

Vitamin D and Hypocalcemia in the Dairy Cow

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Introduction

Vitamin D is a common nutrient in the diets of dairy cows that does not receive much attention because it is inexpensive, and it uses little space in the ration. Cows are efficient at cutaneous vitamin D₃ synthesis when outdoors during summer months (Hymoller and Jensen, 2010) and they acquire some vitamin D₂ from forages (Horst and Littledike, 1982) but for practical purposes (i.e., housing and seasonal variation) nearly all dairy cows in the U.S. receive supplemental vitamin D₃. The hormonal activity of vitamin D is required for normal Ca homeostasis and, accordingly, there have been many attempts to prevent or treat hypocalcemia using vitamin D metabolites. Dietary vitamin D₃ recommendations were recently updated for dairy cattle (NASEM, 2021) but the revisions have few practical implications for formulating diets. The 25-hydroxyvitamin D₃ [25(OH)D₃, calcidiol] metabolite, commercially marketed as Hy-D®, has recently been approved for use ruminant diets. Feeding 25(OH)D₃ during late gestation increases total tract Ca absorption and has positive effects for transition cow performance (Martinez et al., 2018, Poindexter et al., 2023a). Herein, vitamin D physiology and its role in prevention of hypocalcemia will be discussed along with updates for vitamin D nutrition and nutritional interventions that benefit vitamin D metabolism of transition dairy cows.

Vitamin D Physiology

Vitamin D is the collective term for a class of seco-steroid molecules originally discovered to have anti-rachitic activity. Vitamin D₃ and its metabolites are derived from 7-dehydrocholesterol in animals through a process of photoconversion in the skin (Holick et al., 1981). The process is efficient in cattle and the vitamin D₃ metabolites represent 90 to 95 % of vitamin D in cattle. Vitamin D₂ is derived from ergosterol of fungi and represents a small but appreciable fraction of vitamin D metabolites in cattle. Vitamin D₂ and vitamin D₃ must undergo subsequent enzymatic oxidation steps to become activated and exert most activity through intracellular vitamin D receptors. Although the rates and transport of vitamin D₂ and vitamin D₃ metabolism differ somewhat in cattle (Sommerfeldt et al., 1983, Hymøller and Jensen, 2017), they share the same enzymes and receptors for activity and the focus will be on vitamin D₃ from here on.

An overview of the vitamin D pathway and some outcomes of endocrine and intracrine vitamin D signaling are depicted in Figure 1. Oxidation of vitamin D₃ to 25(OH)D₃ is catalyzed by several hepatic 25-hydroxylases. Subsequent oxidation of 25(OH)D₃ to 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃, calcitriol], the active metabolite, is catalyzed by the 25-hydroxyvitamin D₃ 1 α -hydroxylase (Fraser and Kodicek, 1973). Finally, oxidation of 25(OH)D₃ and 1,25(OH)₂D₃ metabolites to 24,25-dihydroxyvitamin D₃

and 1,24,25-trihydroxyvitamin D₃, respectively, is catalyzed 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 (Sakaki et al., 2005).

