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Research shows that cows with subclinical hypocalcemia had a 3.24-fold greater risk of developing metritis and an 11-fold increase in the risk of developing puerperal metritis within the first 12 days in milk compared with normocalcemic cows.

John Maday

SEEKING BALANCE

Calcium balance affects immune competency of transition dairy cows.

By Angela Rowson, DVM, and Erin Flood, DVM

Periparturient dairy cows are immunosuppressed, making them more susceptible to diseases such as mastitis, metritis and retained placental membranes (RP). This immunosuppression is broad in its scope, affecting functions of numerous immune-cell types involved in both innate and adaptive immunity.

The exact cause of periparturient immunosuppression is unknown, but it appears multifactorial with endocrine, nutritional and metabolic factors all contributing.

For example, increases in estrogen and cortisol which occur prior to parturition may negatively impact immune function. Nutritional deficiencies also have been correlated with depressed immunity and increased disease susceptibility. Specifically, negative energy and protein balance, as well as lower blood concentrations of vitamins A, D and E, have all been found to impair immune responses. Hypocalcemia, which is common in periparturient cows, also contributes significantly to immunosuppression.

HYPOCALCEMIA AND NEUTROPHIL FUNCTION

One immune-cell type negatively impacted by hypocalcemia is the neutrophil. Neutrophils are the first cells attracted to sites of inflammation, where they bind, phagocytose and kill microorganisms. After neutrophils emigrate from blood vessels, they crawl directly toward invading pathogens by following a chemical gradient, a process called chemotaxis. The neutrophils then injest the pathogens and destroy them with a variety of potent oxidants.

These oxidants are produced through a process called the oxidative (or respiratory) burst. During the oxidative burst, hydrogen peroxide is generated from two superoxide anions. It is then converted to hypochlorous acid by the enzyme myeloperoxidase (MPO). Hypochlorous acid, also the active ingredient in household bleach, kills bacteria by oxidizing their lipids and unfolding and aggregating their proteins. Chemotactic, phagocytic, MPO and oxidative burst activities are commonly assessed *in vitro* as indices of gen-

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eral killing capabilities of neutrophils and have been shown to be altered in hypocalcemic cows.

HYPOCALCEMIA AND UTERINE DISEASE

In a 2012 study by Martinez *et al.*, the association between subclinical hypocalcemia (SCH) and both neutrophil function and risk of uterine disease was assessed. The authors collected neutrophils from normocalcemic and SCH cows and analyzed their phagocytic and oxidative burst activities after challenging them with *Escherichia coli.* It was determined that cows with SCH had a significant decrease in the proportion of neutrophils undergoing phagocytosis and reduced number of bacteria phagocytosed per neutrophil compared with normocalcemic cows.

There was also a significant reduction in the percentage of neutrophils with oxidative burst activity in cows with SCH compared to normocalcemic cows. Furthermore, cows with SCH had a 3.24-fold greater risk of developing metritis and an 11-fold increase in the risk of developing puerperal metritis within the first 12 days in milk compared with normocalcemic cows. Subsequently, cows with SCH had a reduced rate of pregnancy and extended days open by 15 days. The authors concluded that SCH compromises neutrophil function and increases the risk of uterine disease.

The negative effects of hypocalcemia on neutrophil function are not unexpected, as calcium is critical for effective immune-cell activation and function. Specifically, calcium is required for the initiation of phagocytosis and helps control the fusion of secondary granules with phagosomal membranes during bactericidal activity. In 2006, Kimura *et al.* hypothesized that the increased demand for calcium in periparturient cows may negatively affect intracellular calcium stores within immune cells, which could blunt intracellular calcium release, thereby contributing to the immunosuppression observed in these animals.

The researchers measured calcium released from intracellular stores within peripheral blood mononuclear cells (PBMC), primarily lymphocytes and monocytes. Cells were collected from normocalcemic and clinically hypocalcemic cows. The authors determined that hypocalcemia is associated with decreased intracellular calcium stores in PBMC, and depletion of these stores likely starts several days prior to parturition. Additionally, the release of calcium from these intracellular stores declines as calcium demand for lactation intensifies and then recovers as plasma calcium normalizes. Therefore, it was concluded that the decrease in PBMC intracellular calcium stores before parturition and the development of hypocalcemia contribute to periparturient immunosuppression in dairy cows.

HYPOCALCEMIA AND OTHER PERIPARTURIENT DISEASES

Cows with hypocalcemia are at an increased risk for developing immune-related diseases other than just metritis. For example, it has been reported that cows with clinical hypocalcemia are much more likely to develop RP (odds ratio: 4.0) and mastitis (odds ratio: 5.4) within the first 30 days in milk (Curtis *et al.*, 1985).

