Transition Cow Immune Function and Interaction with Metabolic Diseases

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Abstract

Nearly all dairy cows experience some degree of immune suppression during the 2 to 3 weeks before and after calving. Typically, this is in the area of a 25 to 40% decline in both neutrophil function (innate immunity) and lymphocyte function (acquired immunity). Metabolic diseases, such as milk fever and ketosis, exacerbate immune suppression at this time; often resulting in closer to 60 to 80% loss of immune function. Several other so called metabolic disorders, such as retained placenta and metritis, may actually be manifestations of a poorly functioning immune system. Maintaining feed intake in the days before and after calving appears to be critical to early lactation immune competence. Preventing milk fever and ketosis can help prevent major loss of immune function and therefore reduce incidence of diseases, such as retained placenta, that occur secondary to a poorly functioning immune system. The immune system of the periparturient cow is also sensitive to inadequate dietary vitamin E, selenium, and trace minerals, such as zinc and copper.

Introduction

Most of the metabolic diseases of dairy cows - milk fever, ketosis, retained placenta, and displacement of the abomasum - occur within the first 2 weeks of lactation. And, the etiology of many of those metabolic diseases that are not clinically apparent during the first 2 weeks of lactation, such as laminitis, can be traced back to insults that occurred in early lactation. In addition to metabolic disease, the overwhelming majority of infectious disease, in particular mastitis, becomes clinically apparent during the first 2 weeks of lactation. Three basic physiologic functions must be maintained during the periparturient period if disease is to be avoided. These are: 1) A strong immune system; 2) Normocalcemia; and 3) Maintaining feed intake during the days before and after calving. Both metabolic disease and infectious disease incidence are greatly increased whenever one or more of these physiological functions is impaired. Each of these 3 physiological factors influence the risk for development of the most common diseases encountered during the periparturient period. And a cow failing in any one of these 3 areas is also more likely to suffer a failure in another vital function. Because other speakers at this conference will address management issues affecting maintenance of feed intake in periparturient cows (cow comfort, grouping, social factors, high straw vs. higher starch, monensin, number of days in the close-up group, etc.), these factors will not be discussed in this review.

Does Milk Production Affect Immune Status?

Neutrophil and lymphocyte function is diminished in the periparturient period, especially in the dairy cow (Kehrli et al., 1989 ab; Detilleux et al., 1994). The onset of milk production imposes

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tremendous challenges to the mechanisms responsible for energy, protein, and mineral homeostasis in the cow. Negative energy, protein, and/or mineral balance and hormonal fluxes associated with the onset of lactation may be responsible for the immunosuppression observed in periparturient dairy cattle. Mastectomy of pregnant dairy cows removes the impact of milk production while maintaining endocrine and other changes associated with late pregnancy and parturition. Mastectomy would be expected to improve immune function in the periparturient dairy cow, if milk production is an immunosuppressive factor. Using 10 mastectomized and 8 intact multiparous Jersey cows (all intact cows developed milk fever) we assessed the ability of neutrophils to kill microbes as assessed by neutrophil myeroperoxidase activity during the periparturient period (Kimura et. al., 1999). Neutrophil myeroperoxidase activity decreased equally before parturition in both groups. While there was a quick recovery of neutrophil myeloperoxidase activity in mastectomized cows, there was no recovery in intact cows after parturition throughout the study, which lasted until day 20 postpartum. Lymphocyte production of gamma-interferon in vitro declined significantly at parturition for intact cows but did not decrease significantly in the mastectomized cows. For intact cows, all T cell subset populations (i.e. CD3, CD4, CD8, and gamma-delta positive cells; CD = cluster of differentiation) decreased as a percentage of total peripheral blood mononuclear cells **(PBMC)** at the time of parturition, while the percentage of monocytes increased. These population changes have previously been shown to be associated with the immune suppression commonly observed in periparturient cows. Mastectomy eliminated these changes in leukocyte subsets (Kimura et al., 2002a).

These results suggest: 1) the mammary gland may produce substances which directly affect immune cell populations, or 2) metabolic demands associated with the onset of lactation negatively impact the composition and functioning of circulating PBMC populations. Two metabolic factors were greatly impacted by mastectomy. Mastectomy eliminated hypocalcemia at parturition. Plasma non-esterified fatty acid **(NEFA)** concentration rose dramatically for intact cows at calving and did not return to baseline level for > 10 days. In contrast, NEFA concentration in mastectomized cows plasma rose only slightly at calving and returned to baseline level the day after calving. It is clear that the intact cow mobilizes a larger amount of body fat than the mastectomized cow, suggestive of a severe negative energy balance at the onset of lactation.

