Vitamin D: Support for Immunity and Transition Cow Performance

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Introduction

Cost effective prepartum dietary interventions that support a smooth transition for pregnancy to lactation provide a large return on investment because adverse events in the first week of lactation have substantial negative consequences on lactation and reproductive performance. For example, cows that experience metritis produce about 8 lbs. less milk per day in the first 10 weeks of lactation compared with cows without metritis. Optimal vitamin and mineral nutrition during the transition period is considered important for transition cow performance, but recommendations and practices are often variable. Herein, the focus will be on the influence of vitamin D in immunity of dairy cows and the effects of prepartum vitamin D nutrition on transition cow performance.

Transition Cow Immunity and Inflammation

The transition from pregnancy to lactation presents major challenges to immune processes, such as initiation of parturition, uterine involution, and increased exposure of mammary glands and uterus to opportunistic pathogens. Moreover, metabolic demands of the immune system coupled with metabolic adaptations at the start of lactation may impair immune cell functionality. The resilience and robustness of the cow's immune system is quite remarkable considering the challenges faced upon the onset of lactation.

The mammalian immune system is far more complex than what is generally described. It contains multiple levels of redundancy to compensate for failures of one component. Only a generalized description of the initial innate immune response to an opportunistic pathogen in cases of metritis or mastitis will be provided here because those are two of the most prominent diseases affecting early lactation. A healthy mammary or uterine environment will normally have very little to no microbial load, but the process of parturition and the onset of lactation exposes each respective organ to microbes that cause infections of the mammary gland, leading to mastitis, or uterus, leading to metritis.

The first line of defense against microbes is the epithelial barrier. The epithelial barrier consists of epithelial cells knit together by tight junction proteins. Many epithelial barriers, like those of the intestines, lungs, and uterus produce mucus that includes antimicrobial factors. Epithelial barriers also include intraepithelial immune cells that, along with epithelial cells, have innate pattern recognition receptors (i.e., toll-like receptors) to survey the environment for microbial associated molecular patterns (i.e., lipopolysaccharide). Activation of the pattern recognition receptors triggers a cascade of signaling events that includes production of antimicrobial proteins, chemokines,

cytokines, and eicosanoids. These molecules represent the beginning of an inflammatory response that increases blood flow and recruitment of immune cells to the site of infection.

In the context of intramammary and intrauterine infections, the chemokines attract other immune cells like monocytes and neutrophils. Cytokines, like interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) provide local cell-to-cell signaling that stimulates defense responses of immune cells. Monocytes and neutrophils are phagocytes that engulf microbes and, within intracellular compartments, oxidative enzymes produce reactive oxygen and nitrogen molecules that are lethal to microbes. Monocytes and neutrophils also produce copious amounts of antimicrobial proteins like defensins, cathelicidins, and proteases that are involved in elimination of the microbe. It is important to recognize that functional capacities of immune cells circulating in blood are somewhat diminished in the first few days postpartum. The cause for a change in immune cell functionality postpartum is likely multifactorial and not explicitly the reason for increased risk of postpartum disease.

The immune response involves an intricate system of checks and balances to eliminate infections while also protecting host tissues. A potent proinflammatory response is necessary to defend against infection. The inability of initial defenses to contain infectious microbes will require an increasingly greater pro-inflammatory response to prime the whole body for defense against the infection. Hallmark indicators of a systemic defense response include elevated body temperature and concentrations of haptoglobin, serum amyloid A, and leukocytes in blood. However, the highly oxidative environment created to eliminate an infection can cause tissue damage and necrosis. Therefore, antioxidant and anti-inflammatory mediators are necessary for effective resolution of infection. Nutrients like vitamin E and omega-3 fatty acids that have antioxidant properties, and selenium that is required for enzymatic reduction, play a role in maintaining a balanced immune response.

Vitamin D and Immunity

Vitamin D is best known for its role in skeletal, Ca and P homeostasis, however, it is involved in many biological functions, including immunity. Vitamin D refers to a class of seco-steroid molecules that largely function through an intracellular protein receptor called the vitamin D receptor. An overview of the vitamin D pathway and some outcomes of endocrine and intracrine vitamin D signaling are depicted in Figure 1. Vitamin D_3 is naturally produced from 7-dehydrocholesterol in animals through a process of photoconversion in skin exposed to UVB light. Vitamin D_3 serves as the precursor to the 25-hydroxyvitamin D_3 [25(OH)D_3] metabolite that is the major form of vitamin D circulating in blood. The 25(OH)D has a half-life of approximately two weeks, its concentration in plasma serves as the best indicator of vitamin D status.

