Vitamin D Metabolism in Dairy Cattle and Implications for Dietary Requirements

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Introduction

Vitamin D was originally discovered nearly a century ago as a factor in butterfat that prevented rickets (McCollum et al., 1922). In the years to follow it was also found to be synthesized in the skin exposed to sunlight and to be critically involved in calcium homeostasis. The role of vitamin D in calcium homeostasis initiated research on its use for milk fever prevention in dairy cattle, and that research has largely contributed to the minimization of milk fever (Horst et al., 2005). The solution for milk fever, however, was not simply to ensure dairy cattle were supplied with sufficient vitamin D. The reason being, vitamin D itself does not have biological activity. It must first be metabolized in the animal to 1,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$), and the 1,25$(OH)_2D_3$ then activates a receptor within the cell that controls gene expression (Haussler et al., 2013). Knowledge of how vitamin D metabolism is regulated and how it affected physiological functions was key to the solution of milk fever (Horst et al., 2005). Likewise, understanding the dynamics of the vitamin D pathway is critical for solving the issue of subclinical hypocalcemia that is still prevalent in dairy herds today.

Besides its contribution to bone formation and maintaining the calcium balance, vitamin D also contributes to several other physiological processes critical for dairy cattle production and well-being. Long-before the discovery that vitamin D prevented rickets, sunlight and cod liver oil were prescribed as a therapy for rickets; both are sources of vitamin D. Those same therapies were also prescribed for tuberculosis. As it turns out, the receptor for vitamin D is present in activated immune cells and controls several immune responses (Hewison, 2010). In cattle, 1,25$(OH)_2D_3$ strongly enhances production of nitric oxide and β-defensin antimicrobial peptides, molecules that are toxic to bacteria (Nelson et al., 2012). Sufficient evidence indicates vitamin D also contributes to reproductive performance and mammary development of cattle (Kemmis et al., 2006; Panda et al., 2001; Ward et al., 1971). Thus, determination of vitamin D requirements for dairy cattle must consider more than its contribution to maintaining calcium balance.

In the classical vitamin D endocrine system, the concentration of 1,25$(OH)_2D_3$ in blood is controlled in the kidneys in response to calcium phosphate needs of the body (Horst et al., 2005). The circulating 1,25$(OH)_2D_3$, in turn, acts on target tissues such as the bones, kidneys, and intestines to control the flow of calcium. However, vitamin D

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metabolism is regulated in an intracrine and paracrine manner for many of the non-calcemic functions of vitamin D (Hewison, 2010). For example, in the immune system $1,25(OH)_2D_3$ is produced in activated macrophages, and acts in the macrophage and surrounding cells to influence immunity (Hewison, 2010; Nelson et al., 2010b). Regulation of $1,25(OH)_2D_3$ synthesis in the immune system is, for the most part, independent of that in the endocrine system. The dynamics of vitamin D metabolism in each system differ, and as a consequence, the requirements of each system for vitamin D also may differ.

For dairy cattle nutrition, the goal is to supply the animal with an amount of vitamin D_3 that achieves a serum 25-hydroxyvitamin D (25(OH)D) concentration that supports the multiple outcomes of vitamin D. The 7th edition of Nutrient Requirements of Dairy Cattle published in 2001 recommends 21,000 IU of vitamin D_3 per day for lactating Holstein cows (NRC, 2001). In a limited survey of current practices, however, most cows receive 1.5 to 2.5 times that amount, and had serum $25(OH)D_3$ concentrations between 60 and 70 ng/mL. Based on all available evidence, that range is adequate for maintaining the calcium balance in dairy cattle. Is that range optimal for immunity, reproduction, or the transition period? Do calves and beef cattle receive adequate amounts of vitamin D_3? Future work should consider those questions along with further exploration of factors that affect vitamin D metabolism in cattle.

