normal skeletal growth and development; rather, it served as a precursor to an intricate endocrine mechanism that maintained Ca and P concentrations in blood (Lund and DeLuca, 1966; Fraser and Kodicek, 1970; DeLuca, 1971). More recently, vitamin D has been shown to have multiple physiological roles, such as control of cellular differentiation and proliferation and activation of innate immune defenses (Norman, 2008; Adams and Hewison, 2010; Nelson et al., 2012). The newfound roles of vitamin D, along with its critical role in Ca and P homeostasis, highlight the need to examine

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current practices for vitamin D supplementation in the dairy industry and question whether current recommendations are adequate for dairy cattle.

Cattle naturally acquire vitamin D as vitamin D₂ from plant-associated fungi or as vitamin D₃ that is synthesized endogenously in sun-exposed skin from 7-dehydrocholesterol. Cattle acquire appreciable amounts of vitamin D₂ from forages. Alfalfa hay, for instance, can contain as much as 2,500 IU of vitamin D₂/kg of DM and corn silage can contain approximately 500 IU of vitamin D₂/kg of DM (Wallis et al., 1958; Horst et al., 1984). The vitamin D₂ content within forage types is highly variable (160 to 2,500 IU/kg of DM for alfalfa hay), however, which excludes forages as a consistent source of vitamin D. Vitamin D₂ also is not metabolized as efficiently as vitamin D₃ (Sommerfeldt et al., 1983; Hymøller and Jensen, 2011); rather, vitamin D₃ metabolites are the major form circulating in blood of cattle exposed to summer sun (Horst and Littledike, 1982), and supplemental vitamin D is most often provided to cattle as vitamin D₃.

Vitamin D₂ and vitamin D₃ are readily converted by 25-hydroxylases to 25-hydroxyvitamin D [**25(OH)D**; refers to D₂ or D₃ metabolite if no subscript is given]. The concentration of 25(OH)D in serum or plasma is the best indicator of the vitamin D status of an animal. Similar to humans, circulating 25(OH)D concentrations of 20 to 50 ng/mL of serum have traditionally been described as normal for cattle, with concentrations below 10 ng/mL indicative of vitamin D deficiency (Horst et al., 1994; Norman, 2008). However, humans and cattle that receive abundant exposure to summer sun (i.e., lifeguards or cattle on pasture) without vitamin D supplementation have serum 25(OH)D concentrations between 40 to 100 ng/mL (Hollis, 2005; Casas et al., 2015; Nelson et al., 2016).

The 25(OH)D metabolite is the precursor to the active hormone, 1,25-dihydroxyvitamin D [**1,25(OH)₂D**] that acts to upregulate expression of genes related to calcium binding and transport, bone remodeling, and innate host-defense, among others. Concentrations of 1,25(OH)₂D in circulation are tightly controlled between 20 to 100 pg/mL, even at low (i.e., 10 to 20 ng/mL) 25(OH)D concentrations (Rajaraman et al., 1997; Nonnecke et al., 2009), in response to the endocrine system that controls Ca and P homeostasis. In contrast, low concentrations of 25(OH)D impair synthesis of 1,25(OH)₂D by immune cells in response to pathogens (Liu et al., 2006; Nelson et al., 2010; Merriman et al., 2015). The minimal 25(OH)D concentration required for optimal immune function in cattle has not been defined, but 25(OH)D concentrations below 30 ng/mL have been hypothesized to represent a state of vitamin

D insufficiency (Hollis, 2005; Norman, 2008; Adams and Hewison, 2010; Nelson et al., 2012).

In the absence of data defining the 25(OH)D concentrations required for optimal health and productivity, the 25(OH)D concentrations of animals on summer pasture provide an approximate target. The range of 20 to 50 ng/mL that was originally defined as normal for cattle was based on the 25(OH)D concentrations of a few seemingly healthy cattle (Horst and Littledike, 1982). In contrast, more recent reports have shown that serum 25(OH)D concentrations of beef calves coming off summer pasture without vitamin D supplementation are between 50 to 60 ng/mL, on average, whereas those of beef cows are between 70 to 80 ng/mL. Those values are based on nearly 300 calves and 30 cows from multiple locations in the United States and suggest that normal serum 25(OH)D concentrations of cattle in natural settings are somewhat higher than those originally reported.

Just as the serum 25(OH)D concentrations required for health of cattle are not well-defined, so are the recommendations of supplemental vitamin D. Wallis (1946) proposed that lactating cattle should receive 12,000 to 15,000 IU of supplemental vitamin D. The NRC (2001) recommends 21,000 IU supplemental vitamin D₃/d (~800 to 1,000 IU/kg of DM) for lactating Holstein cows (calculated for 680 kg of BW). Dairy producers instead typically provide lactating cows with 30,000 to 50,000 IU of vitamin D₃ (Weiss, 1998). The NRC (2001) recommends about 300 IU of vitamin D₃/d (600 IU/kg of DM) for dairy calves, the minimum necessary to prevent rickets in calves (Bechdel et al., 1937). Milk replacers, on the other hand, typically contain 11,000 IU/kg of DM. The NRC recommendation and current practices for cows and calves, however, are not based on dose titration studies or corresponding serum 25(OH)D concentrations. Whether or not the recommendations and practices for vitamin D supplementation of dairy cattle are adequate or excessive is largely unknown.