The 1,25-dihyroxyvitamin D_3 [1,25$(OH)_2D_3$] metabolite is the biologically active metabolite and its concentration in plasma is typically 20 to 50 pg/mL in lactating and dry cows, and upwards of 100 to 300 pg/mL in 2 to 3 DIM. Plasma 1,25$(OH)_2D_3$ concentrations do not correspond to vitamin D intake (Poindexter et al., 2020). The main

points of control in the vitamin D metabolic pathway center around regulating the concentration of $1,25(OH)_2D_3$ via the activities of 1α-hydroxylase (activation) and 24-hydroxylase (inactivation) enzymes. Most 1α- hydroxylase activity occurs in the kidneys under strict hormonal control, but a small fraction also occurs in adipocytes, immune cells, mammary epithelial cells, and reproductive tissues under control of various signal processes. The $1,25(OH)_2D_3$ induces its own catabolism by upregulation of 24-hydroxylase in a classical feed-back manner. The 24-hydroxylase can be expressed in nearly every cell that has vitamin D receptors, which serves to control vitamin D activity at the cellular level. Collectively, the balance of 1α-hydroxylase and 24-hydroxylase activity serve to regulate vitamin D activity. Most biological activity of vitamin D occurs through intracellular vitamin D receptors (VDR). The DNA-binding domain of the VDR recognizes short, specific sequences of DNA referred to as vitamin D response elements. The elements are located promoter or enhancer segments upstream, downstream, and within vitamin D target genes.

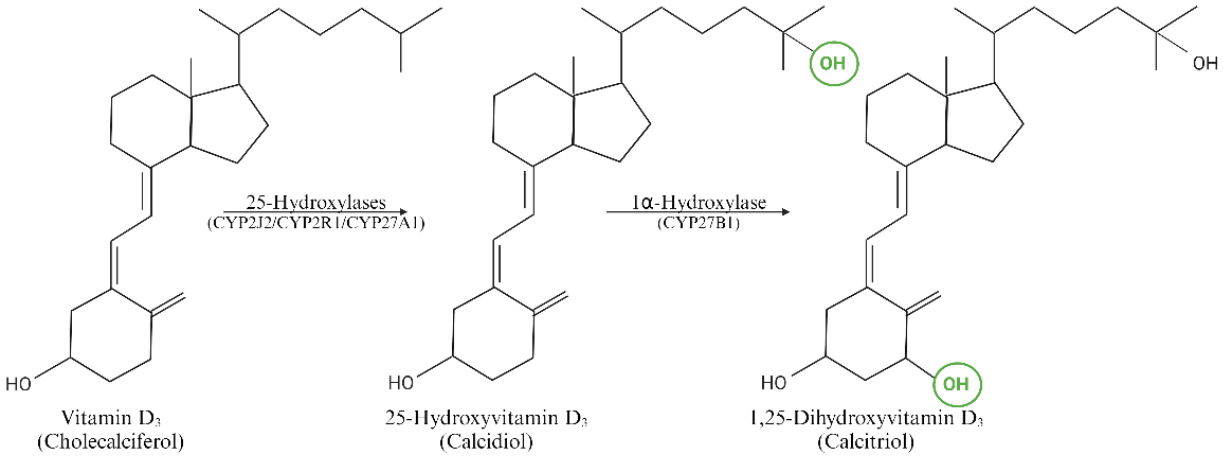

Figure 1. Oxidation of vitamin D_3 to 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3. Oxidation of vitamin D_3 to $25(OH)D_3$ is catalyzed by several hepatic 25-hydroxylases. Subsequent oxidation of $25(OH)D_3$ to 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$, calcitriol], the active metabolite, is catalyzed by the 25-hydroxyvitamin D_3 1α-hydroxylase. Not shown is the oxidation of $25(OH)D_3$ and $1,25(OH)_2D_3$ metabolites to 24,25-dihydroxyvitamin D_3 and 1,24,25-trihydroxyvitamin D_3, respectively, by the 25-hydroxyvitamin D-24-hydroxylase. The 24-hydroxyvitamin D metabolites are reported to have some biological activity, but are generally regarded as degradation products of vitamin D.