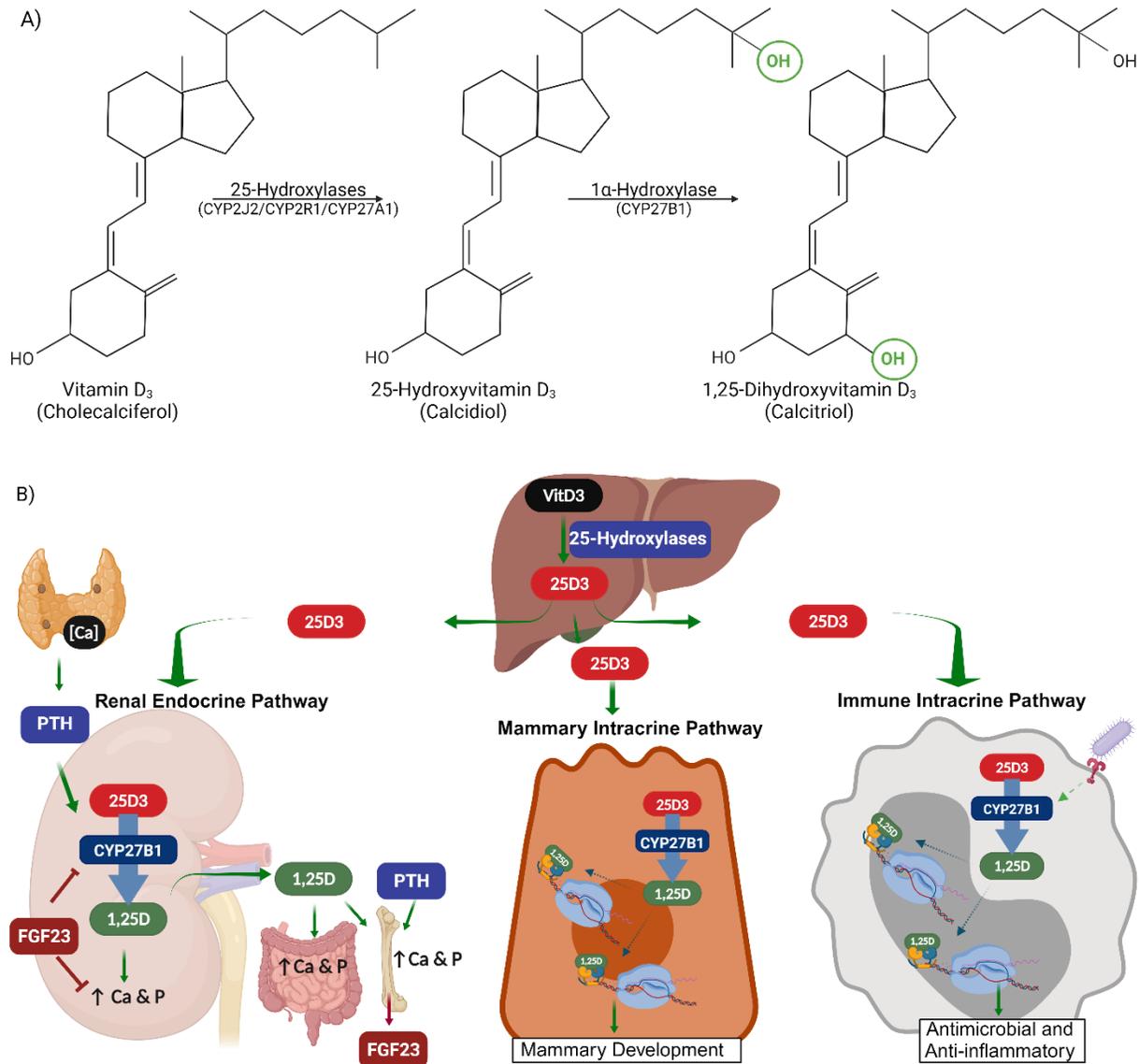


Figure 1. Oxidation of vitamin D₃ to 25-hydroxyvitamin D₃ and 1,25-dihydroxyvitamin D₃ (A) and examples of vitamin D pathways (B).

The main points of control in the vitamin D metabolic pathway center around regulating the concentration of 1,25(OH)₂D₃. The 1,25(OH)₂D₃ concentration of 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)₂D₃ does not correspond to vitamin D intake (Poindexter et al., 2023b). Most 1 α -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 $1,25(\text{OH})_2\text{D}_3$ 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.

In comparison to $1,25(\text{OH})_2\text{D}_3$, the $25(\text{OH})\text{D}_3$ metabolite is the major form of vitamin D circulating in blood and, with a half-life of approximately two weeks, its concentration in plasma serves as the best indicator of vitamin D status. Plasma $25(\text{OH})\text{D}_3$ concentrations from 40 to 100 ng/mL are typically observed in dairy cows fed standard rations (Nelson et al., 2016a). Similar concentrations are observed for beef cows on summer pasture (Nelson et al., 2016b). Some control of the $25(\text{OH})\text{D}_3$ concentration also exists, albeit to a lesser extent than control of $1,25(\text{OH})_2\text{D}_3$. Cutaneous vitamin D_3 synthesis is limited by feedback signals effectively limiting the concentration of $25(\text{OH})\text{D}_3$ in circulation (Holick et al., 1981). In cattle, the upper limit of serum $25(\text{OH})\text{D}_3$ derived from sun exposure seems to be near 100 ng/mL based on observed serum $25(\text{OH})\text{D}_3$ concentrations in beef cows (Nelson et al., 2016b). Some feedback inhibition also seems to exist in oxidation of vitamin D_3 to $25(\text{OH})\text{D}_3$ in dairy cows (Rodney et al., 2018, Poindexter et al., 2020, Poindexter et al., 2023b). For example, increasing the rate of dietary vitamin D_3 from 1 mg/d (40,000 IU/d) to 3 mg/d (120,000 IU/d) did not increase plasma $25(\text{OH})\text{D}_3$ concentrations even though it increased plasma vitamin D_3 concentrations (Figure 2). Consequently, there is little benefit, and potentially detrimental consequences, to overfeeding vitamin D_3 (Fraser, 2021).

Nearly all vitamin D metabolites circulate in blood bound to the vitamin D binding protein (DBP), an abundant member of the albumin family of proteins (Haddad et al., 1993). Significant genetic variation exists for the group-specific component (GC) globulin gene encoding the vitamin D binding protein and it, along with genetic variants of genes encoding 25-hydroxylases, is another key determinant in circulating concentrations of $25(\text{OH})\text{D}_3$ (Delanghe et al., 2015). In regard to hypocalcemia of dairy cows, polymorphisms linked to the GC gene in cattle are associated with risk of postpartum hypocalcemia (Cavani et al., 2022). The basis for the association between GC variants and hypocalcemia is not yet understood, but knowledge of the association allows for selection of animals with lower risk of hypocalcemia.