The objective of our study was to assess serum 25(OH)D concentrations of dairy cattle fed and managed according to typical industry practices with the goal of ascertaining the appropriateness of current practices in the dairy industry. Serum samples were collected from dairy cattle from 12 dairy herds across different management practices and locations in the United States and analyzed for concentrations of 25(OH)D. Serum 25(OH)D concentrations from experiments with dairy calves also were analyzed to develop predictions of serum 25(OH)D in response to supplemental vitamin D as a guide for vitamin D supplementation of calves. Altogether, our study provides a formative assessment of serum 25(OH)D concentrations of dairy cattle fed

and managed according to typical industry practices and identifies needs and opportunities for improvement of health and productivity through vitamin D supplementation.

MATERIALS AND METHODS

All procedures were performed according to animal care protocols approved at each respective institution involved in accordance with accepted principles and guidelines for care and use of animals in research (FASS, 2010).

Survey of Serum 25(OH)D Concentrations

Blood samples were collected from dairy cows in 12 dairy herds across different management practices and locations in the United States (Table 1). Information regarding housing, time spent outdoors, season of sample collection, and estimated supplemental vitamin D₃ were collected. All of the herds consisted of Holstein cows, except herd 6, which consisted of approximately 50% Holstein cows and 50% Jersey cows. Blood samples were collected in April from 2 herds in southeastern United States from Holstein heifers approximately 1 yr of age, along with samples from preweaned Holstein calves from 6 farms using various management and housing practices. Blood samples from preweaned calves were collected after colostrum consumption.

Blood samples were either transported or shipped to the laboratory where serum was collected and stored at -20°C until analysis. Serum 25(OH)D concentra-

tions were measured using validated RIA (DiaSorin, Stillwater, MN) or ELISA methods (VID3-K01, Eagle BioScience, Nashua, NH). The assays were performed as previously reported using standards prepared in vitamin D-deficient bovine calf serum (Hollis et al., 1993; Nelson et al., 2016). The data were analyzed using GraphPad Prism (GraphPad Software Inc., La Jolla, CA) to determine the mean and variance of serum 25(OH)D of cows and heifers. Comparisons of 25(OH)D across stage of lactation, season of collection, supplemental vitamin D, or age of calves were performed by using the mixed procedure of SAS (version 9.3, SAS Institute Inc., Cary, NC) with animal within herd as a random variable.

Effects of Dietary Supplemental Vitamin D₃ on Serum 25(OH)D of Calves

Two experiments were conducted at the USDA National Animal Disease Center in Ames, Iowa, where Holstein bull calves were fed milk replacer containing 1,700 or 17,900 IU of vitamin D₃/kg of milk replacer powder in one experiment, and 400 or 11,000 IU of vitamin D₃/kg of milk replacer powder in a second experiment. The milk replacer was formulated to meet NRC recommendations for dairy calves and fed to achieve a medium rate of growth according to model predictions (NRC, 2001). The calves received colostrum at birth and were housed indoors and raised strictly on a milk replacer diet from 2 to 3 d of age up to 10 wk in the first experiment and 6 wk of age in the second experiment. Serum samples were collected from the calves

Table 1. Serum 25-hydroxyvitamin D [25(OH)D] concentrations of dairy cows¹

Herd	Herd size	No. of samples	Herd location	Supplemental vitamin D ² (kIU)	Time outside ³ (h)	Months of collection ⁴	Serum 25(OH)D ⁵ (ng/mL)
1	100	92	WI	49	2	3, 6, 9, 12	72 ± 23
2	90	11	MN	30	8	9	69 ± 16
3	200	271	WI	35	<1	3, 6, 9, 12	63 ± 22
4	50	26	WI	31	<1	3, 6, 9, 12	71 ± 24
5	60	29	MN	30	8	9, 12	71 ± 18
6	160	139	WI	43	4	3, 6, 9, 12	69 ± 16
7	120	20	OH	30	<1	12	104 ± 18
8	400	12	IA	35	<1	5, 10	71 ± 10
9	4,000	16	FL	47	<1	4	81 ± 17
10	5,000	22	FL	47	<1	4	85 ± 14
11	2,500	24	GA	36	<1	4	81 ± 15
12	500	32	FL	20	<1	2	42 ± 15
Total		702					68 ± 22

¹Data represent lactating cows from 0 to 300 DIM and pregnant, nonlactating cows.

²Estimated amount of supplemental vitamin D₃ each cow received per day based on amount of vitamin-mineral mix fed at the herd level and concentration of vitamin D₃ in the mix.