Vitamin D Metabolic Pathway

There are two forms of vitamin D, vitamin D_2 and vitamin D_3. Metabolites of both forms are found in plasma of cattle (Horst and Littledike, 1982). Vitamin D_2 is derived from ergosterol in plants and vitamin D_3 is derived from 7-dehydrocholesterol in animals. Vitamin D_2 and vitamin D_3 metabolism occurs through the same pathway in cattle, with exceptions in digestion in the rumen and side chain catabolism (Horst et al., 1994). Both forms contribute to the overall signaling events of vitamin D, but vitamin D_3 is the predominant form in cattle (Horst and Littledike, 1982). The metabolic pathway of vitamin D_3 is shown in **Figure 1**. Vitamin D is hydroxylated to 25-hydroxyvitamin D (25(OH)D) in the liver by cytochrome P450 enzymes. The enzymes CYP2R1, CYP27A1, and CYP3A4 have demonstrated 25-hydroxylase activity in mammals (Jones et al., 2014). The CYP2J2 gene in cattle is correlated with 25(OH)D, implicating that CYP2J2 catalyzes 25-hydroxylation of vitamin D in cattle as well (Casas et al., 2013).

Conversion of vitamin D_3 to $25(OH)D_3$ is not tightly regulated; so most vitamin D_3 that is acquired in the diet or synthesized in the skin is quickly converted to $25(OH)D_3$ (Horst et al., 1994). The $25(OH)D_3$ is the most abundant vitamin D metabolite in plasma of cattle, and is relatively stable over time (Sommerfeldt et al., 1983). Consequently, the concentration of $25(OH)D_3$ in plasma serves as a suitable marker of vitamin D status. Normal serum concentrations of 25(OH)D [$25(OH)D_2$ and $25(OH)D_3$] for cattle are typically defined as 20 to 50 ng/mL (Horst et al., 1994). Most mid-lactation dairy cattle in a recent survey of several Midwest dairies were supplemented with 30 to 50 KIU of vitamin D_3 and had serum concentrations between 40 to 100 ng of 25(OH)D/mL regardless of time in sun or season of sample collection (Lippolis 2012, unpublished).

The $25(OH)D_3$ metabolite serves as the precursor to the biologically active metabolite, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$). The conversion is catalyzed by the 25-hydroxyvitamin D 1α-hydroxylase (1α-OHase/CYP27B1), a mitochondrial cytochrome P450 enzyme that is tightly regulated. The concentration of $1,25(OH)_2D_3$ in blood is tightly regulated and typically ranges from 5 to 20 pg/mL in serum of cattle, but is elevated to > 300 pg/mL during severe hypocalcemia (Horst et al., 1994). The biological function of $1,25(OH)_2D_3$ is to regulate gene expression by activating the vitamin D receptor (**VDR**) . The VDR is a nuclear hormone receptor that forms a heterodimer with the retinoid X receptor (**RXR**). The DNA binding domains of the VDR/RXR heterodimer recognize DNA sequences, known as vitamin D response elements (**VDRE**), in the promoter regions of vitamin D responsive genes (Haussler et al., 2013). The human and murine genomes are predicted to have nearly 1,000 genes with potential VDRE (Wang et al., 2005). Regulation of each gene would depend on the presence of the VDR and accessibility of the promoter, but the wide distribution of VDRE does suggest that $1,25(OH)_2D_3$ has multitude of effects throughout the body.

Both $25(OH)D_3$ and $1,25(OH)_2D_3$ are substrates for CYP24A1. The CYP24A1, or 24-hydroxylase (24-OHase), is a cytochrome P450 enzyme that adds a hydroxyl group at the 24 position of both $25(OH)D_3$ and $1,25(OH)_2D_3$ (Horst et al., 1994). The expression of 24-OHase is under control of multiple VDRE and as such is highly responsive to increases in $1,25(OH)_2D_3$ concentrations in most cells that have the VDR (Haussler et al., 2013). The 24-hydroxyvitamin D metabolites are inactive, so 24-OHase serves as a feedback regulator of $1,25(OH)_2D_3$ synthesis (Reinhardt and Horst, 1989). The 24-hydroxyvitamin D metabolites undergo further side chain oxidation in the kidney to eventually form more polar metabolites, which are excreted in the bile (Horst et al., 1994).

