

HYPOCALCEMIA: BIOLOGICAL EFFECTS AND STRATEGIES FOR PREVENTION

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Milk fever is a disorder affecting about 6% of dairy cows each year in the United States. Sub-clinical milk fever, defined as blood calcium concentration falling below 8 mg Ca /dl, occurs in up to 50% of older cows during the days immediately following calving (see Horst, et al.,2004 in these proceedings). The decline in blood calcium concentration around parturition represents a breakdown in the calcium homeostatic mechanisms of the body. Blood Ca in the adult cow is maintained around 8.5-10 mg / dl. There are 3 g Ca in the plasma pool and only 8-9 g Ca in all the extracellular fluids (outside of bone) of a 600 kg cow. The fluid within the canaliculi of bone may contain another 6-15 g Ca; the size of this Ca pool being dependent on the acid-base status of the animal; larger during acidosis and smaller during alkalosis. Dairy cows producing colostrum (containing 1.7-2.3 g Ca / kg) or milk (containing 1.1 g Ca / kg) withdraw 20 -30 g Ca from these pools each day in early lactation. In order to prevent blood Ca from decreasing, which has a variety of severe consequences to life processes beyond parturient paresis, the cow must replace Ca lost to milk by withdrawing Ca from bone or by increasing the absorption of dietary Ca. While this is potentially damaging to bones (lactational osteoporosis typically results in loss of 9-13% of skeletal Ca in dairy cows, which is reversible in later lactation), the main objective - to maintain normocalcemia - can be achieved. Bone Ca mobilization is regulated by parathyroid hormone (PTH) which is produced whenever there is a decline in blood Ca. Renal tubular reabsorption of Ca also is enhanced by PTH. However the total amount of Ca that can be recovered is usually relatively small. A second hormone, 1,25-dihydroxyvitamin D, is required to stimulate the intestine to efficiently absorb dietary Ca. This hormone is made within the kidney from vitamin D in response to an increase in blood PTH. Put simply, hypocalcemia and milk fever occur when cattle do not extract enough Ca from their bones and diet to replace the Ca lost to milk. Several nutritional factors are involved in the breakdown of Ca homeostasis that results in milk fever.

Metabolic Alkalosis causes disruption of Parathyroid Hormone Function

Metabolic alkalosis predisposes cows to milk fever and subclinical hypocalcemia¹. Metabolic alkalosis blunts the response of the cow to PTH²⁻⁴. In vitro studies suggest the conformation of the PTH receptor is altered during metabolic alkalosis rendering the tissues less sensitive to PTH^{5,6}. Lack of PTH responsiveness by bone tissue prevents effective utilization of bone canaliculi fluid Ca, sometimes referred to as osteocytic osteolysis, and prevents activation of osteoclastic bone resorption. Failure of the kidneys to respond to PTH reduces renal

reabsorption of Ca from the glomerular filtrate. More importantly, the kidneys fail to convert 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D. Therefore enhanced intestinal absorption of dietary Ca that normally would help restore blood Ca to normal, fails to be instituted. Metabolic alkalosis is largely the result of a diet that supplies more cations (K, sodium (Na), Ca, and Mg) than anions (chloride (Cl), sulfate (SO₄), and phosphate (PO₄)) to the blood. In simplest terms, a disparity in electrical charge occurs in animals fed these diets because a greater number of positively charged cations enter the blood than negatively charged anions. To restore electroneutrality to this positively charged blood, a positive charge in the form of a hydrogen ion (H⁺) is lost from the blood compartment and the pH of the blood is increased ⁷.

It must also be remembered that magnesium plays a role in calcium homeostasis. Hypomagnesemia affects Ca metabolism in two ways. 1. By reducing PTH secretion in response to hypocalcemia and 2. by reducing tissue sensitivity to PTH. PTH secretion is normally increased greatly in response to even slight decreases in blood Ca concentration. However hypomagnesemia can blunt this response ¹⁰. Hypomagnesemia is also capable of interfering with the ability of PTH to act on its target tissues. When PTH binds its receptor on bone or kidney tissues, it normally initiates activation of adenylate cyclase, resulting in production of the second messenger, cyclic AMP. In some tissues, PTH-receptor interactions should also cause activation of phospholipase C, resulting in production of the second messengers diacylglycerol and inositol 1,4,5-triphosphate. Both adenylate cyclase and phospholipase C have a Mg⁺⁺ binding site which must be occupied by a Mg ion for full activity ⁸. Field evidence suggests that blood Mg concentrations below 1.6 mg/dl in the periparturient cow will increase the susceptibility of cows to hypocalcemia and milk fever ⁹.

