A review of vitamin D and its importance to the health of dairy cattle

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Abstract

This Research Reflection short review will discuss vitamin D metabolism, its role in nutrition, disease prevention, and welfare of dairy cattle, as well as its toxicity. Vitamin D is an important fat-soluble vitamin. However, some researchers regard it as a hormone due to its function in the organism. Its role is not limited just to Ca homoeostasis and bone metabolism but is also associated with immunity. In dairy cattle it is known for preventing milk fever. Cows can acquire vitamin D in many ways for example through feed, parenteral injections or through UVB irradiation from the sun or artificial lighting. The vitamin D in feed can either be plant-/fungi- based ergocalciferol or animal-based cholecalciferol. There is currently only one registered feed vitamin D supplement for cattle in the European Union and it is cholecalciferol. Animals can also synthesize their own vitamin D when 7-dihydrocholesterol in the skin is irradiated with UVB light resulting in cholecalciferol production. Despite its importance, many cattle are deficient in vitamin D due to inadequate supplementation or insufficient sun exposure. In a study performed at the Veterinary Faculty in Slovenia 12 high producing Holstein Friesian cows at a commercial dairy farm were blood tested for vitamin D status for three succeeding months and all but one were vitamin D insufficient in all testings. The cows were not exposed to direct sunlight and the content of vitamin D_3 in feed was <400 IU/kg dry matter, which is less than half of the NRC (2001) recommendation. Deficiency can also occur due to diseases affecting the gastrointestinal tract, such as paratuberculosis, which lower the absorptive capacity of the gut. Vitamin D can be toxic if cows are over-supplemented or consume large quantities of plants like *Trisetum flavescens*, which contain an active form of vitamin D-calcitriol or its glycosides, that are activated by digestion in the rumen.

Introduction

Vitamin D is exposed to many structural changes before it reaches its active form called calcitriol. Calcitriol is regarded as a hormone by many as it is primarily synthesized in the kidneys but acts on remote tissues. Vitamin D can enter the body in two ways, either orally through feed or parenterally with intramuscular administration. In addition, most mammals can synthesize their own vitamin D in the skin with the help of UVB irradiation from the sun or artificial UV lights. Vitamin D is important in bone metabolism and Ca homoeostasis. However, new research suggests it also has an important effect on immunity and cell differentiation (Nelson *et al.*, 2012). In this short review vitamin D metabolism, vitamin D toxicity and its role in nutrition, disease prevention and welfare of dairy cattle will be discussed.

Vitamin D metabolism

Vitamin D in feed can be of plant-/fungi- origin (ergocalciferol/vitamin D_2) or animal-origin (cholecalciferol/vitamin D_3) (Ferrari *et al.*, 2017). Cattle can synthesize their own cholecalciferol in the skin, upon cutaneous UVB exposure of 7-dehydrocholesterol, which is an intermediate of cholesterol synthesis (Herrmann *et al.*, 2017). The optimal wavelength of UVB light for cholecalciferol synthesis is between 295 and 300 nm (Jakobsen *et al.*, 2015). With prolonged UVB exposure previtamin D_3 is subjected to photodegradation into inactive substances. After intestinal absorption and synthesis in the skin vitamin D_2 and D_3 are carried to the liver, where they are hydroxylated for the first time to form 25-hydroxy-vitamin D (25-OHD)/calcidiol. The second hydroxylation (by 1α-hydroxylase) takes place in the proximal tubules of kidneys to form an active form of vitamin D called calcitriol/1,25-dihydroxy-vitamin D $(1,25\text{-}(OH)_2D)$. Hydroxylation of 25-OHD can also take place in other tissues including bone, placenta, prostate, keratinocytes, macrophages, T-lymphocytes, epithelial cells of the colon and islet cells of the pancreas. However, calcitriol produced in extra-renal tissues can only act locally (Herrmann *et al.*, 2017). Most of the vitamin D metabolites in blood are bound either to vitamin D binding proteins (VDBP) or albumins (Herrmann *et al.*, 2017).

Vitamin D catabolism starts in the proximal tubules of kidneys to form $24,25\text{-}(OH)_2D$. The end product of vitamin D catabolism is calcitroic acid, which is excreted in bile

(Herrmann *et al.*, 2017). Detailed descriptions of vitamin D metabolism can be found elsewhere (Dusso *et al.*, 2005; Herrmann *et al.*, 2017).