Biological activity of vitamin D is exerted primarily through intracellular vitamin D receptors (VDR). The VDR is a member of the nuclear hormone family of receptors, which have DNA-binding and ligand-binding domains and largely function as transcription factors (Pike et al., 2007). 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. Thousands of genes are under control of vitamin D response elements, but binding of the VDR to a given element is largely limited by cell-type, differentiation stage, and co-signaling molecules (Carlberg, 2014). Some well-known gene targets of the VDR include those involved in Ca binding and transport like the genes encoding intestinal transient

receptor potential family vanilloid subgroup 6 (TRPV6) and calbindin-D9k proteins. Additionally, there are the many extra-calcemic vitamin D target genes such as those related to anti-inflammatory and anti-microbial functions in immune cells and mammary gland development (Nelson et al., 2018).

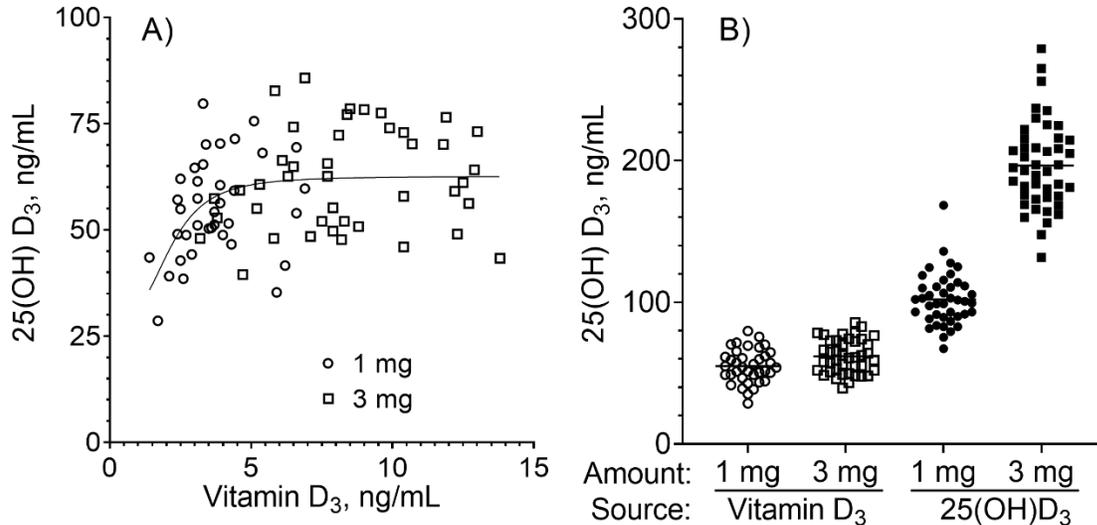


Figure 2. Holstein cows ($n = 173$) were fed either 1 or 3 mg/d of vitamin D₃ or 25(OH)D₃ for the last 4 weeks of gestation. A) Plot of serum 25(OH)D₃ vs. serum vitamin D₃ in cows fed 1 or 3 mg/d vitamin D₃. Effect of amount on serum vitamin D₃, $P < 0.01$; effect of amount on serum 25(OH)D₃, $P > 0.1$. B) Plot of serum 25(OH)D₃ concentrations for cows fed 1 or 3 mg/d of vitamin D₃ or 25(OH)D₃. Effect of source, $P < 0.01$; effect of amount, $P < 0.01$; Interaction between source and amount, $P < 0.01$.

Non-genomic actions of vitamin D also are reported and may have particular significance for gastrointestinal Ca absorption. Rapid influx of Ca in response to 1,25(OH)₂D₃ was observed in intestinal epithelial cells, and non-genomic responses were confirmed by knockdown of nuclear VDR in bone cells (Nemere et al., 2012). Membrane-associated VDR and membrane protein disulfide isomerase family A member 3 (PDIA3), which has several aliases [1,25(OH)₂D₃-MARRS, GRP58 and ERp57] are reported to be responsible for the non-genomic actions of 1,25(OH)₂D₃. The non-genomic vitamin D pathway involves activation of protein kinase A (PKA) and PKC intracellular pathways and results in rapid uptake of Ca and P. Despite the potential relevance of the non-genomic vitamin D pathway for postpartum Ca and P absorption, it has yet to be defined in cattle.

Vitamin D Endocrine Response to Hypocalcemia

The classical vitamin D endocrine system exists as part of the intricate endocrine system that controls blood and skeletal Ca and P economies (Figure 1). Case in point, 200 µg of 1,25(OH)₂D₃ within 6 h of parturition increased increase in plasma Ca by 0.3 mM and plasma P by 2 mM compared with control (Vieira-Neto et al., 2021b). The 1,25(OH)₂D₃ is one of several hormones that function in a concerted manner to maintain

blood Ca and P concentrations within a very narrow range and support skeletal development and homeostasis (Horst et al., 2005). Control of Ca is especially critical because of the various (nerve, muscle, etc.) signaling processes that involve the Ca^{2+} ion. Accordingly, small deviations in blood Ca from normal have severe, even fatal, consequences.