Hypocalcemia and Mastitis Susceptibility

Milk fever cows are at increased risk of developing mastitis (Curtis et al., 1983). Why? Calcium is necessary for proper contraction of muscle. Severe hypocalcemia prevents skeletal muscle contraction to the point that the clinical syndrome known as milk fever occurs. Muscle contraction is reduced by any decrease in blood calcium concentration. However, it must be severe before we observe the "downer cow". Daniel et al. (1983) demonstrated that contraction rate and strength of the smooth muscle of the intestinal tract is directly proportional to blood calcium concentration. Hypocalcemia reduces abomasal contraction which causes the abomasum to fill with gas and become displaced. Of course, lack of effective fiber is the other contributor to displacement of the abomasum. A major reason for the current trend toward high straw close-up diets is the ability of these types of diets to prevent displacement of the abomasum.

It should be kept in mind that the sphincter at the end of the teat is also a muscle. The teat sphincter smooth muscle must contract if closure of the teat end is to occur. If low blood calcium reduces teat sphincter contraction, the teat canal may remain open inviting environmental pathogens to enter the mammary gland. While we associate milk fever with

the day of calving, we have demonstrated that many cows remain subclinically hypocalcemic for the first week of lactation (Goff et al., 1996). Hypocalcemic cows tend to spend more time lying down than do normocalcemic animals, which could increase teat end exposure to environmental opportunists.

Hypocalcemia also acts as a stressor to the cow. Cows typically exhibit a 3 to 4 fold increase in plasma cortisol as part of the act of initiation of parturition. However, subclinically hypocalcemic cows may have 5 to 7 fold increases in plasma cortisol on the day of calving and the typical milk fever cow may exhibit plasma cortisol concentrations that are 10 to 15 fold higher than pre-calving plasma cortisol concentration (Horst and Jorgensen, 1982). Cortisol is generally considered a powerful immune suppressive agent and likely exacerbates the immune suppression normally observed in the periparturient period. Exacerbates rather than causes because most studies suggest that immune suppression begins 1 to 2 weeks before calving (Kashiwazaki et al., 1985; Ishikawa, 1987; Kehrli et al., 1989ab), and the cortisol surge is fairly tightly confined to the day of calving and perhaps the day after calving. Dystocia, twin calvings, overcrowded conditions, and movement into new pens just before calving are other factors that contribute to excessive cortisol levels at calving.

Recently, we have found a direct association between hypocalcemia and failure of immune cells to become activated when they encounter a stimulus such as bacteria (Kimura et al., 2006). When an immune cell, such as a lymphocyte, encounters a bacterial antigen at its surface, it triggers the release of calcium from organelles within the cell. This begins the process by which the lymphocyte will produce antibodies, bactericidal peptides, etc., to kill the bacteria. A rise in intracellular calcium is a key early feature in immune cell activation. Peripheral blood mononuclear cells were obtained from multiparous dairy cows spanning a period of 2 weeks before

and 2 weeks after parturition. The cells were exposed to activating stimuli in vitro, and intracellular calcium release from intracellular stores was measured. The intracellular calcium released in response to the activation signal declined as calcium demand for lactation became more intense and recovered as plasma calcium normalized. Hypocalcemia, which is common in periparturient dairy cows, was causing decreased intracellular calcium stores in peripheral mononuclear cells. This caused a blunted intracellular calcium release response to an immune cell activation signal and helps explain how hypocalcemia contributes to periparturient immune suppression.

Ketosis and Mastitis Susceptibility

Ketosis is diagnosed whenever there are elevated levels of ketones in the blood, urine, or milk of a cow. The disease is always characterized by a decline in blood glucose as well. In lactation, the amount of energy required for maintenance of body tissues and milk production exceeds the amount of energy the cow can obtain from her diet, especially in early lactation when dry matter intake is still low.