Strategy to prevent milk fever in confined cows:Reducing Diet Cation-Anion Difference

In theory all the cations and anions in a diet are capable of exerting an influence on the electrical charge of the blood. The major cations present in feeds and the charge they carry are Na (+1), K (+1), Ca (+2), and Mg (+2). The major anions and their charges found in feeds are Cl (-1), SO₄ (-2), and phosphate (assumed to be -3). Cations or anions present in the diet will only alter the electrical charge of the blood if they are absorbed into the blood.

The difference between the number of cation and anion particles absorbed from the diet determines the pH of the blood. The cation-anion difference of a diet is commonly described in terms of mEq/kg (some authors prefer to use “mEq/100 g” diet) of just Na, K, Cl, and SO₄ (traditionally calculated on S% reported when diet is analyzed by wet chemistry) as follows:

Dietary Cation-Anion Difference (DCAD) = (mEq Na⁺ + mEq K⁺) - (mEq Cl⁻ + mEq S⁻).

While DCAD equations provide a theoretical basis for dietary manipulation of acid-base status they are not necessary for formulation of mineral content of prepartum dairy cow rations because, with the exception of K and Cl, the rate of inclusion of the other macrominerals can be set at fixed rates.

For example, the NRC¹⁰ requirement for Na in the diet of a late gestation cow is about 0.12%. That is the amount that should be fed. At least two studies have clearly demonstrated that inclusion of Ca in the diet at NRC required levels or several fold above NRC required levels does not influence the degree of hypocalcemia experienced by the cow at calving ^{11,12}. It appears

from these studies that close-up diet Ca concentration should be maintained between 0.85 and 1.0% Ca.

To ensure adequate concentrations of Mg in the blood of the periparturient cow the dietary Mg concentration should be 0.35-0.4% to take advantage of passive absorption of Mg across the rumen wall.

Dietary P concentration should be fed at a level to meet the NRC requirement for P in the late gestation cow. This is generally about 0.4% P for most cows, though recent studies suggest this may overestimate the true requirement of the cow for dietary P¹³. A diet supplying more than 80 g P/day, or utilizing dietary P in the form of phosphoric acid as an anion to acidify the blood of the cow¹⁴, will block production of 1,25-dihydroxyvitamin D and cause milk fever. Dietary S must be kept above 0.22% (to ensure adequate substrate for rumen microbial amino acid synthesis) but below 0.4% (to avoid possible neurological problems associated with S toxicity).

Now, with the exception of K and Cl, the “variables” in the various proposed DCAD equations have become “fixed”. The key to milk fever prevention (at least with Holstein cows) is to keep K as close to the NRC requirement of the dry cow as possible (about 1.0% diet K). The key to reduction of subclinical hypocalcemia, not just milk fever, is to add Cl to the ration to counteract the effects of even low diet K on blood alkalinity. For formulation purposes the concentration of Cl required in the diet to acidify the cow is approximately 0.5% less than the concentration of K in the diet. In other words, if diet K can be reduced to 1.3% the Cl concentration of the diet should be increased to 0.8%. If dietary K can only be reduced to 2.0% the diet Cl would need to be roughly 1.5% to acidify the cow. This level of Cl in the diet is likely to cause a decrease in dry matter intake. Chloride sources differ in their palatability and since achieving low dietary K can be difficult it is prudent to use a palatable source of Cl when formulating the diet. Ammonium chloride (or ammonium sulfate) can be particularly unpalatable when included in rations with a high pH. At the higher pH of some rations the ammonium cation is converted to ammonia, which is highly irritating when smelled by the cow. Prilling the Cl (and SO₄) salts can reduce the unpleasant taste of the salts. In our experience hydrochloric acid has proved the most palatable source of anions. As with sulfuric acid, hydrochloric acid can be extremely dangerous to handle when it is procured as a liquid concentrate. Several companies now manufacture hydrochloric acid based anion supplements, which are safe to handle.