Nearly all vitamin D metabolites in serum are bound by the vitamin D binding protein (**DBP**). The DBP is a member of the albumin family of serum proteins and is produced in the liver (Haddad, 1995). It has multiple functions besides vitamin D binding, including actin binding, macrophage activation, and fatty acid transport (Speeckaert et al., 2006). The DBP is very abundant in serum and has a high affinity for vitamin D metabolites. As a result, over 99.9% of $25(OH)D_3$ and 99% of $1,25(OH)_2D_3$ in serum are bound by DBP (White and Cooke, 2000). The DBP has not been studied in cattle, but its concentration may contribute to $25(OH)D_3$ and $1,25(OH)_2D_3$ concentrations in serum and greatly impact function of the vitamin D system.

Targets of the Vitamin D Receptor

As noted above the biological activity of vitamin D is carried out by the activation of the VDR with $1,25(OH)_2D_3$. Several of the genes upregulated by the activated VDR are shown in **Figure 2.** The activated VDR serves to regulate transcription of genes under control of accessible VDREs. Classical targets of the VDR in the kidneys and intestines are genes that code for calcium transport and calcium binding proteins; examples are calbindin-D(9k), calbindin-D(28k), and TRPV6 (Haussler et al., 2013).

The $1,25(OH)_2D_3$ also increases osteocyte RANKL and fibroblast growth factor 23 (**FGF23**) production and promotes both bone resorption and mineralization (Haussler et al., 2013). In bovine monocytes, $1,25(OH)_2D_3$ enhances iNOS, RANTES, and several β-defensin genes (Nelson et al., 2012). It also dampens the antigen-specific IFN-γ and IL-17 responses of T cells (Nelson et al., 2011). Mammary epithelial cell proliferation also is inhibited by $1,25(OH)_2D_3$, likely through cell cycle regulators p21 and p27 (Welsh, 2007). In bovine mammary epithelial cells, $1,25(OH)_2D_3$ upregulates β-defensin 4 gene expression, but down regulates several other of the β-defensins (Merriman and Nelson, unpublished).

The transcriptional response is proportional to the concentration of $1,25(OH)_2D_3$ and VDR in the cell. The concentration of $1,25(OH)_2D_3$ required to elicit a response depends on the abundance of the VDR and accessible VDREs in the target gene (Haussler et al., 2013). For instance, CYP24A1, one of the most responsive vitamin D target genes, responds to picomolar concentrations of $1,25(OH)_2D_3$ in the kidneys and intestinal epithelial cells. In contrast, genes such as iNOS or RANTES in bovine monocytes require nanomolar concentrations of $1,25(OH)_2D_3$ to elicit a meaningful response (Nelson et al., 2011; Nelson et al., 2010b). That contrast is a key difference between the vitamin D endocrine system, that maintains blood calcium and phosphorous, and the intracrine and paracrine mechanism in the immune system. The calcium binding and transport genes in the intestines and kidneys, and consequently blood calcium, are influenced by the concentration of $1,25(OH)_2D_3$ circulating in the blood. That blood concentration normally ranges from 20 to 50 pg/mL (50 to 125 pM) in cattle, and reaches 100 to 200 pg/mL in serum of cows post-partum during periods of hypocalcemia (Horst et al., 1994). The vitamin D responsive genes of the immune system, in contrast, are not influenced by circulating $1,25(OH)_2D_3$. The mechanisms that influence circulating and localized $1,25(OH)_2D_3$ are considered next.

Regulation of Renal Vitamin D Metabolism

The concentration of $1,25(OH)_2D_3$ in blood is primarily determined by renal expression of 1α-OHase (synthesis) and 24-OHase (degradation). Those enzymes are tightly regulated in response to parathyroid hormone (**PTH**), FGF-23, and $1,25(OH)_2D_3$ at a ratio that keeps circulating $1,25(OH)_2D_3$ at a concentration that maintains blood concentrations of calcium and phosphate (Haussler et al., 2013; Horst et al., 2005). If blood calcium decreases, calcium sensing receptors in the parathyroid gland stimulate PTH production. The PTH subsequently elevates renal 1α-OHase expression and inhibits renal 24-OHase. In contrast, FGF-23 inhibits renal 1α-OHase expression and stimulates 24-OHase expression (Haussler et al., 2013). The FGF-23 is produced by bone cells in response to $1,25(OH)_2D_3$ and phosphorous levels. It suppresses renal sodium-phosphate co-transporters to decrease phosphate reabsorption. Finally, $1,25(OH)_2D_3$ directly represses renal 1α-OHase and stimulates 24-OHase to regulate its own concentration in a feed-back manner.