Regulation of vitamin D synthesis takes place at multiple levels. First, the synthesis is already regulated in the skin by the production of inactive products under excessive UVB exposure. Second and most important is regulation in the kidneys at 1α-hydroxylation. 1,25-$(OH)_2$D acts through a negative-feedback loop on the expression of 1α-hydroxylase. 1,25-$(OH)_2$D also lowers the secretion of parathyroid hormone (PTH), which is responsible for increasing 1α-hydroxylase transcription. Raised concentrations of 1,25-$(OH)_2$D also raise fibroblast growth factor 23 (FGF23) expression which suppresses 1α-hydroxylase activity. In addition, dietary calcium and phosphate intake influence 1α-hydroxylase activity: increasing intakes reduce 1α-hydroxylase activity. 1,25-$(OH)_2$D and FGF23 cause up-regulation of expression of CYP24A1, which is an important enzyme in vitamin D catabolism (Herrmann *et al.*, 2017). Cholecalciferol can be stored in adipose tissue, skeletal muscles, lungs, liver, heart and plasma (Mawer *et al.*, 1972).

Physiological function of vitamin D

Calcitriol increases circulating calcium levels by multiple mechanisms. Firstly, through the upregulation of the intestinal absorption of calcium. Next, it upregulates the formation and activation of osteoclasts through the stimulation of the ligand for receptor activator for nuclear factor κ B (RANKL). In addition, it suppresses the transcription of calcitonin and PTH and it also induces the reabsorption of calcium in the kidney distal tubules. Calcitriol also regulates phosphate levels by upregulating intestinal absorption (Colotta *et al.*, 2017).

Immune and inflammatory cells can convert 25OHD into calcitriol which acts locally (Colotta *et al.*, 2017). It is suspected that calcitriol may have a role in the activity, mitosis and differentiation of some immune cells (Nelson *et al.*, 2012). Calcitriol increases phagocytosis and enhances the secretion of H_2O_2 which are important in the microbicidal and tumoricidal function of macrophages (Reinhardt and Hustmyer, 1987). Calcitriol also reduces the production of type 1 proinflammatory cytokines like IL-12, IFN-γ, IL-6, IL-8, tumour necrosis factor-α, IL-17, IL-9 and increases the production of type 2 anti-inflammatory cytokines for example IL-4, IL-5, and IL-10 (Colotta *et al.*, 2017).

Vitamin D in disease prevention

Vitamin D can be important for the prevention of some diseases (Lean *et al.*, 2014). It is best known for its role in prevention of rickets, osteomalacia and hypocalcaemia. A reasonable practice for hypocalcaemia prevention is to supplement the dry cow with 20–30 000 IU vitamin D/day in the diet. Earlier studies often recommended feeding or injecting massive doses (up to 10 million units of vitamin D) 10–14 d prior to calving to prevent milk fever. These vitamin D doses pharmacologically increased intestinal Ca absorption, and most times prevented milk fever (Goff, 2008). Unfortunately, the dose of vitamin D that effectively prevents milk fever is very close to the dose causing irreversible metastatic calcification (Littledike and Horst, 1980). However, in a recent Slovenia study the use of high dose vitamin D parenteral supplementation (10 million IU of vitamin D_3) 8 to 2 d before calving proved to be very effective in preventing milk fever and other periparturient diseases in Slovenian cattle rearing conditions (Starič, 2010). Parenteral treatment with lower doses (500

000–1 million IU of vitamin D) can induce milk fever in some cows because high levels of 25-OH D and 1,25$(OH)_2$D result in treatment-suppressed PTH secretion and renal synthesis of endogenous 1,25$(OH)_2$D. These animals become hypocalcaemic once the exogenous source of vitamin D is cleared from the body (Littledike and Horst, 1980). By preventing hypocalcaemia, other diseases associated with hypocalcaemia are also prevented for example mastitis, metritis, abomasum displacement, ketosis, retained placenta and uterine prolapse (Erb *et al.*, 1985; Stevenson and Call, 1988; Starič, 2010; Lean *et al.*, 2014). Martinez *et al.* (2018a) found that feeding calcidiol in the transition period reduced the incidence of retained placenta, metritis and reduced the proportion of cows affected with multiple diseases in early lactation, which tended to improve fertility. They also found that cows fed calcidiol produced more colostrum with more IgG antibodies, which benefits the health of calves (Martinez *et al.*, 2018b).