Calcium sensing receptors in the parathyroid glands serve as the primary thermostat for regulating the extracellular pool of Ca^{2+} . Decreased blood Ca^{2+} triggers the release of parathyroid hormone (PTH) as evident by the increased serum PTH concentration of postpartum dairy cows (Rodney et al., 2018). The PTH stimulates renal $1,25(\text{OH})_2\text{D}_3$ synthesis and, along with $1,25(\text{OH})_2\text{D}_3$, stimulates osteoclasts to release Ca and P from bone. The $1,25(\text{OH})_2\text{D}_3$, meanwhile, stimulates gastrointestinal Ca and P absorption and renal reabsorption. The calcitropic and phosphotropic activities of PTH and $1,25(\text{OH})_2\text{D}_3$ are counteracted by calcitonin and fibroblast growth factor 23 (FGF23). Calcitonin and FGF23 have not been studied much in cattle, so most of what we know is from other species. Calcitonin is secreted by C-cells of the thyroid gland in response to elevated blood Ca. Calcitonin suppresses intestinal Ca absorption acts in the kidneys to suppress Ca reabsorption. During normocalcemic conditions calcitonin promotes bone mineralization by stimulating renal $1,25(\text{OH})_2\text{D}_3$ synthesis and inhibiting bone resorption but during hypercalcemic conditions calcitonin inhibits 24-hydroxylase activity (Beckman et al., 1994). FGF23 is released from bone cells in response to elevated P and $1,25(\text{OH})_2\text{D}_3$ (Shimada et al., 2004). FGF23 suppresses renal phosphate transporters and $1,25(\text{OH})_2\text{D}_3$ synthesis, thereby counteracting elevated P. In addition to the classical Ca and P regulating hormones described above, serotonin and PTH-related protein (PTHrp) from the mammary gland also have a significant contribution in Ca and skeletal homeostasis (Weaver and Hernandez, 2016). It is important to keep in mind these hormones function as a delicate system among multiple organs to maintain Ca, P, and skeletal homeostasis.

The irreversible loss of Ca to milk at the onset of lactation require a tremendous shift in Ca and P economies that rely on appropriate hormone response for the cow to adapt. Failure of any aspects of the hormone system results in hypocalcemia and hypophosphatemia. The consequences of clinical hypocalcemia resulting from maladaptation to the onset of lactation are evident. Subclinical hypocalcemia also impairs immunity, smooth muscle function, and insulin signaling in cows. Delayed or chronic subclinical hypocalcemia specifically are associated with poor lactation and reproductive performance (Neves et al., 2018, Wilkens et al., 2020). Complicating the situation even more are the perturbations caused by inflammation. Inflammation associated with diseases like metritis result in temporal sequestration of Ca by tissues, decreased feed intake and impaired gastrointestinal function (Horst et al., 2021). Careful management of transition cows and transition cow diets are necessary for prevention of maladaptation to the demand of Ca at the onset of lactation.

Nutritional Strategies to Enhance Vitamin D Metabolism

The NASEM 2021 Nutrient Requirements for Dairy Cattle updated the recommendations for supplemental vitamin D₃. The recommended amounts of supplemental vitamin D₃ were increased slightly for dairy calves, heifers and cows. A summary of the updated recommendations is provided in Table 1. It should be noted that data from dose-response experiments were not available to develop the vitamin D recommendations; instead, the recommendations represent the committee's best estimate of adequate intake. The revised recommendations have little to no impact on formulating diets because most nutritionists have been formulating diets to include more than the recommended amount of vitamin D₃ for many years (Weiss, 1998). Most lactating cow diets provide cows 30,000 to 50,000 IU (0.75 to 1.25 mg) supplemental vitamin D₃ per day. Likewise, dry cow and closeup cow diets usually provide at least 20,000 to 30,000 IU supplemental vitamin D₃ per day.

The average serum 25(OH)D concentration of 700 samples collected from cows in multiple herds under various management and geographical locations in the U.S. was 68 ng/mL with 90% of those samples between approximately 40 and 100 ng/mL regardless of season, housing or geographical location (Nelson et al., 2016a). Under these practices, it is unlikely that postpartum hypocalcemia results from inadequate vitamin D. Nonetheless, uncertainty still exists for the optimal amount of vitamin D₃, particularly for transition cows, because there is a lack of experimental data to form solid recommendations. Supplementing high (> 50,000 IU/d, or 1.25 mg/d) amounts of vitamin D₃ is not beneficial, and potentially detrimental, because there is a limit in the uptake and conversion of vitamin D₃ to 25(OH)D₃ in cows (Figure 2). Whether an optimum amount between 20,000 to 50,000 IU (0.5 to 1.25 mg) of vitamin D₃ per day remains to be determined.