The ratio of 1α-OHase:24-OHase in the kidneys is critical in the transition dairy cow (Horst et al., 2005). The higher the 1α-OHase:24-OHase ratio the better suited is

the cow to increase circulating $1,25(OH)_2D_3$. Conditions that promote PTH production and PTH receptor signaling are expected to increase the 1α-OHase:24-OHase ratio. Greater PTH sensitivity is achieved through feeding a diet low in dietary cation-anion difference (**DCAD**) (Horst et al., 2005). The acidic conditions achieved with a low DCAD diet alter the conformation of renal PTH receptors slightly to make them more sensitive (Goff and Horst, 2003). In theory, keeping the FGF-23 concentration low also will increase the 1α-OHase:24-OHase ratio. The FGF-23 was recently discovered and so far has not been studied in cattle, but limiting excess intake of phosphorus is expected to inhibit FGF-23 production.

The $25(OH)D_3$ concentration also affects levels of 1α-OHase and 24-OHase in the kidneys. If the $25(OH)D_3$ concentration is low, the body compensates by producing more PTH (Lips, 2004), thereby stimulating 1α-OHase and depressing 24-OHase. Under normal conditions in humans, PTH rises to compensate for serum $25(OH)D_3$ concentrations < 30 ng/mL (Vieth et al., 2003). Conversely, as $25(OH)D_3$ concentrations rise, less 1α-OHase and more 24-OHase are required to keep circulating $1,25(OH)_2D_3$ in the correct balance (Engstrom et al., 1984). Consequently, circulating $1,25(OH)_2D_3$ does not correlate with the $25(OH)D_3$ concentration.

Extra-renal Vitamin D Metabolism

In contrast to the genes related to calcium and phosphate balance, vitamin D responsive genes in the immune system are controlled by locally produced $1,25(OH)_2D_3$ (Nelson et al., 2010a; Nelson et al., 2010b). Macrophages are major sources of the $1,25(OH)_2D_3$ that controls vitamin D-mediated immune responses. The 1α-OHase is stimulated in bovine macrophages via toll-like receptor (**TLR**) recognition of pathogen associated molecular patterns such as lipopolysaccharide, peptidoglycan, and mycobacterial lipopeptides. The macrophage 1α-OHase enables conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$, and subsequently activation of vitamin D-mediated immune responses. The response of genes *in vitro* such as iNOS, RANTES, and β-defensins is correlated with the concentration of $25(OH)D_3$. That correlation is in contrast with the vitamin D endocrine system, where calcium and phosphate do not correlate with $25(OH)D_3$.

The 1α-OHase is expressed in the udder during mastitis in dairy cattle (Nelson et al., 2010a). The majority of 1α-OHase in the infected mammary gland is present in the $CD14^+$ cells (macrophages) secreted in the milk. Induction of 1α-OHase in the udder in response to bacterial infection enables conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$ as indicated by upregulation of 24-OHase in the mammary gland. Normally milk $25(OH)D_3$ is < 5ng/mL (McDermott et al., 1985), but intramammary administration of 100 µg of $25(OH)D_3$ inhibited mastitis in dairy cattle (Lippolis et al., 2011). The effects of intrammamary $25(OH)D_3$ presumably occurred via 1α-OHase conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$, and $1,25(OH)_2D_3$-mediated induction of nitric oxide and β-defensin production. Meanwhile, the intramammary $25(OH)D_3$ infusion did not affect serum $25(OH)D_3$ or $1,25(OH)_2D_3$ concentrations. In addition, circulating $1,25(OH)_2D_3$ does not

increase during mastitis, indicating that vitamin D signaling is limited to the infected mammary gland.