³Estimated time the herd spent outdoors each day during daylight hours.

⁴Represents the months within the calendar year in which serum samples were collected from each herd.

⁵Serum 25(OH)D was measured by RIA for herds 1 to 8, and ELISA for herds 9 to 12. Data represent mean ± SD of all cows sampled within each herd regardless of DIM. Some cows were sampled multiple times for herds where samples were collected more than once.

every 3 to 4 d and 25(OH)D was measured by RIA, as previously described (Hollis et al., 1993). The serum 25(OH)D data were analyzed by using a mixed model with experiment, dose of vitamin D₃, age, dose × age, and dose × age² were included as fixed effects and calf nested within treatment as a random effect. The effect of experiment was not significant, so it was removed from the final version of the model. Serum 25(OH)D data of the calves at ≥30 d of age, where serum 25(OH)D concentrations reached a plateau, were also analyzed without age in the model to determine the main effect of dose on serum 25(OH)D.

Effects of Subcutaneous Vitamin D₃ Injection on Serum 25(OH)D of Calves

The response of calves to a bolus injection of vitamin D₃ at birth was modeled by using data from multiple experiments. In the first experiment, 9 Holstein bull calves 0 to 2 d of age at the University of Florida Dairy Unit (Hague, FL) were subcutaneously injected with either 0 or 30,000 IU of vitamin D₃ (Cayman Chemical, Ann Arbor, MI; diluted in 1 mL of ethanol). In the second experiment, 16 Holstein bull calves 0 to 2 d of age at the University of Florida Dairy Unit were subcutaneously injected with either 0, 80,000, or 120,000 IU of vitamin D₃. In the third experiment, 14 Holstein calves at the University of Idaho (Moscow, ID) dairy herd were administered 4 mL of saline or a commercial product containing 300 IU of vitamin E, 10,000 IU of vitamin D₃, and 100,000 IU of vitamin A (VITAL E-A+D, Stuart Products, Bedford, TX) at the time of birth. In all experiments, calves received colostrum, were housed under shade, and fed pasteurized waste milk; serum samples were collected at 0 and 7 d after injection. Serum 25(OH)D was measured by using an ELISA (Eagle Biosciences, Nashua, NH) as previously described (Nelson et al., 2016). Serum 25(OH)D data at 7 d of age was combined from all experiments and analyzed for effect of the dose of vitamin D₃ injected to determine the dose response relationship between vitamin D₃ injected and serum 25(OH)D at 7 d of age.

RESULTS AND DISCUSSION

Vitamin D Status of Dairy Cows

The 25(OH)D concentrations of 702 serum samples collected from cows on 12 different dairy herds were measured to determine the typical serum 25(OH)D values for dairy cows. Descriptive data for each herd, including amount of supplemental vitamin D, estimated time spent outdoors during daylight hours, and average serum 25(OH)D concentration, are provided in Table

Figure 1. Frequency distribution of serum 25-hydroxyvitamin D [25(OH)D] concentrations for mature dairy cows. The frequency of serum samples within each 10-ng/mL range of 25(OH)D concentrations is plotted for 702 samples collected from cows in the 12 different dairy herds listed in Table 1 (includes lactating cows 0 to 300 DIM, and pregnant, nonlactating cows). The dotted line represents the threshold for vitamin D insufficiency and the shaded area represents the limits of the 10th and 90th percentiles.

1. The average 25(OH)D concentration of all samples collected was 68 ± 22 ng/mL of serum (mean $\pm$ SD). The interquartile range was 53 to 82 ng/mL, and the 10th and 90th percentiles were 42 and 96 ng/mL, respectively (Figure 1).

Serum 25(OH)D concentrations were not affected by season within herds where samples were collected during multiple seasons ($P > 0.1$, data not shown; herds located approximately 43°N, 89°W). Serum 25(OH)D concentrations also were very similar between herds, with average serum 25(OH)D of mid- to late-lactation cows in 10 of the 12 herds between approximately 70 to 85 ng/mL (Figure 2). The serum 25(OH)D concentrations were also similar between Holstein and Jersey cows sampled in herd 6 (71 ± 18 and 66 ± 15 ng/mL, respectively, $P > 0.1$). All but herd 12 supplemented vitamin D₃ at a rate of 30,000 to 50,000 IU/d in the lactating cow ration (Table 1). For cows receiving 30,000 to 50,000 IU/d and managed similarly (freestall barn with limited sun exposure), we found no relationship between rate of supplementation and average serum 25(OH)D ($P > 0.1$). The average serum 25(OH)D of mid- to late-lactation cows from herd 12 that were supplemented with 20,000 IU of vitamin D₃/d, however, was significantly lower than those from other comparable herds (herd 12 vs. herds 9, 10, and 11; $P < 0.001$, Figure 2). Furthermore, 22% of the cows receiving 20,000 IU/d, the approximate NRC requirement, had serum 25(OH)D below 30 ng/mL; whereas, 95% of cows receiving 30,000 IU/d or more had serum

Figure 2. Serum 25-hydroxyvitamin D [25(OH)D] concentrations of mid- to late-lactation cows (100–300 DIM) within each herd listed in Table 1. The dashed line represents the proposed 30-ng/mL cutoff for vitamin D insufficiency. Boxes represent the inner quartile range of serum 25(OH)D concentrations with bars extending to the 5th and 95th percentile and dots representing outliers. Herd 12 supplemented cows with 20,000 IU of vitamin D₃/d, all others were between 30,000 to 50,000 IU/d. The mean serum 25(OH)D of herd 12 was significantly lower than those of comparable herds (herds 9, 10, and 11; $P < 0.001$).

