INTRODUCTION

Vitamin D is critical for normal development and growth of cattle (Rupel et al., 1932; Bechdel et al., 1937). Recent reports also have documented a positive role for vitamin D in immune function of cattle (Nelson et al., 2010a,b; Merriman et al., 2015). Despite its roles in growth and health, the vitamin D needs of beef cattle have largely been ignored because it is commonly assumed that cattle on pasture receive adequate vitamin D either from photoconversion of 7-dehydrocholesterol to vitamin D₃ in the skin or ingestion of dietary sources.
of vitamin D₃ from forages (NRC, 2000; Vasconcelos and Galyean, 2007). Indeed, cattle can efficiently synthesize enough vitamin D₃ to meet their requirements when exposed to adequate summer sun (Hymøller and Jensen, 2010, 2012) and appreciable amounts of vitamin D₂ are obtained from sun-cured hay (i.e. alfalfa or grass hay; Moore et al., 1948; Newlander, 1948; Wallis et al., 1958; Horst and Littledike, 1982). However, cutaneous synthesis of vitamin D₃ decreases through the winter months (Hymøller et al., 2009) and vitamin D₂ is not metabolized as efficiently as vitamin D₃ in cattle (Hymøller and Jensen, 2011). The assumption that beef cattle rarely require supplemental vitamin D is based on historical observations that rickets and osteomalacia, obvious clinical signs of vitamin D deficiency, could be prevented with feeding high-quality hay or being raised outdoors (Huffman et al., 1935; Wallis, 1946; Thomas and Moore, 1951). Yet the absence of visual signs of vitamin D deficiency does not mean the animal has adequate vitamin D supplies for optimal growth and health.

The 25-hydroxyvitamin D₂ (25(OH)D₂) metabolite (which refers to both 25-hydroxyvitamin D₂ [25(OH)D₂] and 25-hydroxyvitamin D₃ [25(OH)D₃] metabolites when no subscript is given) is the best indicator of vitamin D status (Hollis and Horst, 2007). It is readily converted from vitamin D by 25-hydroxylases in the liver and serves as the precursor for the active vitamin D hormone, 1,25-dihydroxyvitamin D (1,25(OH)₂D). Serum 25(OH)D concentrations above 20 ng/mL have generally been considered adequate for cattle whereas concentrations < 10 ng/mL are representative of deficiency and put the animal at risk for rickets and weak bones (Hollis, 2005). Recent studies also have shown that low in vitro 25(OH)D concentrations impair innate immunity of cattle (Nelson et al., 2010b, 2011; Merriman et al., 2015). Bovine macrophages convert 25(OH)D to 1,25(OH)₂D on activation and the 1,25(OH)₂D stimulates nitric oxide production and β-defensin antimicrobial peptide gene expression. In light of vitamin D’s role in immunity and other nonclassical functions, it has been proposed that circulating 25(OH)D concentrations of at least 30 ng/mL are needed to promote optimal health (Adams and Hewison, 2010; Nelson et al., 2012); however, the 25(OH)D concentrations that truly achieve optimal health and productivity of cattle have yet to be defined.

Little attention has been given to the vitamin D status of beef cattle until recently. Casas et al. (2015) reported that serum 25(OH)D concentrations of fedlot cattle drop below 15 ng/mL over the winter from a high of 50 to 60 ng/mL when they were off pasture at the end of summer and entered a fedlot. Serum 25(OH)D concentrations of those calves also were below 30 ng/mL when they were 2 to 3 mo old, suggesting that young calves may have inadequate vitamin D supplies. The typical vitamin D status of newborn beef calves, however, has not been examined. Therefore, the objective of this study was to evaluate the serum 25(OH)D concentrations of calves from the time of birth to weaning, along with that of beef cows on pasture, as an indicator of vitamin D status.

**MATERIALS AND METHODS**

**Animals**

Retrospective samples were collected from completed or ongoing studies conducted in research herds located in Florida, Idaho, and Minnesota. The experiment was conducted in accordance with acceptable practices as outlined by the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010) and was approved by the respective institutional animal care and use committees. The Florida herd was located at the University of Florida Santa Fe River Ranch Beef Unit in Alachua, FL, at a latitude of 30° N. The herd consisted of approximately 250 cows of Angus and Brangus breeds fed on bahia grass pasture and hay. The cows received a trace mineral supplement during pregnancy at a rate of 0.45 kg/d that provided 6,220 IU vitamin D₃/kg of supplement, thereby resulting in an estimated intake of 2,800 IU vitamin D₃/d. Actual intake, however, was not determined.

