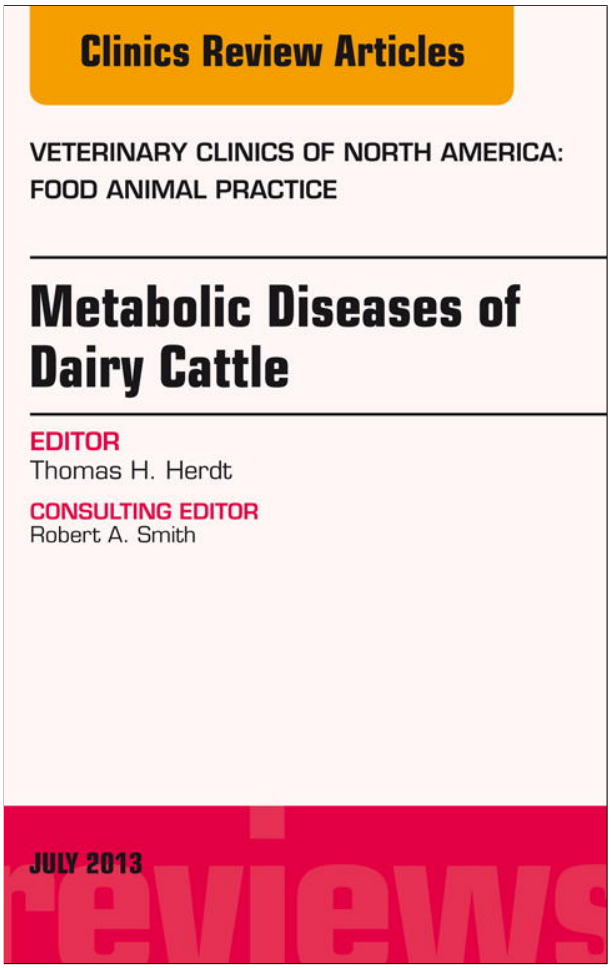

Mineral and Antioxidant Management of Transition Dairy Cows

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KEYWORDS

• Transition • Calcium • Skeleton • Antioxidant • Vitamins

KEY POINTS

- Effective transition management requires an integrated approach to nutritional and environmental management to provide cows with freedom from rumen disruption, mineral deficiencies, immunosuppression, disorders of lipid metabolism, and other forms of stress (eg, toxic feeds, social disruption).
- The skeleton is an important regulator of energy and protein metabolism.
- Although calcium is pivotal in the pathogenesis of milk fever, the most significant factor influencing risk of milk fever is the magnesium content of the diet.
- Vitamin and mineral status of cattle should not be considered in isolation from other antioxidants or from the level of oxidative challenge. Adequacy is a function of these interactions, not just a single vitamin or mineral, and increased concentrations of 1 of these may also not be better.

INTRODUCTION

Although controlling disorders of macromineral metabolism, and in particular milk fever, forms a small part of the overall management of the transition cow, it is often the focus at a producer level. As a result, it is critical to ensure that any transition cow program is effective in controlling macromineral disorders. Further, recent developments in understanding of the role of calcium in metabolism and bone as an integrator of metabolism reinforce the need to ensure that there is careful attention to calcium metabolism. The concept of milk fever and hypocalcemia being central to the interactions of other diseases has been well understood since the pivotal studies of Curtis and colleagues.[1] Recent understandings of the role of bone in integrated

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Vet Clin Food Anim 29 (2013) 367–386
http://dx.doi.org/10.1016/j.cvfa.2013.03.004 vetfood.theclinics.com
0749-0720/13/$ – see front matter

metabolism (reviewed later) provide a basis on which to understand the gateway role of milk fever in other disorders and reproduction.

The pathophysiology of hypocalcemia and dietary manipulations to control the risk of milk fever have been extensively reviewed,[2–5] and insights from these reviews are incorporated in this article. Further, the understanding that transition management needs to be fully integrated to be effective[6] is discussed in the context of review of a study that integrated these priniciples.[7–9] Micronutrient needs are addressed in the context of vulnerability of cattle to oxidative stress and inflammatory disorders. This article concludes with a series of practical approaches to improving transition diets.

Approach to transition management: correcting 1 area of challenge is not enough. Effective solutions are derived from ensuring freedom from rumen disruption, mineral deficiencies, immunosuppression, and disorders of lipid metabolism and that further other forms of stress (eg, toxic feeds, social disruption) are reduced and cows are comfortable.

Milk Fever Control

The following recommendations for the dietary control of hypocalcemia are based on 4 meta-analyses examining factors influencing the risk of milk fever.[10–13] These meta-analyses showed that the risk of milk fever can be predicted from dietary levels of calcium, magnesium, phosphorus, dietary cation-anion difference (DCAD) (as calculated by $[Na^+ + K^+] - [Cl^- + S^{2-}]$), breed of cattle, and duration of exposure to the diet. To effectively prevent these disorders, careful attention is needed to concentrations of calcium, magnesium, and phosphorus as well as the DCAD of the prepartum diet (**Table 1**).

What is meta-analysis?: a form of study design that uses previous studies to provide a pooled estimate of effect of an observation or intervention. Well-conducted meta-analyses are the gold standard for assessing these effects and provide more precise estimates of the effect of interventions. Ideally, these studies are based on randomized controlled studies.