Hypocalcemia contributes to the development of mastitis both through its negative effects on immune cells and on muscle contraction. Low blood calcium suppresses contraction of the teat sphincter muscle responsible for closure of the teat end orifice after milking, thereby allowing entry of pathogens into the mammary gland and further increasing the likelihood of mastitis. Additionally, hypocalcemia acts as a stressor to dairy cows and can result in significantly

elevated plasma cortisol concentrations. Cortisol compromises neutrophil chemotaxis and bactericidal activity, further exacerbating the immunosuppression cows experience during the periparturient period.

MINIMIZING PERIPARTURIENT HYPOCALCEMIA

Several different pre-partum feeding strategies may be utilized to prevent clinical and subclinical hypocalcemia. One of the most thoroughly researched and efficacious of these is feeding a negative dietary cation-anion difference (DCAD) diet prior to parturition. DCAD is defined as the difference in milliequivalents between cations (primarily potassium [K+] and sodium [Na+]) and anions (primarily chloride [Cl-] and sulfur [S-2]) in the diet.

Calculating the DCAD concentration of pre-fresh diets is important because the number of cation and anion charges absorbed from the gastrointestinal tract into the bloodstream determine blood pH. Metabolic alkalosis results when a diet that consists of more cations than anions (a positive DCAD diet) is fed. Recently, Goff *et al.*, (2014) suggested that metabolic alkalosis initiates a state of pseudohypoparathyroidism with a reduced response of calcium-regulating tissues to parathyroid hormone (PTH) by altering the structure of PTH receptors.

This altered structure reduces their affinity for PTH and prevents cows from successfully acclimating to the high calcium requirements of colostrum and milk synthesis that occurs around the time of parturition. Feeding a diet with more anions than cations (a negative DCAD diet) results in a compensated metabolic acidosis. This physiologic state restores tissue responsiveness to PTH and allows calcium homeostasis to continue normally (Goff *et al.*, 2014). This allows the cow to compensate for calcium losses, preventing or reducing clinical and subclinical hypocalcemia.

DCAD DIET GUIDELINES

Following are recommended guidelines for feeding a negative DCAD diet pre-partum:

- Feed a fully acidified, pre-fresh diet for at least three weeks prior to parturition. One strategy for effectively feeding anionic rations is to reduce the DCAD concentration to a point where urine pH values are maintained between 5.5 and 6.0. This recommendation is supported by data demonstrating that cows acidified to a mean urine pH of 5.69 more quickly replaced calcium into the blood compartment than cows acidified to a mean urine pH of 6.86 (Oba *et al.*, 2011). Oftentimes, the DCAD concentration used to reach this target urine pH range is -10 to -15 mEq/100 grams of dry matter (DM). Anions may be increased or decreased from this initial amount as needed to keep urine pH values within the recommended range. Negative DCAD diets are typically fed for 21 days prior to parturition. However, recent work by Wu *et al.* (2014) has demonstrated they can be fed for six weeks pre-partum without negatively affecting cow health or performance.

Phibro Animal Health

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- Ensure that proper dietary concentrations and amounts of macrominerals are included in the pre-fresh diet (Table 1).

Nutrient	% DM basis	Grams/day*
Calcium	1.52 – 1.60	180 – 190
Phosphorus	0.36 – 0.42	42 – 50
Magnesium	0.45 – 0.50	53 – 59
Sodium	0.10 – 0.20	12 – 24
Sulfur	0.40 – 0.47	47 – 55
Chloride	0.80 – 1.00	94 – 118
Potassium	< 1.30	

* grams/day based on a dry-matter intake of 26.0 lbs.

- Measure urine pH values of pre-fresh cows regularly. This is an inexpensive and quick way to gauge the optimal level of anion supplementation. Recommended urine pH range is between 5.5 and 6.0 for fully acidified diets. Measure urine pH on a weekly basis (ideally four to six hours after feed is delivered) and approximately two to three days after every significant ration change. Midstream urine should be collected from approximately 10 to 15 percent or at least eight cows in the close-up pen.
- Determine the incidence of SCH through blood calcium testing. SCH affects 25 percent of all first-lactation and approximately 50 percent of all second and greater lactation dairy cows (Reinhardt *et al.*, 2011). SCH is commonly diagnosed by collecting blood from cows within 48 hours of parturition and before any supplemental intravenous or oral calcium is administered. Traditionally, the threshold for total calcium concentration within the first 48 hours after parturition for SCH has been 8.0 mg/dL. However, recent research challenges this value and instead suggests a new target threshold greater than 8.5 mg/dL.
- Identify and correct management and environmental factors that might elicit stress and further exacerbate periparturient immunosuppression. Providing adequate bunk space in the pre-fresh group; minimizing pen moves and group changes near calving dates; ensuring optimal freestall size, cushion and cleanliness; mitigating heat stress; and avoiding over-conditioned cows will minimize the negative effects of stress during transition which may further impair dairy cow immune function.