Technical note for units of measure: Vitamins were historically quantified based on biological activity (anti-rachitic activity in the case of vitamin D) and international units were defined to standardize activity. Unfortunately, international units are still widely used today even though the unit of measure results in much confusion and misuse. For example, vitamin D₂ is metabolized somewhat differently in cattle and although it contributes to overall vitamin D activity 1 mg vitamin D₂ does not have the same activity as 1 mg vitamin D₃ (Sommerfeldt et al., 1983). Therefore, units for vitamin D₃ in Table 1 are provided as micrograms and international units. Either mass or molar units should be used for comparison of vitamin D₃ with other sources of vitamin D.

Supplemental 25(OH)D₃ was recently approved for ruminant diets in the U.S. Also referred to as calcidiol and commercially marketed as Hy-D®, this vitamin D metabolite has been fed to poultry for many years because it is more readily absorbed compared with vitamin D₃. In addition to more efficient absorption, direct feeding of 25(OH)D₃ bypasses the initial hepatic oxidation (Figure 1) step making it much more effective at increasing serum 25(OH)D₃ compared with feeding vitamin D₃. For example, feeding 3 mg of 25(OH)D₃ to closeup cows increased serum 25(OH)D₃ from 60 ng/mL to 200 ng/mL, whereas feeding 3 mg vitamin D₃ did not cause serum 25(OH)D₃ to increase above 100

ng/mL (Figure 2; Poindexter et al., 2023b). Feeding 3 mg of 25(OH)D₃ per day for the last 3 to 4 weeks of gestation increased total tract digestibility of Ca, colostrum yield, and milk yield compared with feeding vitamin D₃ (Martinez et al., 2018, Silva et al., 2022, Poindexter et al., 2023a). Although feeding 25(OH)D₃ did not increase serum Ca at 0 or 1 DIM compared with vitamin D₃, it did increase average serum Ca from 2 to 11 DIM (Poindexter et al., 2023b). The increased serum Ca at 2 to 11 DIM from feeding 25(OH)D₃ was correlated with serum 25(OH)D₃ and milk yield of cows (Poindexter et al., 2023a).

Table 1. Vitamin D₃ recommendations for dairy and beef cattle¹.

Stage ²	µg/kg BW	µg/kg DM	IU/kg DM	IU/lb. DM	IU/d
Dry	0.75	40	1,600	726	22,500
Closeup	0.75	50	2,000	908	22,700
Fresh	1.0	45	1,800	817	28,000
Lactating	1.0	30	1,200	545	28,000
Calves	0.8	80	3,200	1,453	3,200
Heifers	0.75	50	2,000	908	12,000
Beef cows	0.165	6.9	275	125	5,000

¹ Based on NASEM Nutrient Requirements for Dairy Cattle (2021) and NRC Nutrient Requirements for Beef Cattle (2016).

² Estimated for typical lactating, dry, closeup or fresh Holstein cow, pre-weaned Holstein calf and growing Holstein heifers. 1,000 IU = 25 µg vitamin D₃.

The benefit of feeding 25(OH)D₃ for transition cow performance also may be attributed to the immunomodulatory role of vitamin D. Vitamin D signaling promotes anti-inflammatory, antimicrobial and antioxidant functions of immune cells (Nelson et al., 2018). Feeding 25(OH)D₃ compared with vitamin D₃ had wide-spread effects on expression of genes with cell-signaling, host-defense and leukocyte recruitment in immune cells of dairy cows (Vieira-Neto et al., 2021c). Importantly, feeding 25(OH)D₃ decreased severity of mastitis (Poindexter et al., 2020) and serum 25(OH)D₃ concentrations were associated with decreased risk for retained placenta and metritis (Wisnieski et al., 2020). Besides immunity, the VDR is expressed in mammary cells and vitamin D signaling is necessary for normal mammary development (Zinser and Welsh, 2004). Ongoing research should provide more clarity on the benefits of feeding 25(OH)D₃ in closeup diets, but for the time being it is a promising nutrient for improving transition cow performance.

Another recent development for prevention of hypocalcemia is use of low P diets or inclusion of aluminosilicate to bind Ca and P in closeup diets. The idea of low P diets originated many years ago (Barton et al., 1987), but it was only recently that it has been implemented. The concept underlying this approach is based on the function of FGF23. FGF23 release from bone in response to elevated P suppresses renal Pi reabsorption and 1,25(OH)₂D₃ synthesis (Shimada et al., 2004). Ultimately, FGF23 counteracts the

calcitropic and phosphotropic actions of PTH and $1,25(\text{OH})_2\text{D}_3$. Specific evidence in support of this concept is still lacking in cows but practically the concept is supported by evidence from experimental manipulation of dietary P. Feeding prepartum acidogenic diets with 0.21 or 0.31% dietary P increased serum Ca during the last week of gestation and first 12 h postpartum compared with feeding 0.44% dietary P (Peterson et al., 2005). Likewise, feeding 0.16% dietary P prepartum increased serum Ca on days 1, 2, and 4 postpartum compared with feeding 0.30% P (Wächter et al., 2022). Inclusion of aluminosilicate in prepartum diets effectively decreases dietary P availability and it also results in improved postpartum Ca status (Kerwin et al., 2019). Restriction of postpartum dietary P is detrimental to cows, but prepartum dietary P should be restricted to the extent that is economically and practically feasible.