Bone metabolism, which is strongly influenced by vitamin D, is also linked to energy metabolism in cows through osteocalcin (OC). OC is produced and deposited by osteoblast into bone matrix and is decarboxylated during osteoclast bone resorption to the active (uncarboxylated-uOC) form. uOC promotes β-cell proliferation, insulin secretion, insulin sensitivity and stimulates adiponectin secretion by adipose cells. Adiponectin increases bone deposition and glucose uptake. Insulin inhibits bone formation and promotes its resorption and thus increases the release of uOC. Bone is most intensely resorbed during the transition period to the peak of lactation. Adipocytes also act on bone metabolism through leptin (a satiety hormone), which indirectly inhibits osteoblast activity and thus OC production (Lean *et al.*, 2014). Heuer *et al.* (1999) found that cows with a body condition score of >4.5/5 had a higher incidence of milk fever. A link between 25(OH)D_3 and energy metabolism in cattle was demonstrated by Rodney *et al.* (2018) who showed that insulin growth factor 1 (IGF1) is influenced by vitamin D supplementation. Therefore, vitamin D supplementation undoubtedly improves cattle welfare.

Vitamin D and paratuberculosis

Paratuberculosis or Johne's disease, which is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) infection, is a chronic intestinal inflammation of ruminants and is manifested as diarrhoea and wasting that results in reduced milk production and premature culling of affected cattle. The severity of MAP infection is thought to be associated with vitamin D levels in blood (Sorge *et al.*, 2013). Cows are more likely to develop clinical Johne's disease shortly after calving and with increasing age which coincides with a decrease in vitamin D receptor concentration in the intestine around calving and with increasing age. In addition, Jersey cows, that are known to be more susceptible to paratuberculosis, have lower levels of intestinal vitamin D receptors than Holstein cows (Goff *et al.*, 1995). Although these observations are only circumstantial, they nonetheless point towards a potential association between vitamin D and paratuberculosis. Clinical signs of paratuberculosis are also more common in cows living in areas receiving less sunshine and therefore less vitamin D, in a similar manner to the north-south gradient of Crohn's disease incidence in humans (Loftus, 2004). In a mouse model vitamin D was beneficial in reducing clinical signs of Crohn's disease which is a human disease associated with MAP (Cantorna *et al.*, 2000). In a study performed by Sorge *et al.*

(2013) a statistical difference was observed in the levels of vitamin D between cows that were ELISA positive and negative for paratuberculosis. They proposed three possible explanations for their results. First, lower vitamin D levels could predispose the cow to a MAP infection. Second, in the later stages of Johne's disease less vitamin D is absorbed from the intestine. Third, more 25 (OH)D is used up to maintain adequate $1,25(OH)_2D$ levels to modulate the hyperactive immune response in the MAP infected gut, or a combination of all the above (Sorge *et al.*, 2013).

Assessment of vitamin D status in dairy cows

Vitamin D status of cattle is assessed by measuring 25OHD. 25OHD is the most abundant vitamin D metabolite in blood and is considered the best indicator of vitamin D status. There is substantial evidence that serum 25OHD is associated with clinical outcomes of diseases. Because of a long half-life of 2–3 weeks, serum levels vary little within short periods of time. Serum 25-OHD levels show a response to both sun exposure, as evidenced by the seasonal variation of levels, as well as to vitamin D supplementation. To measure 25OHD automated immunoassays, radioimmunoassay, binding-protein or chromatographic assays like LC-MS/MS and HPLC with UV can be used (Herrmann *et al.*, 2017). Nelson *et al.* (2016) suggest that a normal vitamin D content in blood of cows should be above 30 ng/mL.