25(OH)D above 40 ng/mL (Figure 2). The lack of association within the 30,000 to 50,000 IU/d range should be viewed cautiously, as total vitamin D and actual intakes were unknown and herd was a confounding factor. Controlled experiments are still needed to establish the relationship between serum 25(OH)D and supplemental vitamin D₃ for dairy cows.

Within herds where samples were collected from cows in both early (0 to 30 DIM) and mid to late lactation (100 to 300 DIM), serum 25(OH)D was lower in early-lactation cows than in mid- to late-lactation cows (57 ± 17 vs. 71 ± 20 ng/mL, includes herds 1–6 and 11; $P < 0.001$; Figure 3). The supplemental rates of vitamin D₃ over the dry period were not collected for most of those herds. Lower vitamin D₃ intake over the dry period could explain the lower serum 25(OH)D in early-lactation cows. However, the close-up dry cow ration for herd 3 was formulated for near the same daily vitamin D₃ intake as the lactating ration (35,000 IU/d) and serum 25(OH)D concentrations of fresh cows (1 to 2 DIM) were lower than that of mid-lactation cows in herd 3 ($P < 0.001$). Increased 25(OH)D metabolism and changes in vitamin D-binding protein concentrations also could explain the lower serum 25(OH)D concentrations observed in fresh cows; this, along with vitamin D intake over the transition period, deserve further investigation as potential causes for decreased serum 25(OH)D in early lactation.

The observations of vitamin D status among dairy cows studied here are similar to those reported by Sorge et al. (2013). Those authors analyzed 25(OH)D in serum samples collected from 160 cows from 6 dairy herds in Minnesota ranging in size from 317 to 710 cows per herd to investigate the relationship between Johne's disease and vitamin D status. The rate of dietary vitamin D supplementation in the lactating rations for those herds, as determined from analysis of vitamin D₂ and vitamin D₃ content, ranged from 25,000 to 52,000 IU/hd/d. The average serum 25(OH)D concentration of 80 *Mycobacterium avium* ssp. *paratuberculosis* antibody negative-cows was 64 ng/mL. The *Mycobacterium avium* ssp. *paratuberculosis*-positive cows tended to have lower serum 25(OH)D (59 ng/mL on average), likely as a consequence of decreased intake and absorption of vitamin D. The overall range of serum 25(OH)D among all samples was from 13 to 100 ng/mL and serum 25(OH)D was not associated with rate of supplemental vitamin D. As in the present study, the early-lactation cows in Sorge et al. (2013) had the lowest serum 25(OH)D concentrations, 54.3 ng/mL on average.

Figure 3. Vitamin D status changes with DIM. Boxes represent the inner quartile range of serum 25-hydroxyvitamin D [25(OH)D] concentrations with bars extending to the 5th and 95th percentile and dots representing outliers for cows within the first 30 DIM ($n = 163$) and cows between 100 to 300 DIM ($n = 424$). Effect of stage of lactation was analyzed with Proc Mixed of SAS (SAS Institute Inc., Cary, NC) with fixed effect of stage and random effect of cow nested in herd.

It should be noted that legume and grass hay can provide a significant amount of vitamin D₂, up to 2,500 IU/kg of DM for sun-dried alfalfa hay (Wallis et al., 1958). Estimations of vitamin D₂ intake could not be made from the data collected, but individual concentrations of 25(OH)D₂ and 25(OH)D₃ were measured by mass spectrometry (Heartland Assays, Ames, IA) in serum samples collected from herds 9 (n = 6) and 10 (n = 8). Concentrations of 25(OH)D₂ and 25(OH)D₃ were 4.5 ± 0.5 and 62.7 ± 8.7 ng/mL, respectively, for herd 9, and 10.3 ± 1.6 and 71.2 ± 11.3 ng/mL, respectively, for herd 10. Hence, the concentrations of 25(OH)D₂ were approximately 7 and 13% of the total 25(OH)D in the samples from the 2 herds. Both herds with data for 25(OH)D₂ were confinement dairies in the southeastern United States (approximately 30°N, 82°W) that fed rations with a low content of hay. The contribution of 25(OH)D₂ to the total 25(OH)D may be greater for cows receiving more high-quality hay than for those observed here; however, that difference may be of little consequence for the total 25(OH)D, as it was quite similar across herds (Table 1).