In addition to stimulation of 1α-OHase, TLR ligands are potent inhibitors of 24-OHase expression in bovine macrophages (Nelson et al., 2010b). In a freshly isolated, resting bovine monocyte, 10 nM of $1,25(OH)_2D_3$ upregulates 24-OHase expression ~50 to 100 fold. However, if the monocytes are stimulated with LPS, the upregulation of 24-OHase by $1,25(OH)_2D_3$ is < 10 fold greater than resting monocytes. The pathogen induced inhibition of 24-OHase seemingly allows for unchecked $1,25(OH)_2D_3$ synthesis in the macrophage. Unchecked production of $1,25(OH)_2D_3$ is a key difference between vitamin D metabolism in the immune system and vitamin D metabolism in the kidneys; the local concentration of $1,25(OH)_2D_3$ is not tightly controlled like the circulating concentration.

Altogether, expression of $1,25(OH)_2D_3$-regulated genes in immune cells is determined by abundance of 1α-OHase, 24-OHase, and $25(OH)D_3$. The strength of the pathogen derived signal (i.e. TLR or IFN-γ) contributes to macrophage 1α-OHase and 24-OHase. The magnitude of vitamin D-regulated responses, such as nitric oxide and β-defensins, will be insufficient if the $25(OH)D_3$ concentration is insufficient. The threshold for $25(OH)D_3$ required to support vitamin D mediated immunity in cattle has not been determined. Epidemiological data from the human population suggests there is a correlation between serum $25(OH)D_3$ and immune function, and that concentrations < 32 ng/mL of serum are insufficient for immunity (Adams et al., 2007).

Besides immune cells, mammary epithelial cells and the placenta are additional sources of $1,25(OH)_2D_3$ synthesis that have significance for dairy cattle. In mice, the 1α-OHase is expressed in mammary tissue during mammary development and involution (Welsh, 2004). Cultured bovine mammary epithelial cells also express the 1α-OHase and respond to $25(OH)D_3$ treatment. The placenta produces enough $1,25(OH)_2D_3$ to affect the circulating pool of $1,25(OH)_2D_3$. Circulating $1,25(OH)_2D_3$ also increases with estrogen therapy in women. However, the function of $1,25(OH)_2D_3$ in pregnancy and reproductive physiology in cattle is unknown. In any case, vitamin D supplementation improved reproductive performance in dairy cattle (Ward et al., 1971), and circulating $1,25(OH)_2D_3$ is elevated during pregnancy (O'Brien et al., 2014). Consequently, reproductive physiology also should be considered in regard to vitamin D metabolism.

Nutritional Implications

Because vitamin D_3 can be synthesized in sun-exposed skin and its biological activity is regulated via tightly regulated processes, a clear dose response to vitamin D supplementation will not occur if the appropriate conditions are not met. As a consequence defining dietary vitamin D_3 requirements has been difficult. Rather than focusing strictly on effects of vitamin D_3 supplementation on a given outcome, emphasis should be placed on identifying serum 25(OH)D concentrations that support the various outcomes of vitamin D metabolism.

The serum concentrations of 25(OH)D required for calcium maintenance in cattle have been studied in depth. Under normal circumstances in calves and lactating cows, serum 25(OH)D concentrations of 20 to 100 ng/mL support a normal calcium and phosphate balance. At the onset of lactation, cows would presumably benefit from having higher serum $25(OH)D_3$ concentrations to support the urgent need for renal $1,25(OH)_2D_3$ synthesis. However, plasma $1,25(OH)_2D_3$ was not greater (~300 vs. 400 pg/mL) in the hours and days postpartum in cows with ~ 175 ng of $25(OH)D_3$/mL of serum compared to cows having ~40 ng of $25(OH)D_3$/mL of serum (Wilkens et al., 2012). Furthermore, cows in that study with the higher $25(OH)D_3$ had lower ionized and total calcium than cows with normal serum $25(OH)D_3$ when not fed a low DCAD diet. There is likely a saturation point of the renal 1α-OHase for $25(OH)D_3$ during the post-partum period. Future experiments should aim to determine the maximum serum $25(OH)D_3$ that benefits the transition cow. Meanwhile, serum $25(OH)D_3$ concentrations over 100 ng/mL serum do not seem to provide the transition cow much benefit compared to concentrations between 20 and 50 ng/mL as regards blood calcium.