The Idaho herd was located near Moscow, ID, at a latitude of 46° N. The herd consisted of pure-bred Charolais cows fed free-choice oat hay. From November to 30 d before calving, the Idaho cows were provided a free-choice mineral supplement that contained 44,000 IU vitamin D₃/kg supplement. The supplement was formulated for 113 g/d intake, approximately 5,000 IU vitamin D₃/d, but actual intake was unknown. From 30 d before calving to 21 d after calving, the Idaho cows were fed a 0.5 kg/d distilled’s dried grains with solubles supplement that contained trace minerals to meet requirements but no vitamin D₃.

The Minnesota herd was located near Grand Rapids, MN, at the University of Minnesota North Central Minnesota Research Center at a latitude of 47° N. The herd consisted of 120 purebred Angus cows that rotationally grazed grass and legume pastures from May to September and were fed grass and legume hay from October to April. The cows received a free-choice mineral supplement containing 110,000 IU vitamin D₃/kg of supplement through the winter that was formulated for a maximum estimated intake of 113 g/d (12,500 IU of vitamin D₃). In April, the cows were switched to a free-choice supplement containing 66,000 IU vitamin D₃/kg of supplement.
that was formulated for an estimated intake of 57 g/d (3,750 IU of vitamin D$_3$).

**Sample Collection**

Samples were collected from 43 cow–calf pairs near the time of calving, normally after calves had consumed colostrum, from animals in the Florida ($n = 12$), Idaho ($n = 15$), and Minnesota ($n = 16$) herds. Calving dates ranged from March 2014 to April 2014 for cows in the Florida herd, from January 2014 to March 2014 for cows in the Idaho herd, and from March 2014 to April 2014 for cows in the Minnesota herd. Samples were collected from each of the cow–calf pairs in the Florida herd again in June 2014, July 2014, and September 2014 at weaning. Samples were collected again from the cow–calf pairs in the Minnesota herd in July 2014.

Additional calves in each herd also were sampled. Samples were collected from 16 calves in the Florida herd near the time of birth in January 2014 and again in February, July, and September 2014. In 2015, samples were collected from calves in the Florida herd at the time of birth in March and again in April, May, July, and August. Samples also were collected from calves in the Minnesota herd in 2015 at the time of birth in March and again in May, June, and October. For each of those groups of calves, the cows were fed in the same way as described above and samples collected near birth were generally after consumption of colostrum.

**Effects of Vitamins A, D, and E Injection on Serum 25-Hydroxyvitamin D**

Thirty-two male and female calves in the Idaho herd were randomly assigned to receive a subcutaneous injection of 4 mL of saline or 4 mL of a commercial product containing 100,000 IU retinyl palmitate as vitamin A, 10,000 IU vitamin D$_3$, and 300 IU of RRR-α-tocopherol as vitamin E (Vital E-A+D; Stuart Products, Inc., Bedford, TX) at the time of birth. The dams of those injected calves were randomly selected to be analyzed for vitamin D$_3$ combined) were determined using a commercially available ELISA for serum 25(OH)D (Human VD$_3$ ELISA; Eagle BioSciences, Nashua, NH). The kit was used according to the manufacturer’s protocol except that a custom standard prepared in vitamin D–deficient bovine calf serum was used in place of the standard provided in the kit. The calf serum standard was prepared by addition of 25(OH)D$_3$ (Cayman Chemical, Ann Arbor, MI) dissolved in ethanol. The concentration of 25(OH)D$_3$ in ethanol was verified by absorbance at 264 nm and then added to calf serum to give final concentrations of 5, 15, 25, 45, 85, and 125 ng/mL. The spiked serum was incubated for 2 h at 37°C and then aliquoted and stored at −20°C. The concentrations of 25(OH)D$_2$ and 25(OH)D$_3$ in the calf serum standard were validated by liquid chromatography coupled to tandem mass spectrometry at the University of Florida health laboratories (Gainesville, FL).

The concentrations of 25(OH)D in serum samples determined using the ELISA were obtained by measurement of absorbance of 450 nm wavelength light with a BioTek Synergy plate reader (BioTek Instruments, Inc., Winooski, VT). Absorbance values were then fitted to a standard curve using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA). The inter- and intra-assay CV of the assay were 9.1 and 6.1%, respectively.