A concern of some producers and dairy consultants is the vitamin D status of cows housed in barns with limited access to sun exposure. Based on the present data, supplementing between 30,000 to 50,000 IU of vitamin D₃/d reliably achieves serum 25(OH)D concentrations between 60 to 80 ng/mL regardless of housing system. Whether supplementing cows with less than 30,000 IU/d is adequate is not yet certain, as serum 25(OH)D concentrations of 22% of the cows sampled in the herd receiving 20,000 IU of vitamin D₃/d were below 30 ng/mL (Figure 2). That observation alone does not warrant changes for vitamin D recommendations, but does highlight a need for further research. Conversely, consequences of over supplementation of vitamin D₃ should be considered. Approximately 10% of the cows sampled had serum 25(OH)D concentrations over 100 ng/mL, with some samples approaching 150 ng/mL. The serum 25(OH)D threshold for vitamin D toxicity (i.e., calcification of tissues) has generally been considered to be 200 ng/mL of serum 25(OH)D based on observations of cows that received very high doses (15×10^6 IU) of vitamin D₃ intramuscularly (Littledike and Horst, 1982; Horst et al., 1994). Weiss et al. (2015), however, did not observe hypercalcemia in cows with serum 25(OH)D concentrations near 270 ng/mL, on average, as a result of feeding 6 mg of 25(OH)D/d. Thus, the concentrations between 100 to 150 ng/mL observed here are likely not a concern for overt toxicity, but negative subclinical consequences of excess vitamin D cannot be ruled out and need to be explored.

Likewise, more investigation is needed on vitamin D status around calving. Lower vitamin D intake over the

dry period, increased metabolism of 25(OH)D at calving, and decreased vitamin D-binding protein concentrations are all possible explanations for the decreased serum 25(OH)D in fresh cows. A lack of data remain for the relationship between supplemental vitamin D₃ and serum 25(OH)D in lactating cattle, as well as whether a relationship exists between vitamin D status and health and production of dairy cattle. Considering the widespread expression of the vitamin D receptor and newfound roles for vitamin D signaling in physiology of the dairy cow, more information on vitamin D nutrition of lactating cows is needed.

Vitamin D Status of Dairy Heifers

The serum 25(OH)D concentrations of 12-mo-old Holstein heifers from herds 3 and 9 are listed in Table 1. The heifers from those herds were housed outdoors on dry lots. Serum samples were collected during the month of April (n = 20/herd) and the estimated amounts of daily supplemental vitamin D₃ were near 11,000 IU and 12,000 IU for each herd. The corresponding serum 25(OH)D concentrations on average were 69 ± 8 and 82 ± 18 ng/mL, respectively. The NRC recommendation for dairy heifers is roughly 1,200 IU of vitamin D₃/kg of DM, or near 9,000 IU/d for a yearling heifer. From our data, the NRC rate for dairy heifers seems to achieve serum 25(OH)D concentrations similar to what a lactating cow achieves with 1,200 to 2,000 IU of supplemental vitamin D₃/kg of DM.

The importance of supplemental vitamin D₃ for dairy heifers is underscored by a recent study of serum 25(OH)D concentrations of feedlot calves (Casas et al., 2015). Serum 25(OH)D concentrations were between 50 and 60 ng/mL, on average, in nearly 200 beef calves coming off summer pasture as they entered the feedlot. In the following month (March), their serum 25(OH)D had dropped to below 20 ng/mL, on average. The calves were in an open feedlot in Nebraska and received between 800 to 1,200 IU of vitamin D₃/d. In another study, serum 25(OH)D of feedlot steers housed indoors without supplemental vitamin D₃ dropped below 10 ng/mL, whereas those fed 1,860 IU of vitamin D₃/kg of DM achieved serum 25(OH)D concentration near 70 ng/mL (Pickworth et al., 2012). It is often assumed that endogenous vitamin D₃ synthesis of cattle housed outdoors along with vitamin D₂ from forages is adequate for cattle, but that assumption could be detrimental to dairy heifers. Besides a role for vitamin D in immunity and growth, recent work using rodent models has shown that vitamin D deficiency in the prepubertal period negatively affects reproductive performance (Dicken et al., 2012). As with lactating cows, a lack of data exists on the dose response of serum 25(OH)D of

dairy heifers to supplemental vitamin D₃, but, according to available data, feeding between 1,200 to 1,500 IU vitamin D₃/kg of DM achieves adequate serum 25(OH)D in dairy heifers.