Urine pH of the cows provides a cheap and fairly accurate assessment of blood pH and can be a good gauge of the appropriate level of anion supplementation¹⁵. Urine pH on high cation diets is generally above 8.2. Limiting dietary cations will reduce urine pH only a small amount (down to 7.5-7.8). For optimal control of subclinical hypocalcemia the average pH of the urine of Holstein cows should be between 6.2 and 6.8, which essentially requires addition of anions to the ration. In Jersey cows the average urine pH of the close-up cows has to be reduced to between 5.8 and 6.3 for effective control of hypocalcemia. If the average urine pH is between 5.0 and 5.5, excessive anions have induced an uncompensated metabolic acidosis and the cows will suffer a decline in dry matter intake.

Strategy to Prevent Milk Fever in Pastured Cows: Ca Deficient Diet To Stimulate PTH Secretion

Those areas of the world where grazing is the most efficient means of producing milk also tend to be areas where soil potassium is high (which promotes grass growth) which means that metabolic alkalosis is going to be a factor that will not be easily overcome. There is no practical way to add enough anions to the diet of these cows to overcome the metabolic alkalosis created by the high potassium forages. A different strategy can be employed successfully in some areas.

When cows are fed a diet that supplies less Ca than they require, the cows are in negative Ca balance. This causes a minor decline in blood Ca concentration stimulating PTH secretion, which in turn stimulates osteoclastic bone resorption and renal production of 1,25-dihydroxyvitamin D. The key is that prolonged (several weeks) exposure to elevated PTH will overcome the PTH resistance in the tissues associated with metabolic alkalosis. This increases bone Ca efflux and the intestine is ready to absorb Ca efficiently once it becomes available in the lactating cow ration. At parturition the cow's osteoclasts are already active and in high numbers and the lactational drain of Ca is more easily replaced from bone Ca. If provided with Ca in the lactation ration, the previous stimulation of enterocytes by 1,25-dihydroxyvitamin D will allow efficient utilization of dietary Ca and the cow avoids hypocalcemia²⁷⁻²⁹.

The absorbable Ca requirement¹⁰ of the late gestation cow is from 14 g / day in Jerseys to about 22 g in large Holsteins. A truly low Ca diet capable of stimulating PTH secretion supplies considerably less absorbable Ca than required by the cow. A 600 kg cow consuming 13 kg DM, typical of confinement cow dry matter intakes, must be fed a diet that is less than .15% absorbable Ca if it is to provide less than 20 g available Ca/ day. Low Ca diets have proved more practical under grazing situations. The availability of Ca from forages is just 30% according to the 2001 NRC. In these cases the total dry matter intake of pasture was 6-7 kg DM/ day and the grasses being grazed were less than 0.4% Ca, which would provide < 28 g total Ca and somewhere around 9-10 g absorbable Ca / day. It is important to note that after calving the animal is switched to a high Ca diet. Grasses grown under tropical conditions may have lower calcium content than the same grasses grown in temperate conditions. For example, common grasses grown in Costa Rica under tropical conditions such as Kikuyu, African Star, and Brachiaria grasses are 0.37, 0.32, and 0.35% Ca respectively. Kikuyu grass grown under temperate conditions, such as New Zealand experiences, is typically 0.60 % Ca. This makes institution of a low calcium diet for prevention of milk fever more practical under tropical conditions.

Recently two methods have been developed to reduce the availability of dietary Ca for absorption. The first method involves incorporation of zeolite (a silicate particle) into the ration, which binds Ca and causes it to be passed out in the feces. At present the method is unwieldy because very large amounts of zeolite must be ingested each day (1 kg) and the effects of zeolite on P and trace mineral absorption are not clear¹⁶. By chemically modifying the zeolite it is theoretically possible to increase the affinity and the specificity of the zeolite for Ca, which may allow its practical use. The second method involves administration of vegetable oils which bind Ca to form an insoluble soap preventing absorption of diet Ca¹⁷. These have been successfully used in cattle fed reduced Ca diets containing 30-50 g / day. However they irreversibly bind enough dietary Ca to cause the reaction typically seen when diet Ca is <15 g absorbable Ca/day.