Vitamin D supplementation in dairy cows

Since more and more cows are being raised indoors with no exposure to direct sunlight vitamin D deficiency is becoming a cause for concern. In a study performed at the Veterinary Faculty, University of Ljubljana, Slovenia, 12 high producing dairy cows of the Holstein Friesian breed at a commercial dairy farm housed in closed barns year-round were tested for vitamin D status for three successive months (October, November, December). All but one were vitamin D insufficient in all testings (mean all cows 22.26 ng/mL, range 17.2–23.9 ng/mL except in one cow where it was over 30 ng/mL in October and November, but just 18.9 ng/mL in December). The cows were not exposed to direct sunlight and the content of vitamin D_3 measured in total mixed ration was <400 IU/kg dry matter, which is too low, even though it was supplemented with mineral and vitamin mixture, calculated to meet NRC (2001) recommendation (30 IU/kg of body weight in feed).

This finding suggests that more attention should be paid to vitamin D supplementation in dairy cattle. Deficiency is even more likely to occur in subpolar or polar regions especially in the winter months. Vitamin D can be supplemented in two ways: feed additives and parenterally. Only cholecalciferol is administered parenterally to food producing animals (EMEA/MRL, 1998). There is currently only one registered vitamin D supplement in the EU for cattle and this is cholecalciferol, although calcidiol is a registered supplement elsewhere. Cholecalciferol can be mixed with cattle feed in a form of a powder with a maximal dose of 4000 IU/kg of complete ration with 12% moisture content. It can also be added to milk replacers for calves (OJ, 2017). Cholecalciferol can also be given by intramuscular and subcutaneous routes at the recommended dose of 500 to 2000 IU/kg body weight in cattle, sheep, horses, pigs, rabbits and chickens (EMEA/MRL, 1998). However, in dairy cows this practice can increase risk of milk fever if employed at the end of pregnancy

(Littledike and Horst, 1980). In a study performed by Jakobsen *et al.* (2015) a special UV light that emitted UV light with a wavelength between 250 and 400 nm was used for irradiating cows. They showed that 25-OHD levels could be raised in blood as well as in milk with UV light exposure. Of course, vitamin D synthesis could also be stimulated by exposing cow to natural sunlight.

Vitamin D toxicity

Vitamin D toxicity can be the result of over supplementation or exposure to calcinogenic plants and it presents with the calcification of soft tissues. However, over supplementation is rare. 25 $(OH)D_3$ was shown to have a large safety margin, exceeding 400 ng/mL in plasma (Celi *et al.*, 2018; Tomkins *et al.*, 2020). The NRC (2001) states that cows tolerated feed with 2200 IU D_3/kg of diet (recommended 30 IU/kg of body weight) for a longer period (60 d) and a dose of 25 000 IU D_3/kg of feed for short periods. The parenteral dose of 15 million IU D_3 32 d before parturition and a second injection of 5 million IU D_3 7 d later, were toxic in pregnant Jersey cows (Littledike and Horst, 1982). The most important calcinogenic plants are *Solanum malacoxylon*, *Cestrum diurnum*, *Trisetum flavescens* and *Nierembergia veitchii*. They contain $1,25-(OH)_2D_3$ glycosides or even an active form of the vitamin. Calcitriol glycosides are activated with microbial digestion in the rumen which cleaves the glycosides from the vitamin. Clinical symptoms of calcinosis are emaciation, extended lying, locomotion disorders, raised pulse and respiratory rate, impaired fertility as well as decreased vitality, altogether resulting in high economic losses. On postmortem examination extensive calcification of the endocardium, large vessels tissues, lungs, kidneys, tendons and ligaments are noticeable. However, some calcinogenic plants can also be used in the prevention of hypocalcaemia (Mello, 2003).

Conclusions

Vitamin D has many functions in the organism, from calcium homoeostasis to modulation of the immune system. It promotes optimal innate and adaptive immune function, which improves cows' defences against infection. Cow secrete a lot of Ca in their milk in early lactation, which leads to lactational osteoporosis. Therefore, it is important that they replenish their bone reserves in mid- and late-lactation that is aided by vitamin D supplementation. Poor skeletal health or the inability to replenish their Ca reserves leaves cows more vulnerable to subclinical hypocalcaemia, which results in higher susceptibility to infections and other associated diseases. These promote the use of antimicrobials and increase cull rates (McGrath *et al.*, 2018). Because of the complexity of vitamin D metabolism and the diversity of its metabolites we have still much to learn about its role in disease prevention and the regulation of many ongoing processes in cows. Thus, vitamin D supplementation is imperative to sustain welfare, health, longevity, intense milk production and to reduce the reliance on antimicrobials in closed barns with no direct sunlight exposure.

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