Vitamin D Status of Dairy Calves

Adequate vitamin D nutrition for young dairy calves is critical because of their rapid growth and metabolism of 25(OH)D (Rajaraman et al., 1997; Nonnecke et al., 2009). Serum 25(OH)D concentrations of preweaned dairy calves across different husbandry and nutritional practices according to age are shown in Figure 4. The average 25(OH)D concentration of all calves less than 3 d of age was 15 ± 11 ng/mL, with a range of 0 to 39 ng/mL. Newborn calves typically have considerably lower serum 25(OH)D than mature animals (Horst and Littledike, 1982); their serum 25(OH)D also correlates with that of their dams. Thus, the vitamin D status of the newborn calf is a reflection of dry cow nutrition (Goff et al., 1982; Weiss et al., 2015). Despite the expectation that newborn serum 25(OH)D will be lower than that of adult animals at birth, the prevalence of calves with serum 25(OH)D below 20 ng/mL at birth is a concern. More than 25% of the newborn calves had serum 25(OH)D concentrations below 10 ng/mL; which, left untreated, puts them at great risk for impaired health and development.

The vitamin D status of calves that received supplemental vitamin D₃ or were exposed to sun improved steadily over time (Figure 4, filled symbols). The calves that received milk replacer that contained 6,600 IU of vitamin D₃/kg of DM had serum 25(OH)D concentrations near 50 to 60 ng/mL at 4 to 6 wk of age. The calves that received milk replacer containing 11,000 IU/kg of DM had serum 25(OH)D concentrations near 100 ng/mL at 4 wk, which is close to what has been previously reported for that rate of supplemental vitamin D (Nonnecke et al., 2010). Likewise, samples collected in mid-April from calves fed pasteurized whole milk and kept outdoors in Florida had serum 25(OH)D concentrations near 40 to 50 ng/mL at 2 to 6 wk of age. In contrast, serum 25(OH)D of calves from herds in Florida and Idaho that were fed pasteurized waste milk without supplemental vitamin D and without exposure to midday summer sun remained near or below 15 ng/mL through 5 wk of age (Figure 4, open symbols). The same was true for calves from a herd in Iowa, where serum 25(OH)D concentrations were 12 ng/mL on average at approximately 14 d of age (data not shown, samples collected during month of May). Serum 25(OH)D of 6-wk-old calves fed pasteurized waste milk was improved (Figure 4), which would have coincided

with intake of starter grain that contained 5,300 IU of vitamin D₃/kg of DM.

The rapid decline of serum 25(OH)D of calves fed whole milk or pasteurized waste milk without supplemental vitamin D₃ has previously been reported by Rajaraman et al. (1997) and, more recently, by Krueger et al., (2014). Feeding of waste milk and whole milk is a common practice of dairy producers and calf-grower operations (USDA, 2012). Although most of those calves may appear healthy, the consequences of insufficient serum 25(OH)D concentrations are the impaired actions of intracrine and paracrine vitamin D signaling mechanisms that are not readily apparent. Perhaps most notable for the young calf are the innate immune responses that are activated through intracrine vitamin D signaling. Activation of macrophages of calves in response to innate sensing of pathogen-associated molecules triggers conversion of 25(OH)D to 1,25-dihydroxyvitamin D that induces nitric oxide and β -defensin

Figure 4. Serum 25-hydroxyvitamin D [25(OH)D] of Holstein dairy calves according to various housing and nutrition practices. Each point represents the mean and 95% CI of samples from at least 6 calves. The samples collected at 0 wk of age were collected after colostrum consumption. Open triangles (Δ) and dashed line represent calves from a herd in Idaho fed pasteurized waste milk with no supplemental vitamin D₃ limited sun exposure (calves were housed in either hutches or barn and samples were collected in winter). Open circles ($\circ$) and dashed line represent calves from a herd in Florida fed pasteurized waste milk with no supplemental vitamin D₃ and no direct sun exposure. Filled circles ($\bullet$) and solid line represent calves in the same herd that received 150,000 IU of vitamin D₃ at birth via injection and pasteurized waste milk supplement that provided 5,000 IU/d. Filled diamonds ($\blacklozenge$) and solid line represent calves fed whole milk 3 times/d and kept outdoors in Florida (samples collected in mid-April). Filled squares ($\blacksquare$) and solid line represent calves from a herd in Georgia fed milk replacer containing 6,600 IU/kg of DM. The calves received 0.8 kg/d of milk replacer from 0 to 14 d and 1.2 kg/d milk replacer from 15 to 42 d and raised under shade. Filled triangles ($\blacktriangledown$) and solid line represent calves from a herd in Florida kept outdoors in a group pen and fed ad libitum milk replacer containing 11,000 IU of vitamin D₃/kg of DM.

antimicrobial peptide production, innate defenses that are critical for young calves (Nelson et al., 2011; Merri-man et al., 2015). The low 25(OH)D concentrations observed in calves fed milk without supplemental vitamin D could impair efficient and rapid activation of those innate defenses and put them at greater disease risk. The key finding being calves fed milk without summer sun exposure require supplemental vitamin D₃. According to our data, a rate of 6,000 IU/kg of DMI is needed for calves to achieve serum 25(OH)D concentrations of 50 to 60 ng/mL, which is typical serum 25(OH)D concentration for beef calves on summer pasture (Casas et al., 2015; Nelson et al., 2016).