The optimal $25(OH)D_3$ concentration for immunity has not been determined for cattle yet. The effects of $25(OH)D_3$ concentration on macrophage host defense responses *in vitro* suggest a linear benefit to at least 100 ng/mL (Nelson et al., 2010b). Calves with ~175 ng of $25(OH)D_3$/mL of serum, however, did not fair any better than calves with ~30 ng of $25(OH)D_3$/mL of serum in regards to severity of experimental respiratory syncytial virus (**RSV**) infection (Sacco et al., 2012). That study does not indicate whether there is a maximal benefit somewhere in between that range, and clearly further work is needed on the relationship between serum $25(OH)D_3$ and infectious disease outcome in cattle. Insufficient vitamin D conceivably impairs immunity, so until more data is available, serum $25(OH)D_3$ concentrations of at least 30 ng/mL are recommended to support immune function in cattle.

The justification for dietary vitamin D recommendations in the 7th edition of Nutrient Requirements of Dairy Cattle (NRC, 2001) cited a study by Ward et al. (1971) that found cows receiving 300,000 IU of vitamin D_3/week by oral bolus reached estrus 16 days earlier post-partum and conception 37 days earlier than non-treated cows. Serum $25(OH)D_3$ data was not available in that study. The NRC (2001) also cites a study (Hibbs and Conrad, 1983) that milk production and feed intake were greatest for cows supplemented with 40,000 IU of vitamin D_2/d than cows receiving no vitamin D or 80,000 IU of vitamin D_2/day. However, vitamin D_2 was much less effective in raising total serum 25(OH)D [$25(OH)D_2$ and $25(OH)D_3$] than vitamin D_3 in a recent study (Hymoller and Jensen, 2011), so an equivalent amount of vitamin D_3 may not have the same effect on milk production.

Overall, the ideal serum $25(OH)D_3$ concentration for cattle likely lies between 40 and 80 ng/mL. Targeting a lower range, below 40 ng/mL, may result in some animals with serum $25(OH)D_3$ concentrations below 20 ng/mL based on variation observed within dairy herds. Based upon the available data, there appears to be no benefit in exceeding 100 ng/mL. A limited survey of dairy herds in the Midwest indicated producers supply lactating Holstein cows with 30,000 to 50,000 IU of vitamin D_3/d

(Lippolis 2012, unpublished). The average $25(OH)D_3$ concentration of 320 serum samples collected from 100 to 250 DIM over a course of 18 months from those herds was 70 ng/mL. Ninety percent of those samples were between 40 and 100 ng/mL. A significant correlation was not detected between serum $25(OH)D_3$ and dietary vitamin D_3, time outside during the day, or month (March, June, September, or December) of collection in those samples. A conclusion on the effects of those factors on serum $25(OH)D_3$ cannot be made, but in another study serum $25(OH)_3$ did not differ between lactating cows fed 10,000 or 50,000 IU of vitamin D_3/d (McDermott et al., 1985). Therefore, supplying cows with 50,000 IU compared to 30,000 IU/d, or the NRC recommended 21,000 IU/d, may not provide a significant advantage. Regardless, supplying cows with 20,000 to 50,000 IU of vitamin D_3/d should result in serum $25(OH)D_3$ concentrations between 40 and 80 ng/mL.

Calves and beef cattle presumably require serum $25(OH)D_3$ concentrations between 40 and 80 ng/mL as well. Calves housed indoors and fed milk replacer supplying 1700, 11,000, or 17,900 IU of vitamin D_3/kg of diet had approximately 30, 90, and 180 ng/mL of serum $25(OH)D_3$, respectively (Nonnecke et al., 2010; Sacco et al., 2012). Close attention should be paid to vitamin D status of calves just receiving cow's milk because serum $25(OH)D_3$ of calves fed whole milk or colostrum declined from 20 ng/mL to < 10 ng/mL in just 7 days (Rajaraman et al., 1997). So, in limited sun conditions, calves should be supplied with at least 2,000 IU/kg of diet DM, but no more than 11,000 IU/kg of diet DM in order to achieve serum $25(OH)D_3$ concentrations of 40 to 80 ng/mL.