The fresh cow is also in negative protein balance shortly after calving. Generally, this is not perceived to be as big a problem as the negative energy balance of early lactation, but the typical cow will lose 37 lb of body protein during the first 2 weeks of lactation. Much of this body protein is being used to support the amino acid and glucose requirements of milk production (Paquay et al., 1972). Therefore, in many respects, the dairy cow in early lactation is in a physiological state comparable to that of humans and rodents with prolonged protein-calorie restriction. Glutamine is the most abundant free amino acid in human muscle and plasma, and it is utilized at high rates by rapidly dividing cells, including leucocytes, to provide energy and optimal conditions for nucleotide biosynthesis.

A few studies have been done on lymphocytes isolated directly from normal cows and cows with clinical and subclinical ketosis and then placed into culture to see how they would respond to stimuli. Leukocytes of cows with clinical symptoms and the highest concentration of ketones and free fatty acids in their blood responded with the lowest levels of interferons alpha and gamma to 3 interferon inducers: Newcastle Disease Virus, phytohemagglutinin, and concanavalin A (Kandefer-Szerszen et al., 1992).

Zerbe et al. (2000) examined the relationship between liver triacyl glycerol **(TAG)** content and functional properties of neutrophils of dairy cows in the peripartum period. Increased liver TAG content, > 40 mg/g, which was considered the upper level of normal, went in parallel with a reduced expression of function-associated surface molecules on blood neutrophils. Moreover, in cows with high liver TAG concentrations, the antibody-independent and -dependent cellular cytotoxicity of blood polymorphonuclear neutrophils **(PMN)** was markedly reduced. The PMN also were less capable of reactive oxygen species generation after stimulation with phorbol myristate acetate. Bottom line – the cow with ketosis has almost no resistance to infectious disease.

What is the Energy Cost to Mount an Immune Response?

Little to no work has been done to examine this issue in cattle. However, in humans suffering from severe infection causing sepsis (various degrees of fever, increased white blood cell **(WBC)** count, and acute phase protein production), the resting energy expenditure (determined by indirect calorimetry) increased progressively over the first week of the infection to around 40% above normal and was still elevated 3 weeks from the onset of illness. As an aside, over a 3-week period, patients lost 13% of their total body protein (Plank and Hill, 2000).

No such measurements have been reported for cattle. However, if we are allowed to extrapolate and speculate, we can go through a few calculations. Maintenance energy for a 600 kg (1320 lb) dairy cow is approximately 9.7 Mcal/day of net energy. If the cow must also increase energy expenditure 40% to mount an inflammatory response as in humans, the energy requirement increases by nearly 4 Mcal/day. This is roughly equivalent to a requirement that the cow consume an additional 2.4 kg (5.3 lb) of diet (assuming a lactating diet that provided 1.65 Mcal NE_L/kg; 0.75 Mcal NE_L lb). Can the periparturient cow, already in negative energy balance, be expected to successfully mount a rapid immune response? If she is in fact in negative protein balance as well, will her immune system produce the immunoglobulins and acute phase proteins necessary to fight an infection while it is still in the acute phase to prevent it from escalating to a clinical infection?

Immune Function and Retained Placenta, Metritis, and Endometritis

There has been some association between the development of retained placenta **(RP)** and the incidence of mastitis (Emanuelson et al., 1993; Peeler et. al., 1994). We have recently confirmed studies begun by Gunnink (1984) that suggest the two disorders are likely linked because both are due to immune suppression in affected cows. Gunnink's theory suggested that the fetal placenta must be recognized as "foreign" tissue and rejected by the immune system after parturition to cause expulsion of the placenta. We hypothesized that impaired neutrophil function causes RP. We examined the ability of neutrophils to recognize fetal cotyledon tissue as assessed by a chemotaxis assay which utilized a placental homogenate obtained from a spontaneously expelled placenta as the chemoattractant. Neutrophil killing ability was also estimated by determining myeloperoxidase activity in isolated neutrophils. Blood samples were obtained from 142 periparturient dairy cattle in 2

herds. Twenty cattle developed RP (14.1%). Neutrophils isolated from blood of cows with RP had significantly lower neutrophil function in both assays prior to calving and this impaired function lasted for 1 to 2 weeks after parturition. Addition of antibody directed against interleukin-8 **(IL-8)** to the cotyledon preparation used as a chemoattractant inhibited chemotaxis by 41%, suggesting one of the chemoattractants present in the cotyledon at parturition is IL-8. At calving, plasma IL-8 concentration was lower in RP cows (51 ± 12 pg/ml) than in cows expelling the placenta normally (134 ± 11 pg/ml) (Kimura et al., 2002b). These data suggest neutrophil function determines whether or not the cow will develop RP. These data also suggest that depressed production of IL-8 may be a factor affecting neutrophil function in cows developing RP. This suppressed immune system could also explain why the same cows are more susceptible to mastitis. Retained placenta probably does not cause mastitis, but it is symptomatic of a depressed immune system.