Serum 25(OH)D Response of Calves to Supplemental Vitamin D₃

The change in serum 25(OH)D of dairy calves over time in response to supplemental vitamin D₃ was predicted using data from multiple experiments (Figure 5). The serum 25(OH)D of calves receiving milk replacer containing only 400 or 1,700 IU of vitamin D₃/kg of DM changed little over time and remained near or below 20 and 30 ng/mL, respectively, throughout the experiments. In contrast, serum 25(OH)D of calves receiving 11,000 or 17,900 IU of vitamin D₃/kg of DM increased significantly over time to approximately 70 and 170 ng/mL, respectively, by the end of the experiments (dose $\times$ age and dose $\times$ age², $P < 0.001$). Regression analysis of serum 25(OH)D concentrations from 30 d of age and older, where age did not have an effect, indicated that serum 25(OH)D of the calves increased 6.6 ± 0.7 ng/mL (slope $\pm$ 95% CI, $P < 0.001$) for every 1,000 IU of vitamin D₃/kg of DM of milk replacer starting from a baseline serum 25(OH)D of about 16 ng/mL. Thus, a supplementation rate of 6,000 to 7,000 IU of vitamin D₃/kg of DMI would achieve serum 25(OH)D concentrations of 50 to 60 ng/mL. This prediction fits well with the data collected from calves on commercial dairies (Figure 4), where calves consuming milk replacer containing 6,600 IU of vitamin D₃/kg of DM had serum 25(OH)D concentrations of 62 ng/mL, on average, at 6 wk of age, and those consuming milk replacer containing 11,000 IU/kg of DM had serum 25(OH)D concentration of 98 ng/mL, on average, at 4 wk of age.

With daily supplemental vitamin D₃ alone, serum 25(OH)D gradually increases over a period of 2 to 3 wk (Figures 4 and 5). A more rapid increase during that critical period of a calf's life would seem beneficial, particularly for those born with extremely low serum 25(OH)D. Similar to what was done for daily supplemental vitamin D₃, the response of calves to a bolus injection of vitamin D₃ at birth was modeled using data from multiple experiments. The regression line for

Figure 5. (A) Predicted (solid lines) and observed (dashed lines) serum 25-hydroxyvitamin D [25(OH)D] concentrations of experimental calves in response to rate of supplemental vitamin D₃ and day of age. Data represent the outcome of 2 experiments where Holstein bull calves were fed milk replacers containing the increasing amounts of supplemental vitamin D₃. In experiment 1, calves were fed milk replacer containing 1,700 (n = 16) or 17,900 IU of vitamin D₃/kg of DM (n = 8). In experiment 2, calves were fed 400 (n = 12) or 11,000 IU of vitamin D₃/kg of DM (n = 12). Data points and error bars represent the mean and 95% CI for each group according to day of age. The equation of the predicted response was $[25(\text{OH})\text{D}] = 16.02 \text{ ng/mL} + (-0.00031 \times \text{IU of vitamin D}_3) + (-0.024 \times \text{d}) + (0.00029 \times \text{IU of vitamin D}_3 \times \text{d}) + (-2.59 \times 10^{-6} \times \text{IU of vitamin D}_3 \times \text{d}^2)$. (B) The solid line represents the slope and intercept of the regression analysis of serum 25(OH)D concentrations of samples collected at 30 d of age and older as a function of supplemental vitamin D₃. The slope (6.6 ng/mL per kIU of vitamin D₃) and intercept (16.1 ng/mL) were significant ($P < 0.001$). The symbols and error bars represent the observed means with 95% CI of samples from calves in each group at 30 d of age and older.

serum 25(OH)D at 7 d after injection with vitamin D₃ predicted an increase of about 30 ± 8 ng/mL (slope $\pm$ 95% CI, $P < 0.001$) for every 100,000 IU of vitamin D₃ administered starting from a baseline serum 25(OH)D of 11 ng/mL (Figure 6).

Figure 6. Prediction of serum 25-hydroxyvitamin D [25(OH)D] in response to subcutaneous vitamin D₃ injection. In 3 separate experiments, calves were administered various amounts of vitamin D₃ via subcutaneous injection at birth and serum 25(OH)D was measured at 7 d of age. In the first experiment, Holstein bull calves received 0 (n = 6) or 30,000 IU of vitamin D₃ (n = 3). In the second experiment, Holstein bull calves received 0 (n = 8), 80,000 (n = 5), or 120,000 IU (n = 3) of vitamin D₃. In the third experiment, Holstein bull and heifer calves received saline injection or 40,000 IU of vitamin D₃ from a commercially available vitamin A, D, and E solution (n = 7/group). In each experiment, calves were fed pasteurized waste milk without any supplemental vitamin D₃. The symbols and error bars represent the means with 95% CI of serum 25(OH)D measured in samples collected at 7 d postinjection for each amount of vitamin D₃ injected. The solid line represents regression line with a slope and intercept of (0.3 ng/mL per kIU of vitamin D₃) and intercept (11.0 ng/mL) that were significant ($P < 0.001$).