The NRC recommendations for beef cattle are 275 IU/kg of diet (NRC, 2000), and for beef cattle in the southern US (below 35°N) that amount should be adequate (Webb et al., 1988). However, beef cattle in the northern states during the winter months, or in conditions with limited sun, may require additional supplementation to keep serum $25(OH)D_3$ above 20 ng/mL (Hymoller et al., 2009). Feedlot steers supplied with the NRC recommended amount of vitamin D_3 had on average ~20 ng/mL of serum $25(OH)D_3$ (Pickworth et al., 2012). Seventy days after removal of supplemental vitamin D and only incidental sun exposure, serum concentration of $25(OH)D_3$ of those steers dropped below 10 ng/mL. Steers in those same conditions supplied with 1,860 IU of vitamin D_3/kg of diet (~15,000 IU/d or 50 IU/kg of BW) for 70 days had on average 67 ng/mL of serum $25(OH)D_3$. In light of that data, beef cattle may require 15 to 50 IU/kg of BW, depending on environmental conditions, to keep serum $25(OH)D_3$ above 30 ng/mL.

Conclusions

Vitamin D contributes to more than calcium and bone formation in cattle. The active vitamin D hormone also contributes to immune, reproductive, and mammary physiology. Multiple tissues and factors also contribute to vitamin D activity. Regulation of renal vitamin D metabolism is fairly well understood, but the contribution of FGF-23 in cattle requires further consideration as to its influence on circulating concentrations of $1,25(OH)_2D_3$. Immune cells utilize $25(OH)D_3$ independent of the kidneys, but the

optimal 25(OH)D_3 concentration for immune function has yet to be determined. Similarly, optimal serum 25(OH)D_3 concentrations for reproduction and lactation have not been determined, even though vitamin D has been shown to affect both. According to available data, moderate serum 25(OH)D_3 concentrations that range from 40 to 80 ng/mL are ideal for cattle. As a general rule of thumb if sun exposure is limited, daily supplemental feeding 30 to 50 IU of vitamin D_3/kg of BW should achieve that range for cattle.

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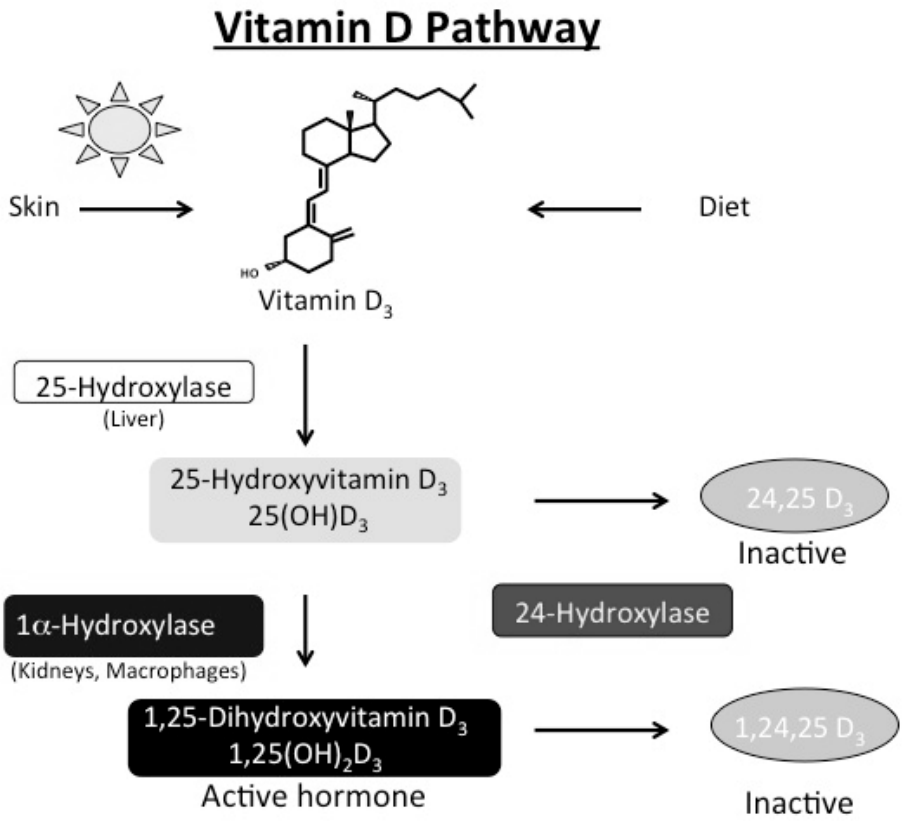