**Statistical Analysis**

The mean, SD, and interquartile range of serum 25(OH)D concentrations for each group of animals were calculated using GraphPad Prism. The Pearson correlation analysis between cow and calf serum 25(OH)D concentrations and repeated measures analysis for effect of time on serum 25(OH)D concentrations were performed using SAS version 9.3 (SAS Inst., Inc., Cary, NC). The GLIMMIX procedure of SAS was used to determine the effect of time where cow or calf was included as a random effect and the fixed effect of time was categorized as early spring/winter (January–April), late spring (May–June), summer (July), and late summer/fall (September–October). Only data from the Florida and Minnesota herds were used for the time analysis as samples from the summer months were not available from the Idaho herd. The model also was run using fixed effects of herd location (Florida vs. Minnesota), calf breed (Angus vs. Brangus within the Florida herd), calf sex, and birth month (January vs. March within the Florida herd). Herd location and breed did not have a significant effect so they were left out of the final model that examined the main effects of time, sex, and time × sex. Likewise, the GLIMMIX procedure of SAS was used to determine the
RESULTS AND DISCUSSION

Descriptive statistics of serum 25(OH)D concentrations of cows and calves are provided in Table 1. The data are confounded by breed, location, and amount of supplemental vitamin D₃ provided, among others; therefore, comparisons between groups and interpretation of the data needs to be done with caution. Overall, the 25(OH)D concentrations for the majority of cows sampled at the time of calving were between 40 and 80 ng/mL with an average of 60 ng/mL. There was an effect of time on serum 25(OH)D of the cows sampled over the summer where, overall, serum 25(OH)D increased over the summer months (*P < 0.001*). Serum 25(OH)D of cows in the Minnesota herd increased from 57 ng/mL on average at calving to 77 ng/mL in mid summer. The average serum 25(OH)D of cows in the Florida herd increased from 69 ng/mL at calving to 89 ng/mL in late summer. There was not an effect of herd location (Florida vs. Minnesota) or location × time interaction for serum 25(OH)D of the cows (*P > 0.05*; the confounding variables should be noted in that comparison).

In contrast to the cows, the serum 25(OH)D concentrations of calves sampled in this study indicate a major prevalence of vitamin D deficiency at the time of birth. The average serum 25(OH)D concentration of calves at birth was 11 ng/mL, with most calves between 3 and 17 ng/mL. Nearly all calves were below the 30 ng/mL mark for serum 25(OH)D at birth, over 80% were below 20 ng/mL, and over half were below 10 ng/mL. The calves in the Idaho herd had the lowest serum 25(OH)D concentrations with many of samples below the assay limit of detection. The Idaho calves were born from January to March but there was no effect of date of birth on serum 25(OH)D concentrations. Even though the overall serum 25(OH)D concentrations were greatest in the Florida herd, the serum 25(OH)D concentrations of the Florida calves born in March were no different than Minnesota calves born in March. Rather, the Florida calves that were born in January had greater serum 25(OH)D concentrations (Fig. 1: 26 ± 12 vs. 12 ± 10 ng/mL) than Florida calves born in March.

No classical signs of vitamin D deficiency were observed in any of the calves; however, the greater concern for calves is the subclinical manifestations of low vitamin D that are difficult to detect. The present study was not designed to examine associations of serum 25(OH)D with health outcomes of calves, and whether or not the low concentrations of 25(OH)D leads to increased disease risk remains to be determined. Given the prevalence of morbidity and mortality of beef calves in the first few weeks of life and data from in vitro experiments showing adequate supply of 25(OH)D is needed for activation of innate antimicrobial defenses of calves (Nelson et al., 2012), the health consequences of low vitamin D status in newborn beef calves observed in the present study deserve further attention.