The most effective and well-documented approach to prevent postpartum hypocalcemia is still the use of prepartum acidogenic diets. A meta-analysis of 42 experiments found that prepartum acidogenic diets increased postpartum DMI, milk and ECM yield, and reduced incidences of retained placenta and metritis compared with alkalogenic diets (Santos et al., 2019). Acidogenic diets are effective at increasing vitamin metabolism and Ca flux. For example, feeding a diet with DCAD of -181 mEq/kg DM increased plasma $1,25(\text{OH})_2\text{D}_3$ and Ca concentrations in response to PTH challenge compared with a diet with DCAD of 188 mEq/kg DM (Goff et al., 2014). Feeding a diet with DCAD of -153 mEq/kg DM for 7 d also increased total tract Ca absorption by 15 g/d (18 vs. 33 g/d; 24 vs. 39 % apparent digestibility) and urinary Ca excretion by 15 g/d (2 vs. 17 g/d) compared with DCAD of 236 mEq/kg DM (Vieira-Neto et al., 2021a).

Use of low K forages, laboratory analysis of forages, and consistent mixing and feeding of diets are key to the effectiveness of prepartum acidogenic diets. Some constraints, like availability of low K forages and number of cows in closeup groups, present a challenge for proper implementation of prepartum acidogenic diets. Regardless, no other approach to managing postpartum hypocalcemia has yet to demonstrate similar health and production benefits as acidogenic prepartum diets.

Conclusion

Vitamin D is part of an intricate endocrine system that regulates Ca, P and skeletal homeostasis. The inability of dairy cows to adapt to the rapid irreversible loss of Ca at the onset of lactation results in hypocalcemia. Consequences of hypocalcemia are further complicated by inflammation and poor digestive function. Supplemental $25(\text{OH})\text{D}_3$ provides a more effective alternative to vitamin D_3 for dairy cows. Adding $25(\text{OH})\text{D}_3$ to closeup diets does not reduce risk of immediate postpartum hypocalcemia but it does help restore Ca faster, thereby reducing the occurrence of delayed and chronic subclinical hypocalcemia. Feeding low P and acidogenic diets prepartum has a greater impact on postpartum Ca than vitamin D supplementation but vitamin D also contributes to many other aspects of physiology, notably immunity and mammary development.

References

- Barton, B. A., N. A. Jorgensen, and H. F. DeLuca. 1987. Impact of prepartum dietary phosphorus intake on calcium homeostasis at parturition. *J. Dairy Sci.* 70(6):1186-1191.
- Beckman, M. J., J. P. Goff, T. A. Reinhardt, D. C. Beitz, and R. L. Horst. 1994. In vivo regulation of rat intestinal 24-hydroxylase: potential new role of calcitonin. *Endocrinology* 135(5):1951-1955.
- Carlberg, C. 2014. Genome-wide (over)view on the actions of vitamin D. *Front. Physiol.* 5:167.
- Cavani, L., M. B. Poindexter, C. D. Nelson, J. E. P. Santos, and F. Peñagaricano. 2022. Gene mapping, gene-set analysis, and genomic prediction of postpartum blood calcium in Holstein cows. *J. Dairy Sci.* 105(1):525-534.
- Delanghe, J. R., R. Speeckaert, and M. M. Speeckaert. 2015. Behind the scenes of vitamin D binding protein: more than vitamin D binding. *Best Pract Res Clin Endocrinol Metab.* 29(5):773-786.
- Fraser, D. R. 2021. Vitamin D toxicity related to its physiological and unphysiological supply. *Trends Endocrinol. Metab.* 32(11):929-940.
- Fraser, D. R. and E. Kodicek. 1973. Regulation of 25-hydroxycholecalciferol-1-hydroxylase activity in kidney by parathyroid hormone. *Nat. New Biol.* 241(110):163-166.
- Goff, J. P., A. Liesegang, and R. L. Horst. 2014. Diet-induced pseudohypoparathyroidism: A hypocalcemia and milk fever risk factor. *J. Dairy Sci.* 97(3):1520-1528.
- Haddad, J. G., L. Y. Matsuoka, B. W. Hollis, Y. Z. Hu, and J. Wortsman. 1993. Human plasma transport of vitamin D after its endogenous synthesis. *J. Clin. Invest.* 91(6):2552-2555.
- Holick, M. F., J. A. MacLaughlin, and S. H. Doppelt. 1981. Regulation of cutaneous previtamin D3 photosynthesis in man: skin pigment is not an essential regulator. *Science* 211(4482):590-593.
- Horst, E. A., S. K. Kvidera, and L. H. Baumgard. 2021. Invited review: The influence of immune activation on transition cow health and performance-A critical evaluation of traditional dogmas. *J. Dairy Sci.* 104(8):8380-8410.
- Horst, R. L., J. P. Goff, and T. A. Reinhardt. 2005. Adapting to the transition between gestation and lactation: differences between rat, human and dairy cow. *J. Mammary Gland Biol. Neoplasia* 10(2):141-156.
- Horst, R. L. and E. T. Littledike. 1982. Comparison of plasma concentrations of vitamin D and its metabolites in young and aged domestic animals. *Comp. Biochem. Physiol. B.* 73(3):485-489.
- Hymoller, L. and S. K. Jensen. 2010. Vitamin D(3) synthesis in the entire skin surface of dairy cows despite hair coverage. *J. Dairy Sci.* 93(5):2025-2029.
- Hymøller, L. and S. K. Jensen. 2017. Plasma transport of ergocalciferol and cholecalciferol and their 25-hydroxylated metabolites in dairy cows. *Domest. Anim. Endocrinol.* 59:44-52.
- Kerwin, A. L., C. M. Ryan, B. M. Leno, M. Jakobsen, P. Theilgaard, D. M. Barbano, and T. R. Overton. 2019. Effects of feeding synthetic zeolite A during the prepartum