Vitamin D activity in immune cells was discovered nearly four decades ago, but it was not until the last two decades that the influence of vitamin D in immunity became appreciated. As with many nutrients that have a role in immunity, there was significant hype regarding vitamin D during the peak of COVID. Many claims regarding vitamin D and immunity may be exaggerated, but substantial evidence indicates it has significant influence in multiple immune processes. As depicted in Figure 2, toll-like receptor recognition of microbial associated molecules like LPS stimulate immune cell 1α-hydroxylase (a.k.a CYP27B1) activity that converts $25(OH)D_3$ to $1,25(OH)_2D_3$ (Nelson et

al., 2010). The upregulation of mRNA for 1α-hydroxylase has been observed in cultures of monocytes and neutrophils cows and in tissues and cells of experimental mammary infections (Merriman et al., 2018). The $1,25(OH)_2D_3$ contributes to antimicrobial, anti-inflammatory, and antioxidant processes of immune cells. For example, vitamin D signaling stimulates nitric oxide production that contributed to the control of *Mycobacterium bovis* growth in monocyte cultures (García-Barragán et al., 2018). Vitamin D signaling also stimulated expression of multiple β-defensins, thioredoxin, and metallothionein genes in monocytes of cows (Merriman et al., 2015 and Kweh et al., 2021). More broadly, vitamin D is known to promote anti-inflammatory processes, such as limit proliferation of pro-inflammatory T cells and promote function of regulatory T cells.

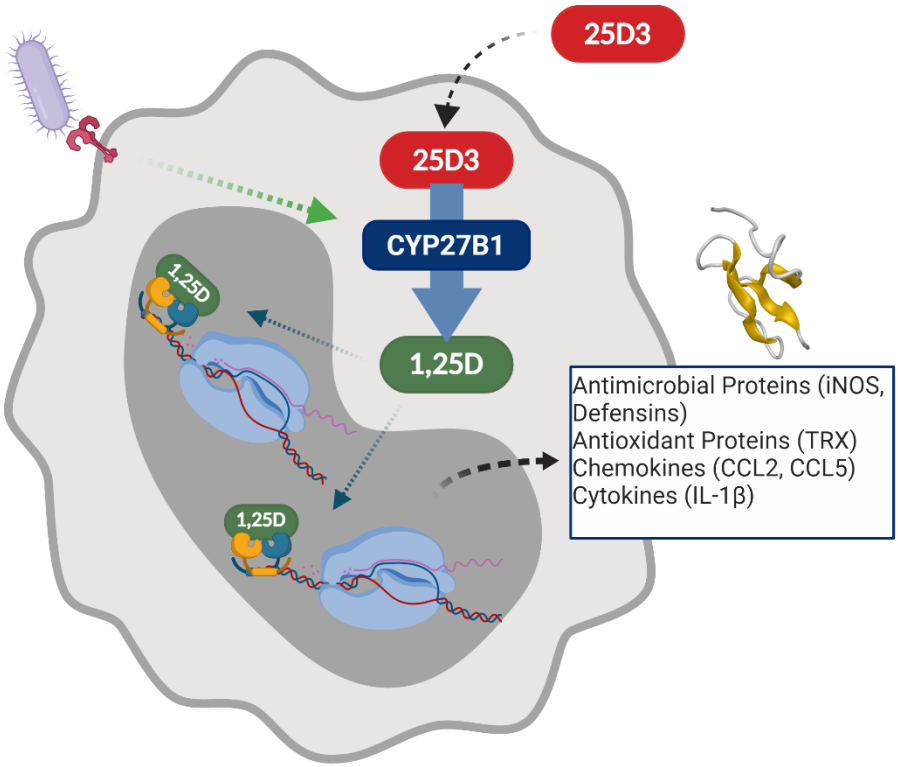

Figure 2. Vitamin D pathway of bovine macrophages. Innate pathogen recognition receptors stimulate expression of 1α-hydroxylase, also known as CYP27B1, and conversion of 25-hydroxyvitamin D_3 to 1,25-dihydroxyvitamin D_3.

Vitamin D Nutrition and Health and Production Outcomes

Recommendations for supplemental vitamin D_3 are largely based on Ca and P-related activity of vitamin D (NASEM, 2021). Most lactating cow diets provide cows 30,000 to 50,000 IU (0.75 to 1.25 mg) supplemental vitamin D_3 per day. Likewise, dry cow and closeup cow diets usually provide at least 20,000 to 30,000 IU supplemental vitamin D_3 per day. Serum 25(OH)D concentrations are typically between 40 and 100 ng/mL regardless of season, housing or geographical location (Nelson et al., 2016). There is scant epidemiological evidence relating vitamin D status to health outcomes, but a study

of 5 dairy herds in Michigan revealed that cows with serum 25(OH)D concentrations below 74 ng/mL were at greater risk of postpartum disease (Wisnieski et al, 2020). Vitamin D deficiency is not common in U.S. dairy cows, so the issue regarding vitamin D is whether increasing vitamin D status provides additional health benefits. At the same time, caution is required for supplemental vitamin D because of risk of vitamin D toxicity (Fraser 2021).