Figure 1. General vitamin D metabolic pathway. Vitamin D_3 is acquired in the skin from photoconversion of 7-dehydrocholesterol, or through dietary supplementation. Vitamin D_3 is readily converted to 25-hydroxyvitamin D_3 (25(OH)D_3). The 25(OH)D_3 is activated to 1,25-dihydroxyvitamin D_3 by the 1α-Hydroxylase, a tightly regulated enzyme expressed in kidneys and macrophages in cattle. The 1,25(OH)$_2D_3$ activates the VDR as shown in Figure 2, and also induces it own catabolism via the 24-hydroxylase. The 24-hydroxylated vitamin D metabolites are further metabolized and excreted in the bile.

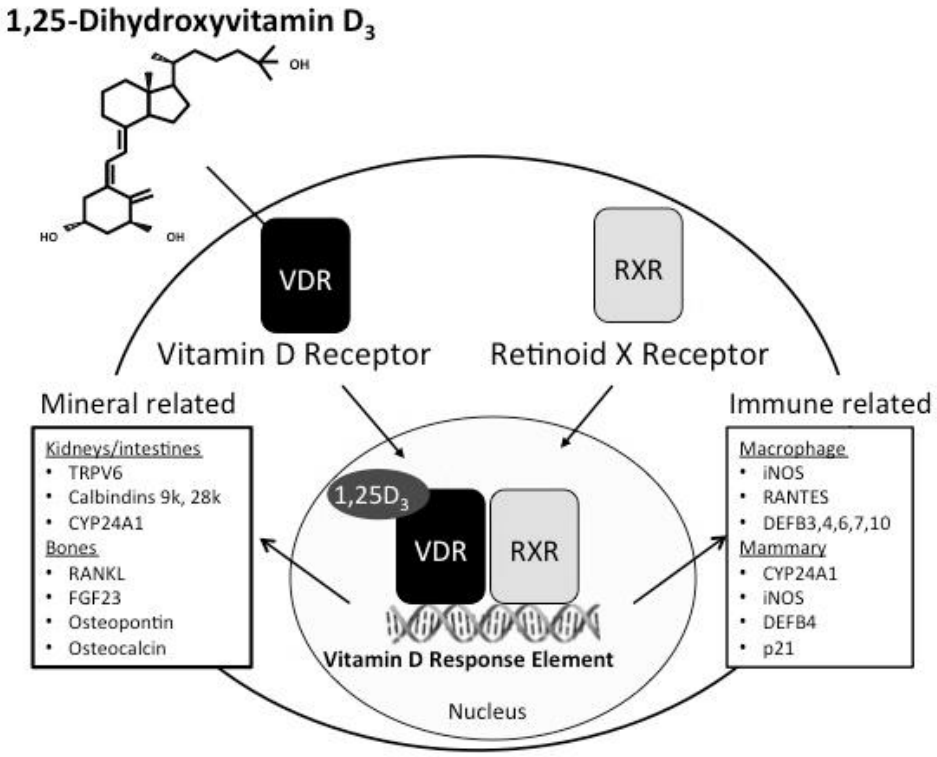

Figure 2. Molecular actions of 1,25-dihydroxyvitamin D_3. The vitamin D receptor (VDR) is the receptor for $1,25(OH)_2D_3$, and once activated it joins with the retinoid X receptor to activate genes with an accessible vitamin D response element. CYP24A1, or 24-hydroxylase, is induced in most cells that have the VDR. Examples of calcium related genes regulated by the VDR are TRPV6 and Calbindins D9k and D28k. In the bone the VDR induces RANKL (bone resorption), FGF23 (decreased renal phosphate reabsorption), osteopontin, and osteocalcin (bone mineralization). The VDR enhances iNOS and DEFB4 (antimicrobial) in macrophages and mammary epithelial cells, and DEFB3,4,6,7,&10 (defensin antimicrobial peptides) in macrophages. The VDR also decreases mammary epithelial cell proliferation via upregulaiton of p21.

SESSION NOTES