Serum 25(OH)D concentrations of calves in the Florida and Minnesota herds increased from near 10 to

Table 1. Descriptive statistics for serum 25-hydroxyvitamin D (25(OH)D)

<table>
<thead>
<tr>
<th>Season/population</th>
<th>No.</th>
<th>Mean (SD) 25(OH)D, ng/mL</th>
<th>Interquartile range, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cows</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter/spring¹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Florida</td>
<td>12</td>
<td>69 (16)</td>
<td>58 to 87</td>
</tr>
<tr>
<td>Idaho</td>
<td>15</td>
<td>54 (8)</td>
<td>49 to 60</td>
</tr>
<tr>
<td>Minnesota</td>
<td>16</td>
<td>58 (8)</td>
<td>54 to 63</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>60 (13)</td>
<td>53 to 65</td>
</tr>
<tr>
<td><strong>Calves</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Florida</td>
<td>35</td>
<td>18 (13)</td>
<td>7 to 26</td>
</tr>
<tr>
<td>Idaho</td>
<td>29</td>
<td>2 (2)</td>
<td>0 to 3</td>
</tr>
<tr>
<td>Minnesota</td>
<td>24</td>
<td>11 (6)</td>
<td>6 to 15</td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td>11 (11)</td>
<td>3 to 17</td>
</tr>
<tr>
<td>Summer²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Florida</td>
<td>10</td>
<td>71 (14)</td>
<td>59 to 86</td>
</tr>
<tr>
<td>Minnesota</td>
<td>16</td>
<td>77 (15)</td>
<td>65 to 88</td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>75 (15)</td>
<td>63 to 86</td>
</tr>
<tr>
<td><strong>Late summer/fall³</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cows</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Florida</td>
<td>30</td>
<td>49 (13)</td>
<td>36 to 62</td>
</tr>
<tr>
<td>Minnesota</td>
<td>16</td>
<td>46 (8)</td>
<td>42 to 51</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>48 (12)</td>
<td>39 to 58</td>
</tr>
</tbody>
</table>

¹Samples were collected from 43 cow–calf pairs at the time of calving. Calving dates ranged from March 2014 to April 2014 for cows in the Florida herd, January 2014 to March 2014 for cows in the Idaho herd, and March 2014 to April 2014 for cows in the Minnesota herd. Samples were collected from an additional 23 calves in the Florida herd that were born in January 2014 (*n* = 16) and March 2015 (*n* = 7), along with 8 additional calves in the Minnesota herd born in March 2015 and 14 additional calves in the Idaho herd (the saline-injected calves from the vitamin A, D, and E injection experiment) that were born from February to March of 2013.

²Samples were collected from cows in July of 2014 and from calves in July of 2014 and 2015.

³Samples were collected at the time of weaning. Florida calves were weaned in late August to early September. Minnesota calves were weaned in October. Samples from Florida cows were collected only in 2014. Samples from Minnesota calves at weaning were collected only in 2015.
15 ng/mL at birth to approximately 50 ng/mL in mid to late summer \((P < 0.001; \text{Fig. 1})\). There was no difference between Florida and Minnesota calves (within calves born in March) or difference between calves born in 2014 and 2015 \((P > 0.05)\). *Means are different \((P < 0.05)\).

Serum 25(OH)D of newborn calves has been shown to be correlated to that of their dams when supplemented with vitamin D\(_3\) \((\text{Goff et al., 1982; Weiss et al., 2015})\), suggesting the low serum 25(OH)D observed in newborn calves here was a reflection of the vitamin D status of their dams. Although there was an association between cows and calves when performing a simple correlation of all pairs regardless of location \((r = 0.37, P < 0.05; \text{Fig. 3})\), there was not an association when location was included as a variable in the analysis. The lack of significant correlation between cow and calf serum 25(OH)D observed here likely stems from the narrow range of serum 25(OH)D in the cow samples. Serum 25(OH)D of the cows in the studies reported by Goff et al. (1982) and Weiss et al. (2015) ranged from 40 to 200 ng/mL and from 20 to 400 ng/mL with correlation coefficients of 0.88 and 0.79, respectively. The data from both of those studies similarly show that a cow serum 25(OH)D concentration of 70 ng/mL will result in a calf serum 25(OH)D concentration of about 20 ng/mL and a concentration of 100 ng/mL in the cow will result in about 30 ng/mL in the calf. Of the cows sampled here, 58, 100, and 88% in the Florida, Idaho, and Minnesota herds, respectively, had serum 25(OH)D concentrations below 70 ng/mL at the time of calving. Although almost all cows were above 40 ng/mL and appear to have sufficient 25(OH)D at calving, increasing supplemental vitamin D\(_3\) of gestating cows to at least achieve the 70 to 80 ng/mL range they have in the summer could help to lessen the prevalence of vitamin D deficiency in newborn calves.