- period on serum mineral concentration, oxidant status, and performance of multiparous Holstein cows. *J. Dairy Sci.* 102(6):5191-5207.
- Martinez, N., R. M. Rodney, E. Block, L. L. Hernandez, C. D. Nelson, I. J. Lean, and J. E. P. Santos. 2018. Effects of prepartum dietary cation-anion difference and source of vitamin D in dairy cows: Lactation performance and energy metabolism. *J. Dairy Sci.* 101(3):2544-2562.
- NASEM. 2021. Nutrient Requirements of Dairy Cattle: Eighth Revised Edition. The National Academies Press, Washington, DC.
- Nelson, C. D., J. D. Lippolis, T. A. Reinhardt, R. E. Sacco, J. L. Powell, M. E. Drewnoski, M. O'Neil, D. C. Beitz, and W. P. Weiss. 2016a. Vitamin D status of dairy cattle: Outcomes of current practices in the dairy industry. *J. Dairy Sci.* 99(12):10150-10160.
- Nelson, C. D., K. E. Merriman, M. B. Poindexter, M. F. Kweh, and L. P. Blakely. 2018. Symposium review: Targeting antimicrobial defenses of the udder through an intrinsic cellular pathway. *J. Dairy Sci.* 101(3):2753-2761.
- Nelson, C. D., J. L. Powell, D. M. Price, M. J. Hersom, J. V. Yelich, M. E. Drewnoski, S. L. Bird, and G. A. Bridges. 2016b. Assessment of serum 25-hydroxyvitamin D concentrations of beef cows and calves across seasons and geographical locations. *J. Anim Sci.* 94(9):3958-3965.
- Nemere, I., N. Garbi, G. Hammerling, and K. J. Hintze. 2012. Role of the 1,25D3-MARRS receptor in the 1,25(OH)2D3-stimulated uptake of calcium and phosphate in intestinal cells. *Steroids* 77(10):897-902.
- Neves, R. C., B. M. Leno, K. D. Bach, and J. A. A. McArt. 2018. Epidemiology of subclinical hypocalcemia in early-lactation Holstein dairy cows: The temporal associations of plasma calcium concentration in the first 4 days in milk with disease and milk production. *J. Dairy Sci.* 101(10):9321-9331.
- Peterson, A. B., M. W. Orth, J. P. Goff, and D. K. Beede. 2005. Periparturient responses of multiparous Holstein cows fed different dietary phosphorus concentrations prepartum. *J. Dairy Sci.* 88(10):3582-3594.
- Pike, J. W., M. B. Meyer, M. Watanuki, S. Kim, L. A. Zella, J. A. Fretz, M. Yamazaki, and N. K. Shevde. 2007. Perspectives on mechanisms of gene regulation by 1,25-dihydroxyvitamin D3 and its receptor. *J. Steroid Biochem. Mol. Biol.* 103(3-5):389-395.
- Poindexter, M. B., M. F. Kweh, R. Zimpel, J. Zuniga, C. Lopera, M. G. Zenobi, Y. Jiang, M. Engstrom, P. Celi, J. E. P. Santos, and C. D. Nelson. 2020. Feeding 25-hydroxyvitamin D3 increases mineral concentrations and decreases severity of mastitis in lactating dairy cows. *J. Dairy Sci.* 103(1):805-822.
- Poindexter, M. B., R. Zimpel, A. Vieira-Neto, A. Husnain, A. C. M. Silva, A. Faccenda, A. Sanches de Avila, P. Celi, C. Cortinhas, J. E. P. Santos, and C. D. Nelson. 2023a. Effect of prepartum source and amount of vitamin D supplementation on lactation performance of dairy cows. *J. Dairy Sci.* 106(2):974-989.
- Poindexter, M. B., R. Zimpel, A. Vieira-Neto, A. Husnain, A. C. M. Silva, A. Faccenda, A. Sanches de Avila, P. Celi, C. Cortinhas, J. E. P. Santos, and C. D. Nelson. 2023b. Effect of source and amount of vitamin D on serum concentrations and retention of calcium, magnesium, and phosphorus in dairy cows. *J. Dairy Sci.* 106(2):954-973.