In theory, vitamin D signaling of immune cells should benefit from increased availability of 25(OH)D_3. Unlike the classical endocrine vitamin D pathway that centers on renal control of vitamin D metabolism, immune cell vitamin D activity seems to be rate limited by availability of 25(OH)D_3. There have yet to be reported experiments that provide convincing evidence that a lack of 25(OH)D_3 results in increased risk of infection of cattle. Holstein bull calves that had serum 25(OH)D_3 < 20 ng/mL had more severe responses to an intravenous LPS challenge compared with calves with serum 25(OH)D_3 concentrations between 30 to 40 ng/mL, but it is unknown whether this outcome translates to a difference in risk of infection (Blakely et al., 2023). On the other hand, increasing serum 25(OH)D concentrations above 100 ng/mL compared with the typical concentrations near 60 ng/mL has been shown to deliver better immune protection. Supplementing 25(OH)D_3 in dairy cow diets is an effective means to elevate serum 25(OH)D_3 and, consequently, availability of 25(OH)D_3 for immune cells. Supplemental 25(OH)D_3 is approved for ruminant diets in the U.S. Direct feeding of 25(OH)D_3 bypasses the initial hepatic oxidation (Figure 1) step making it much more effective at increasing serum 25(OH)D_3 compared with feeding vitamin D_3. For example, feeding 3 mg of 25(OH)D_3 to cows increased serum 25(OH)D_3 from 60 ng/mL to 200 ng/mL, whereas feeding 3 mg vitamin D_3 did not cause serum 25(OH)D_3 to increase above 100 ng/mL (Poindexter et al., 2023). In an experiment with lactating dairy cows, Poindexter et al. (2020) showed that feeding 1 or 3 mg of 25(OH)D_3 increased expression of inducible nitric oxide synthase and IL-1β genes in immune cells of milk compared with cows fed 1 or 3 mg of vitamin D_3. Expression of several immune genes also were correlated with serum 25(OH)D_3 concentrations. Feeding 3 mg of 25(OH)D_3 also decrease the severity of intramammary *Streptococcus uberis* infection compared with feeding 1 mg of vitamin D_3 (Poindexter et al., 2020).

The benefits of feeding 25(OH)D_3 are best realized during the transition period. Vieira-Neto et al. (2021) showed that feeding 25(OH)D_3 to closeup cows altered the expression of more than a dozen genes in blood leukocytes that related to immune cell trafficking, cell signaling, and antimicrobial activity. Martinez et al. (2018) also showed that feeding 25(OH)D_3 to closeup cows increased oxidative burst capacity of neutrophils from blood. Whether or not those effects of 25(OH)D_3 truly lead to better immune protection remain to be determined but feeding 25(OH)D_3 to closeup cows has consistently resulted in improved transition cow performance. Martinez et al. (2018), Silva et al. (2021), Poindexter et al. (2023), and Holub et al. (2023) have reported that feeding 3 mg 25(OH)D_3 to closeup cows resulted in 4 to 8 lbs. more milk compared with feeding vitamin D_3. A plausible explanation for increased milk production is the control of inflammation associated with metritis that is common in fresh cows. For example, Martinez et al. (2018) observed that feeding 25(OH)D_3 decreased risk of retained placenta and metritis. Likewise, *post hoc* analysis of data from Poindexter et al. (2023)

revealed the benefits of 25(OH)D_3 were most apparent in cows that were diagnosed with metritis and feeding 25(OH)D_3 decreased concentrations of haptoglobin in serum indicating better containment of inflammation.

Conclusion

Vitamin D signaling has an influential role in shaping the immune response and containing inflammation. Supplemental 25(OH)D_3 provides a more effective alternative to vitamin D_3 for dairy cows and has been reported to influence various immune processes in dairy cows. Important to the bottom line for dairy producers, feeding 25(OH)D_3 to closeup cows is a cost effective approach to increasing serum 25(OH)D of cows during the critical transition period that results in increased milk production.

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