At calving, the cow's reproductive tract is exposed to bacteria, even in the cleanest of environments. The cow survives because her WBC provides protection from infection. Neutrophils provide the first line of defense, moving out of the blood whenever and wherever bacteria invade body tissue. Once in the infected tissue, the neutrophils ingest the bacteria and release enzymes and free radical compounds onto the bacteria to kill them. Occasionally, the neutrophils don't succeed in killing the intruder. The immune system then calls on macrophages and lymphocytes, which work together to produce antibodies and other anti-bacterial factors. Production of these factors takes a little more time but will eventually eliminate most infections that the neutrophils can't handle.

In immune compromised cows, the bacteria are not kept in check and grow to large numbers in the uterus, causing a condition known as metritis. Around 20 to 30 % of cows will develop metritis, which is characterized by a foul-smelling, red-brown, watery discharge from the uterus within 10 to 14 days after calving. It is often, but not always, accompanied by a fever. Hammon et al. (2006) demonstrated that neutrophils of cows with metritis are significantly less able to kill bacteria (measured by a neutrophil iodination assay) than neutrophils from cows without metritis. The surprise was that poor neutrophil function was evident in these cows the day of calving – before lactation began and before any bacteria could have entered the uterus.

Endometritis is a uterine problem characterized by inflammation of the lining of the uterus, lasting more than 3 to 4 weeks after calving. Studies suggest 40 to 50 % of cows can have endometritis at 4 weeks after calving. These cows are less likely to be successfully bred back. An interesting study by Kim et al. (2005) compared the peripartum immune responses of dairy cows that developed endometritis by 4 weeks postpartum (n = 11) to cows that did not develop this disease (n = 19). Blood samples were collected 1 week before calving, just after or during calving, and then at weeks 1, 2, 3, and 4 postpartum. The leukocytes from cows that developed endometritis were significantly less phagocytic than those from control cows at all sampling time points (P < 0.01). In the study of Hammon et al. (2006), cows with endometritis and subclinical endometritis had poorer neutrophil function than cows with a healthy uterus - from the time they calved until the diagnosis of endometritis was made 4 weeks later. Cows in this study developing metritis, or diagnosed with subclinical or clinical endometritis, had higher NEFA concentrations in their blood than did cows with a healthy uterus. Surprisingly, the NEFA concentrations were significantly higher for at least 2 weeks prior to calving, suggesting these cows were mobilizing body fat even before calving. Individual cow feed intake data revealed those cows that were going to develop uterine health problems ate significantly less feed than cows that would maintain a healthy uterus, and this difference in feed intake existed at least 1 week prior to calving. Urton

et al. (2005) were able to demonstrate that feeding behavior prior to calving was a major risk factor for metritis. In this study, for every 10 min/day reduction in time spent eating in the weeks prior to calving, the risk of developing metritis doubled. Though uterine diseases are diagnosed in early lactation, it now appears that the health of the uterus is greatly influenced by feed intake and avoidance of negative energy at the end of the dry period.

Micronutrients and the Immune System

The biggest culprit affecting the immune system is the hormone changes associated with calving and the negative energy and protein balances of cows in early lactation. The energy and protein imbalances are not easily fixed. Deficiencies of any of the micronutrients will result in immune function loss as well. To that end, the following guidelines are utilized by me to prevent the short term deficiencies in vitamins and minerals that are sometimes seen in dairy cattle.

Unless you are using a pasture-based system, feed 4000 IU/day of vitamin E for the 2 to 3 weeks before calving and the 2000 IU/day of vitamin E during the first 2 weeks of lactation. This recommendation is based on the work of Weiss et al. (1997) and our own observations on plasma vitamin E concentrations at calving (Goff and Stabel, 1990). In the study by Weiss et al. (1997), the addition of vitamin E greatly decreased the incidence of mastitis. If these data are only half correct, the vitamin E is a cheap investment.