- Rodney, R. M., N. Martinez, E. Block, L. L. Hernandez, P. Celi, C. D. Nelson, J. E. P. Santos, and I. J. Lean. 2018. Effects of prepartum dietary cation-anion difference and source of vitamin D in dairy cows: Vitamin D, mineral, and bone metabolism. *J. Dairy Sci.* 101(3):2519-2543.
- Sakaki, T., N. Kagawa, K. Yamamoto, and K. Inouye. 2005. Metabolism of vitamin D₃ by cytochromes P450. *Front. Biosci.* 10:119-134.
- Santos, J. E. P., I. J. Lean, H. Golder, and E. Block. 2019. Meta-analysis of the effects of prepartum dietary cation-anion difference on performance and health of dairy cows. *J. Dairy Sci.* 102(3):2134-2154.
- Shimada, T., M. Kakitani, Y. Yamazaki, H. Hasegawa, Y. Takeuchi, T. Fujita, S. Fukumoto, K. Tomizuka, and T. Yamashita. 2004. Targeted ablation of Fgf23 demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism. *J. Clin. Invest.* 113(4):561-568.
- Silva, A. S., C. S. Cortinhas, T. S. Acedo, M. J. F. Morenz, F. C. F. Lopes, M. B. Arrigoni, M. H. Ferreira, T. L. Jaguaribe, L. D. Ferreira, V. N. Gouvêa, and L. G. R. Pereira. 2022. Effects of feeding 25-hydroxyvitamin D₃ with an acidogenic diet during the prepartum period in dairy cows: Mineral metabolism, energy balance, and lactation performance of Holstein dairy cows. *J. Dairy Sci.* 105:5786-5812.
- Sommerfeldt, J. L., J. L. Napoli, E. T. Littledike, D. C. Beitz, and R. L. Horst. 1983. Metabolism of orally administered [³H]ergocalciferol and [³H]cholecalciferol by dairy calves. *J. Nutr.* 113(12):2595-2600.
- Vieira-Neto, A., I. M. R. Leão, J. G. Prim, A. C. M. Silva, M. Nehme Marinho, R. Zimpel, S. Etheve, C. D. Nelson, and J. E. P. Santos. 2021a. Effect of duration of exposure to diets differing in dietary cation-anion difference on Ca metabolism after a parathyroid hormone challenge in dairy cows. *J. Dairy Sci.* 104(1):1018-1038.
- Vieira-Neto, A., G. Negro, R. Zimpel, M. Poindexter, F. Lopes, W. W. Thatcher, C. D. Nelson, and J. E. P. Santos. 2021b. Effects of injectable calcitriol on mineral metabolism and postpartum health and performance in dairy cows. *J. Dairy Sci.* 104(1):683-701.
- Vieira-Neto, A., M. B. Poindexter, M. Nehme Marinho, R. Zimpel, A. Husnain, A. C. M. Silva, J. G. Prim, C. D. Nelson, and J. E. P. Santos. 2021c. Effect of source and amount of vitamin D on function and mRNA expression in immune cells in dairy cows. *J. Dairy Sci.* 104(10):10796-10811.
- Weaver, S. R. and L. L. Hernandez. 2016. Autocrine-paracrine regulation of the mammary gland. *J. Dairy Sci.* 99(1):842-853.
- Weiss, W. P. 1998. Requirements of fat-soluble vitamins for dairy cows: a review. *J. Dairy Sci.* 81(9):2493-2501.
- Wilkens, M. R., C. D. Nelson, L. L. Hernandez, and J. A. A. McArt. 2020. Symposium review: Transition cow calcium homeostasis-Health effects of hypocalcemia and strategies for prevention. *J. Dairy Sci.* 103(3):2909-2927.
- Wisnieski, L., J. L. Brown, S. J. Holcombe, J. C. Gandy, and L. M. Sordillo. 2020. Serum vitamin D concentrations at dry-off and close-up predict increased postpartum urine ketone concentrations in dairy cattle. *J. Dairy Sci.* 103(2):1795-1806.
- Wächter, S., I. Cohrs, L. Golbeck, M. R. Wilkens, and W. Grünberg. 2022. Effects of restricted dietary phosphorus supply to dry cows on periparturient calcium status. *J. Dairy Sci.* 105(1):748-760.

Zinser, G. M. and J. Welsh. 2004. Accelerated mammary gland development during pregnancy and delayed postlactational involution in vitamin D3 receptor null mice. *Mol. Endocrinol.* 18(9):2208-2223.