The NRC Nutrient Requirements of Beef Cattle \((\text{NRC, 2000})\) recommends 275 IU vitamin D\(_3\)/kg of DM intake for beef cattle, approximately the minimum rate required to prevent rickets in calves \((\text{Bechdel, 1937})\), but it is generally assumed they do not require supplemental vitamin D \((\text{Vasconcelos and Galyean, 2007})\). Seasonal variation of serum 25(OH)D concentrations have been documented for cattle where concentrations drop from a high in late summer to a low in late winter and early spring \((\text{Hymøller et al., 2009})\). Furthermore, serum 25(OH)D concentrations of feedlot cattle dropped below 20 ng/mL in the absence of adequate supplemental vitamin D\(_3\)
Vitamin D status of beef cattle

The vitamin D needs of beef cattle have largely been ignored because it is often assumed that endogenous synthesis or good-quality hay are adequate for them. The study reported by Casas et al. (2015) highlighted the fact that feedlot cattle managed under current practices become vitamin D deficient over the winter months. The data here highlights that vitamin D deficiency also is prevalent in newborn beef calves in the United States regardless of geographical location.

**Conclusions**

Besides increasing vitamin D supplementation of pregnant cows, treating newborn beef calves with injectable products that contain vitamin D is another way to quickly boost serum 25(OH)D concentrations of calves. Serum 25(OH)D of dairy calves that received a one-time injection of 40,000 IU of vitamin D₃ rose from 20 ng/mL up to 40 ng/mL within 1 wk (Krueger et al., 2014). The serum 25(OH)D of a group of calves in the Idaho herd that received 40,000 IU of vitamin D₃ via injection with a commercially available vitamin A, D, and E product increased from 3 ng/mL up to 11 ng/mL, on average, at 48 h after treatment (P < 0.001; Fig. 4). It is expected that serum 25(OH)D of those calves would continue to rise as a result of the treatment because of time needed to convert vitamin D₃ to 25(OH)D₃. Recently, Krueger et al. (2016) reported that dairy calves treated with 150,000 IU of vitamin D₃ at birth along with 5,000 IU of vitamin D₂ daily elevates serum 25(OH)D concentrations to near 60 ng/mL within the first week. Caution, however, must be used for injectable products that also contain vitamin A, as oversupplementation of vitamin A (approximately 2 × 10⁸ IU vitamin A) in combination with vitamin D has been shown to cause premature growth plate closure in calves (Woodard et al., 1997). The health and performance benefits of injectable vitamin D treatments for calves remains to be seen but it does offer a simple and inexpensive solution to low vitamin D supplies in the calf.

**Figure 3.** Relationship between cow and calf serum 25-hydroxyvitamin D (25(OH)D) concentrations. Serum 25(OH)D concentrations of calves were plotted against those of their dams from cow–calf pairs in the Florida (○), Idaho (●), and Minnesota (×) herds at the time of birth. Concentrations were not correlated when adjusted for effect of herd location (P = 0.38). The dashed lines represent the regression analysis within each herd with the bottom line representing the Idaho herd, the middle line representing the Minnesota herd, and the top line representing the Florida herd. The solid line the represents regression analysis without the effect of herd (y = 0.22x – 4.2; P = 0.019); however, when adjusted for the effect of herd location, the slope was not significant (P = 0.27).

**Figure 4.** Effect of supplemental vitamins A, D, and E on serum 25-hydroxyvitamin D (25(OH)D) of calves at birth. Concentrations of 25(OH)D were measured in samples collected at 5 and 48 h after injection from calves in the Idaho herd that were subcutaneously injected at birth with 4 mL of a vitamin A, D, and E injectable product containing 100,000 IU retinyl palmitate, 10,000 IU vitamin D₂, and 300 IU of RRR-α-tocopherol (n = 10; filled bars) or sterile saline (n = 14; open bars). Data were analyzed as repeated measures with the fixed effects of time and treatment. Bars represent average serum 25(OH)D with the 95% confidence interval; ***p < 0.001. Treatment and treatment × time interaction were significant (P < 0.001).
The correlation between serum 25(OH)D of cows and their calves that has been previously shown (Goff et al., 1982; Weiss et al., 2015) indicates that increased vitamin D supplementation of gestating cows over the winter or vitamin D supplementation of newborn calves would be beneficial. Further investigation is needed to determine the rates of supplemental vitamin D that are needed for optimal health and productivity of beef cattle.

**LITERATURE CITED**