Feed 0.3 mg/kg of selenium, the legal limit, and add trace minerals in adequate amounts. Farms should get in the routine habit of assessing the adequacy of their trace mineral program by harvesting liver from a cow that has died rapidly due to trauma, lightning , etc., or is going to slaughter, once or twice each year and having the liver sample analyzed for trace mineral content. Liver is the best indicator of the status of copper, zinc, and

manganese supplementation. Blood is generally unreliable for these minerals. Whole blood selenium is a good indicator of selenium deficiency. In some cases, inorganic trace minerals do not seem capable of meeting the mineral needs of animals. This is often due to the presence of substances in the diet that interfere with absorption of the inorganic trace minerals. Organic sources of trace minerals are less susceptible to antagonists and should be considered when lab tests suggest deficiency in the face of good levels of inorganic minerals. Another strategy is to insure adequate trace mineral status by routine use of the more expensive organic trace minerals, in place of expensive lab tests. Iron can be antagonistic to trace mineral absorption. It is also a strong pro-oxidant; this increases the need for vitamin E. Dietary iron greater than 800 mg/kg (364 mg/lb) of diet and water iron greater than 0.7 to 2 mg/kg is too much. We think this causes oxidative stress in animals and reduces immune cell function.

Maintaining Normal Blood Calcium Concentration

Inadequate blood calcium **(Ca)** concentrations can cause a cow to lose the ability to rise to her feet as Ca is necessary for nerve and muscle function. The result is the metabolic disease known as milk fever, though it is more properly termed periparturient hypocalcemia or periparturient paresis, as an elevated body temperature is not typically observed. Surveys in the USA suggest around 5% of cows will develop milk fever each year and the incidence of subclinical hypocalcemia – blood Ca values between 8 and 5.5 mg/dl during the perparturient period- is around 50% in older cows. Milk fever and subclinical milk fever should be considered gateway diseases that greatly reduce the chance for full productivity in the ensuing lactation. Hypocalcemia reduces rumen and abomasal motility, increasing the risk of abomasal displacement. Hypocalcemia reduces feed intake so that greater body fat mobilization occurs in early lactation. Hypocalcemia reduces all muscle

contraction, including the teat sphincter muscle responsible for closure of the teat orifice after milking, thus increasing the risk of mastitis. And as described earlier, hypocalcemia directly impairs immune cell response to an activating stimulus.

Ca Homeostasis and Monitoring for Hypocalcemia

Blood Ca in the adult cow is maintained between 8.5 to 10 mg/dl (2.1 to 2.5 mmol/L). Typically, the nadir in blood Ca concentration occurs between 12 and 24 hours after calving and blood samples obtained around this time can reveal the extent of hypocalcemia experienced by a dairy herd. Nearly 25% of heifers will have blood Ca concentration below 8 mg/dl. About 50% of older cows will fall into this category.

In order to prevent blood Ca from decreasing at the onset of lactation, the cow must replace extracellular Ca lost to milk. She does this by withdrawing Ca from bone and by increasing the efficiency of absorption of dietary Ca. The dairy cow (as are most mammals) is programmed to go into a state of lactational osteoporosis, mobilizing bone Ca to help her achieve normocalcemia in early lactation. This will typically result in loss of 9 to13% of her skeletal Ca in the first month of lactation (which is reversible in later lactation). Though it might stress her bones, the main objective to maintain normocalcemia can be achieved. Bone Ca mobilization is regulated by PTH which is produced whenever there is a decline in blood Ca. Renal tubular reabsorption of Ca is also enhanced by PTH. However, the total amount of Ca that can be recovered by reducing urinary Ca excretion is relatively small as only small amounts of calcium are typically lost to urine each day. A second hormone, 1,25-dihydroxyvitamin D, is required to stimulate the intestine to efficiently absorb dietary Ca. This hormone is made from vitamin D by the kidney –but only 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.

Factors Impairing Calcium Homeostasis at the Cellular Level

Metabolic Alkalosis

Metabolic alkalosis predisposes cows to milk fever and subclinical hypocalcemia (Craige and Stoll, 1947). Metabolic alkalosis blunts the response of the cow to PTH (Gaynor et al., 1989; Goff et al., 1991; Phillippo et al., 1994). We now believe that the conformation of the PTH receptor is altered during metabolic alkalosis, rendering the tissues less sensitive to PTH. 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 also 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 **(SO$_4$)**, and phosphate **(PO$_4$)**] to the blood. In simplest terms, a disparity in electrical charge in body fluids 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 high cation, positively charged blood, a positive charge in the form of a hydrogen ion (H$^+$) must be lost from the blood compartment. A reduction in H$^+$ concentration is equivalent to an increase in the pH of the blood (Stewart, 1983). Adding readily absorbable anions to the diet increases the total

negative charges in the blood, allowing more H^+ to exist and the blood pH decreases – it is more acidic.