Krueger et al. (2014) reported a similar response where serum 25(OH)D of calves injected with 40,000 IU of vitamin D₃ at birth increased from approximately 20 ng/mL at birth to 40 ng/mL 7 d later. Those calves were fed pasteurized waste milk and, in the absence of continued vitamin D supplementation, their serum 25(OH)D dropped to 30 ng/mL after 14 d and 15 ng/mL at 35 d. In a separate study, those authors showed that calves given 150,000 IU of injectable vitamin D₃ at birth followed by 5,000 IU of supplemental vitamin D₃/d (~7,500 IU of vitamin D₃/kg of DM, in combination with vitamins A and E) increased from an average serum 25(OH)D of 30 ng/mL at birth to near 100 ng/mL at 7 and 14 d of age (Krueger et al., 2016). In contrast, serum 25(OH)D of control calves dropped from 30 ng/mL at birth to less than 20 ng/mL at 14 d of age. Ultimately, those authors demonstrated that a bolus

injection of vitamin D₃ at birth followed by daily supplemental vitamin D₃ is an effective means of increasing serum 25(OH)D of calves. Assuming an initial 25(OH)D concentration of 15 to 25 ng/mL for most calves at birth, and depending on the rate of daily supplemental vitamin D₃, an initial injection of 50,000 to 100,000 IU of vitamin D₃ at birth should be adequate to achieve vitamin D sufficiency. Caution must be used, however, with injectable vitamin formulations, as most products on the market contain vitamins A, D, and E in various combinations. Intramuscular injections of excessive vitamin A (i.e., 2×10^6 IU) caused the development of hyena disease in calves (Takaki et al., 1996; Woodard et al., 1997). Data from the experiments reported by Krueger et al. (2014, 2016) also indicate that bolus vitamin A injections are not necessary if adequate vitamin A is provided in the diet. Consequently, use of injectable vitamin products should carefully consider the background, diet, and management of the calf.

The serum 25(OH)D concentrations that support optimal growth and health of calves are not yet fully known; thus, recommendations for supplemental vitamin D should not be viewed as definitive at this time. Concentrations below 30 ng/mL of serum have been proposed as a good benchmark for insufficiency (Norman, 2008; Adams and Hewison, 2010). Conversely, calves with average serum 25(OH)D above 170 ng/mL were not protected from respiratory syncytial virus infection better than calves with 30 ng/mL serum 25(OH)D. Future experiments need to examine more fully the relationship between serum 25(OH)D, disease incidence (epidemiological and experimental diseases), vitamin D-associated immune functions (nitric oxide and β -defensin production of macrophages), and overall production. For the time being, a moderate range of 40 to 80 ng/mL of serum 25(OH)D seems to be a reasonable range based on serum 25(OH)D concentrations of calves on summer pasture. Milk replacers often contain about 11,000 IU of vitamin D₃/kg of DM, which somewhat exceeds serum 25(OH)D of calves on pasture but is satisfactory based on current state of knowledge. In contrast, producers that raise calves on milk need to adopt the practice of adding supplemental vitamin D₃, as discussed. In addition, a bolus vitamin D supplement at birth would help to quickly increase the vitamin D status of newborn calves.

CONCLUSIONS

The current practices for dietary vitamin D₃ supplementation in the dairy industry seem to be adequate for cows and heifers, with the 25(OH)D concentrations of most animals ranging between 50 to 80 ng/mL of serum. In fact, supplementing cows at rates well above

the NRC recommendation of 21,000 IU of vitamin D₃/d for mature cows, such as 40,000 to 50,000 IU/d, may be more than necessary. In contrast, supplementation with 21,000 IU/d may not be adequate based on limited observations reported here. Future research needs to explore the relationship between supplemental vitamin D₃ and serum 25(OH)D of dairy cows with regards to key endpoints of disease incidence, fertility, and milk production, along with consideration of the long-term effects of high serum 25(OH)D concentrations that occur in a portion of cows under current practices. In regard to dairy calves, the amount of vitamin D₃ in typical milk replacers is adequate but needs to be studied further to determine amounts needed for optimal growth and health. Calves raised on a milk diet, however, are prone to vitamin D deficiency, as milk is very low in vitamin D content. It is recommended that producers raising calves on milk should provide supplemental vitamin D₃ at a rate of 6,000 to 10,000 IU/kg of DM. A 50,000 to 100,000 IU bolus of vitamin D₃ at birth, whether calves are fed milk or milk replacer diets, would also help to quickly achieve vitamin D sufficiency in newborn calves. Altogether, the recommendations provided here are intended to maintain vitamin D sufficiency as defined by serum 25(OH)D concentrations. Additional research is needed to identify whether correlations exist between serum 25(OH)D and health and productivity of cattle.

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