Conclusion

In animals fed large amounts of dry matter pre-calving in a confinement setting the best option for controlling hypocalcemia appears to be to reduce the offending cause = metabolic alkalosis. This is done thru better agronomy to control potassium intake, and by adding anions to the diet to acidify the cow's blood. In animals at pasture prevention of metabolic alkalosis is extremely difficult. In these settings the best approach may be to trick the parathyroid gland into early secretion of PTH to stimulate bone and kidneys prior to calving. The key is to reduce the available Ca in the diet to a level below the requirement of the cow for several weeks before calving. Tropical growing conditions aid this process and new technologies that bind dietary Ca may also be available in the future to make this approach more successful in temperate climates.

1. Craige AH, Stoll IV. Milk fever (parturient paresis) as a manifestation of alkalosis. *Am J Vet Res.* 1947;8:168.
2. Gaynor PJ, Mueller FJ, Miller JK, Ramsey N, Goff JP, Horst RL. Parturient hypocalcemia in jersey cows fed alfalfa haylage-based diets with different cation to anion ratios. *J Dairy Sci.* Oct 1989;72(10):2525-2531.
3. Phillippo M, Reid GW, Nevison IM. Parturient hypocalcaemia in dairy cows: effects of dietary acidity on plasma minerals and calciotropic hormones. *Res Vet Sci.* May 1994;56(3):303-309.
4. Goff JP, Horst RL, Mueller FJ, Miller JK, Kiess GA, Dowlen HH. Addition of chloride to a prepartal diet high in cations increases 1,25-dihydroxyvitamin D response to hypocalcemia preventing milk fever. *J Dairy Sci.* Nov 1991;74(11):3863-3871.
5. Bushinsky DA. Metabolic alkalosis decreases bone calcium efflux by suppressing osteoclasts and stimulating osteoblasts. *Am J Physiol.* Jul 1996;271(1 Pt 2):F216-222.
6. Martin KJ, Freitag JJ, Bellorin-Font E, Conrades MB, Klahr S, Slatopolsky E. The effect of acute acidosis on the uptake of parathyroid hormone and the production of adenosine 3',5'-monophosphate by isolated perfused bone. *Endocrinology.* May 1980;106(5):1607-1611.
7. Stewart PA. Modern quantitative acid-base chemistry. *Can J Physiol Pharmacol.* Dec 1983;61(12):1444-1461.
8. Rude RK. Magnesium deficiency: a cause of heterogeneous disease in humans. *J Bone Miner Res.* Apr 1998;13(4):749-758.
9. van de Braak AE, van't Klooster AT, Malestein A. Influence of a deficient supply of magnesium during the dry period on the rate of calcium mobilisation by dairy cows at parturition. *Res Vet Sci.* Jan 1987;42(1):101-108.

10. National RC. Nutrient Requirements of Dairy Cattle. Washington, D.C.: National Academy Press; 2000.
11. Goff JP, Horst RL. Effects of the addition of potassium or sodium, but not calcium, to prepartum ratios on milk fever in dairy cows. *J Dairy Sci.* Jan 1997;80(1):176-186.
12. Beede DK, Pilbean TE, Puffenbarger SM, Tempelman RJ. Peripartum responses of Holstein cows and heifers fed graded concentrations of calcium (calcium carbonate) and anion (chloride) three weeks before calving. *J Dairy Sci.* 2001;84(Suppl 1):83.
13. Peterson AB, Beede DK. Periparturient responses of multiparous Holstein cows to varying prepartum dietary phosphorus. *J Dairy Sci.* 2002;85(Suppl 1):187.
14. Crill RL, Sanchez WK, Guy MA, Griffel LA. Should phosphorus be included in the dietary cation-anion difference expression? *J Dairy Sci.* 1996;85(Suppl 1):187.
15. Jardon P. Using urine pH to monitor anionic salt programs. *Compend Contin Educ Pract Vet.* 1995;17:860.
16. Thilsing-Hansen T, Jorgensen RJ, Enemark JM, Larsen T. The effect of zeolite A supplementation in the dry period on periparturient calcium, phosphorus, and magnesium homeostasis. *J Dairy Sci.* Jul 2002;85(7):1855-1862.
17. Wilson GF. Development of a novel concept (Calcigard) for activation of calcium absorption capacity and prevention of milk fever. *Acta Vet Scand Suppl.* 2003;97:77-82.