Hypomagnesemia

Cow plasma Mg concentration is normally between 1.8 and 2.4 mg/dl (0.75 and 1.0 mmol/L). Hypomagnesemia affects Ca metabolism in two ways: 1) by reducing PTH secretion in response to hypocalcemia (Littledike et al., 1983) and 2) by reducing tissue sensitivity to PTH (Rude, 1998). The integrity of the interaction between PTH and its receptor is vital to Ca homeostasis. Hypomagnesemia, independent of metabolic alkalosis, can also interfere with the ability of PTH to act on its target tissues. Field evidence suggests that blood Mg concentrations below 0.65 mmol/L in the periparturient cow will increase the susceptibility of cows to hypocalcemia and milk fever (van de Braak et al., 1987).

Maintenance of normal plasma Mg concentration is nearly totally dependent on a constant influx of Mg from the diet. The Mg is well absorbed from the small intestine of young calves and lambs. As the rumen and reticulum develop, these sites become the main, and perhaps the only, sites for net Mg absorption (Martens and Rayssiguier, 1980). The Mg absorption from the rumen is dependent on the concentration of Mg in solution in the rumen fluid and the integrity of the Mg transport mechanism (Martens and Gabel, 1986). Active transport of Mg across the rumen wall is necessary when dietary Mg is not in great supply. Unfortunately, high K concentration in the rumen fluid depolarizes the apical membrane of the rumen epithelium, reducing the electromotive potential needed to drive Mg across the rumen wall (Martens and Schweigel, 2000). Thus, a ration that might otherwise be adequate in Mg results in a Mg deficient state when dietary K is excessive.

A second pathway for absorption of Mg exists that is not affected by K. Unfortunately, this passive transport process only operates at high rumen fluid Mg concentrations, which allows Mg to flow down a concentration gradient into the extracellular fluids of the cow (Martens and Schweigel, 2000). The concentration of Mg in rumen fluid needed to utilize concentration gradient driven absorption of Mg is greater than 4 mmol/ L (Care et al., 1984; Ram et al., 1998). The minimum level of Mg required in the diet to prevent negative Mg balance in the face of high K levels in ruminants is approximately 0.35% (Ram et al., 1998). Thus, Mg content of the close-up dry cow ration and the early lactation ration should be between 0.35 and 0.4% as insurance against the possibility that the active transport processes for Mg absorption are impaired. Be aware that when using magnesium oxide as the source of Mg, there is a good possibility that the Mg is not soluble enough to provide the Mg needed. There is a wide range in availability of Mg from magnesium oxide. Sampling the blood of several cows within 12 hours after calving provides an effective index of Mg status of periparturient cows. If serum Mg concentration is not at least 1.8 mg/dl (0.8 mmol/L), it suggests inadequate dietary Mg absorption and that hypomagnesemia may be limiting productivity as well as contributing to hypocalcemia in the herd.

Reducing Dietary Cation-Anion Difference to Prevent Hypocalcemia

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), SO_4 (-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 general acid-base balance of the body and therefore the pH of the blood. The cation-

anion difference of a diet is commonly described in terms of mEq/kg DM (some authors prefer to use "mEq/100 g" dietary DM) of just Na, K, Cl, and SO$_4$, although it must be kept in mind that Ca, Mg, and P absorbed from the diet will also influence blood pH. Experimental evidence from our laboratory (Goff et al., 2004) and the meta-analysis of Charbonneau et al. (2006) support the concept that dietary sulfate **(S)** is only about 60% as effective as chloride as an acidifying agent in the diet of the prepartum cow, suggesting an appropriate dietary cation-anion difference (DCAD) equation could be (Na + K) – (Cl + 0.6 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 in this author's opinion because, with the exception of K and Cl, the rate of inclusion of the other macrominerals can be set at fixed rates as outlined below.

The USA National Research Council (NRC, 2001) requirement for Na in the diet of a late gestation cow is about 0.12%. A small amount of salt is added to the diet to prevent pica, which often is manifested as a desire to drink urine from the floor. Unlimited access to NaCl is to be avoided in late gestation because it will increase the risk of udder edema but not because it greatly affects acid-base status.

At least 2 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 (Goff and Horst, 1997; Beede et al., 2001). It appears from these studies that close-up dietary Ca concentration should be maintained between 0.85 and 1.0%.

To ensure adequate concentrations of Mg in the blood of the periparturient cow, the dietary Mg concentration should be 0.35 to 0.4%. This higher dietary Mg concentration allows the cow to take advantage of passive absorption of Mg across the rumen wall. Be sure the Mg source you are using is available – many magnesium oxide sources are not!

Dietary P concentration should be fed at a level to meet the NRC requirement for P in the late gestation cow. This is 0.35 to 0.4% P for dry cows. A diet supplying more than 80 g/day of P (Barton, 1978; Kichura et al., 1982) will block renal production of 1,25-dihydroxyvitamin D and will actually cause milk fever.

Dietary S must be kept above 0.22% to ensure adequate S for rumen microbial amino acid synthesis. Corn silage diets are notoriously low in S. Dietary S should be kept below 0.4% to avoid possible neurological problems associated with S toxicity (Gould et al., 1991).

Now, with the exception of K and Cl, the "variables" in the various proposed DCAD equations have become more or less "fixed". The key to milk fever prevention (at least with Holstein cows) is to now keep K as close to the NRC requirement of the dry cow as possible (about 1.0% dietary 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 dietary K on blood alkalinity. For formulation purposes, the concentration of Cl required in the diet to acidify the cow is approximately 0.5 percentage unit less than the concentration of K in the diet. In other words, if dietary K can be reduced to 1.3%, the Cl concentration of the diet should be increased to 0.8%. This is a conservative approach – most farms will find they end up closer to: %K - 0.3% = % Cl needed after assessing urine pH. However, if you start at %K – 0.3% = % Cl, you will overacidify some herds (about 20% of herds). If dietary K can only be reduced to 2.0%, the dietary 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 DM 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 higher pH, a portion of the ammonium cation is converted to ammonia, which is highly irritating when inhaled by the cow. Prilling the Cl (and SO_4) salts reduces the unpleasant taste of the salts. In our experience, hydrochloric acid has proved the most palatable source of anions. Hydrochloric acid can be extremely dangerous to handle when it is procured as a liquid concentrate. Several US companies now manufacture hydrochloric acid based anion supplements, which are safe to handle. Some also include Mg in a highly soluble form to reduce the possibility that hypomagnesemia is present in the herd and contributing to hypocalcemia.

These are simply guidelines for anion supplementation used by this author and are based on inclusion of Ca, Na, S, Mg, and P at the levels outlined above. 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 supplementation (Jardon, 1995). Urine pH on high cation diets is generally above 8.2. Limiting dietary cations will reduce urine pH only a small amount (down to ~7.8). 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 DM intake. Urine pH can be checked 48 or more hours after a ration change. Urine samples should be free of feces and made on midstream collections to avoid alkalinity from vaginal secretions. Anion supplemented diets are generally fed for the last 3 weeks before calving, though the length of time these diets need to be fed to induce a compensated metabolic acidosis is no more than 4 to 5 days.

How do I interpret urine pH? Let's say you check urine pH of 10 close-up cows. You are interested in the average – there is always one odd ball cow. Don't adjust the diet to accommodate the one cow!!

- Scenario 1 - average pH = 6.3 ± 0.6
  - Congratulations, you have induced a compensated metabolic acidosis.

- Scenario 2 - average pH= 7.4 ± 0.5
  - Add anion source in 0.25 lb/cow increments, wait 3 days and check pH.

- Scenario 3 - average pH 5.2 ± 0.5
  - You have induced an uncompensated metabolic acidosis = sick cows! Reduce anion source by 0.5 lb/cow and check urine pH in 3 days.

- Scenario 4 - 4 cows at pH 5.2 and 6 cows at pH 7.8
  - Cows at pH 5.2 today ate well this morning but will not this afternoon due to uncompensated metabolic acidosis. The cows at pH 7.8 went off feed yesterday from over-acidification but will likely eat today and be over-acidifed tomorrow. Reduce anion source by 0.5 lb/cow, wait 4 to 5 days, check pH, and start increasing anion source by 0.25 lb increments as needed.

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