DCAD

The DCAD theory of milk fever prevention has its basis in the strong ion model of acid: base balance,[14] modified in the 1980s[15] and simplified in the late 1990s.[16] Some contention still exists regarding the most appropriate equation for predicting DCAD. Charbonneau and colleagues[13] preferred the equation $(Na^+ + K^+) - (Cl^- + 0.6\ S^{2-})$ on the basis that it was the best equation at predicting blood pH, whereas DeGaris and Lean[3] preferred the equation $(Na^+ + K^+) - (Cl^- + S^{2-})$, because the equations were equivalent for predicting the risk of milk fever. Given that the equations are equivalent for predicting milk fever, we recommend use of the latter and more simple equation.

The simplified strong ion model[17] to predict plasma pH is:

$$pH = pK'_1 + \log \frac{[SID^+] - K_a[A_{TOT}]/(K_a + 10^{-pH})}{S * p_{co2}}$$

where pK'_1 is the ion product of water, K_a is the effective equilibrium disassociation constant for plasma nonvolatile weak acids, $[SID^+]$ is the strong ion difference,

Table 1
Logistic meta-regression analysis of the dietary components and variables that predict the incidence of milk fever (random effects model)

Predictor Variable	Coefficient	Standard Error	*P* Value	Odds Ratio	95% Confidence Interval
Constant	−5.76	1.028	0.001	0.003	0.001–0.024
Breed 1[a]	0.86	0.382	0.024	2.374	1.122–5.023
Breed 2[b]	1.49	0.824	0.071	4.424	0.880–22.235
Ca[c]	5.48	1.729	0.013	239.362	8.082–7089.244
Mg[c]	−5.05	1.618	0.002	0.006	0.001–0.152
P[c]	1.85	0.716	0.010	6.376	1.566–25.958
DCAD 1[d]	0.02	0.007	0.040	1.015	1.001–1.030
Ca * Ca	−2.03	0.819	0.013	0.131	0.026–0.654
Exposure[e]	0.03	0.014	0.030	1.030	1.003–1.058
Trial	−0.01	0.001	0.369	—	—
Variance (σ)	1.33	0.357	—	—	—

[a] Breed 1, Jerseys (Holstein Friesian used as the reference breed).
[b] Breed 2, Norwegian Red and White (Holstein Friesian used as the reference breed).
[c] Ca, Mg, and P expressed as % of DM.
[d] DCAD 1 = (Na + K) − (Cl + S) in mEq/100 g DM.
[e] Exposure is the mean time in days that the cows in a study were exposed to the precalving transition diet.

From Lean IJ, DeGaris PJ, McNeil DM, et al. Hypocalcemia in dairy cows: meta analysis and dietary cation anion difference theory revisited. J Dairy Sci 2006;89:673.

$[A_{TOT}]$ is the plasma nonvolatile weak acid concentration, S is the solubility of CO_2 in plasma, and p_{co2} is the partial pressure of CO_2 in plasma. The implication of this equation is that the major variable factor that can be readily influenced is the strong ion difference and prevention of milk fever involves, in part, the appropriate application of DCAD theory to reduce the strong ion difference ($[SID^+]$) by lowering plasma pH and producing strong ion metabolic acidosis. This goal can be achieved by feeding salts of the strong cations ($CaCl_2$, $CaSO_4$, $MgCl_2$, $MgSO_4$, NH_4Cl, and $(NH_4)_2SO_4$) or acids of the anions (HCl and H_2SO_4). The strong cations Ca^{2+}, Mg^{2+}, and NH_4^+ are absorbed to a lesser extent from the gastrointestinal tract (GIT) than are the strong anions Cl^- and SO_4^{2-}. The differential absorption results in a relative excess of absorbed anions compared with absorbed cations lowering the $[SID^+]$ and subsequently plasma pH. Because Na^+ and K^+ are absorbed with near 100% efficiency in the intestine, NaCl and KCl have a net effect of zero on the $[SID^+]$.

Inducing a mild metabolic acidosis in the prepartum cow reduces milk fever risk through changes in calcium metabolism. Numerous effects of decreasing or increasing the DCAD of precalving diets have been reported. Among the effects reported are:

- Metabolic acidosis in goats[18,19] and cattle[20]
- Decreased renal sensitivity to parathyroid hormone (PTH) in cows fed a strongly positive DCAD precalving diet[20,21]
- Enhanced renal production of 1,25(OH) vitamin D_3 in response to a low-DCAD precalving diet[20,21]
- Increased responsiveness of target tissues to 1,25(OH) vitamin D_3 associated with increased calcium absorption from the intestinal tract[22]

- Increased resorption of calcium from bone stores[23–25]
- Calciuria[20,26–28]
- Increased plasma ionized calcium concentrations[26,28]

Critically, the overall effect is to increase calcium turnover through increased GIT absorption and increased sensitivity of target tissues to homeostatic signals, rather than an improvement in overall calcium balance. The meta-analyses of milk fever risk factors[10–13] have identified that the effect of DCAD on the risk of milk fever is linear and independent of the important effects of dietary Ca, Mg, and P concentrations. Consequently, any reduction in the DCAD decreases the risk of milk fever. This linear relationship should not be confused with the curvilinear relationship between DCAD and urine pH, with DCAD having little impact on urine pH until it reaches approximately 20 mEq/100 g dry matter (DM) (**Fig. 1**). This curvilinear relationship is caused by renal buffering systems that maintain an alkaline urinary pH until overwhelmed. Although recommendations exist for target urine pH to ensure adequate acidification, these assess only effectiveness of DCAD management of the diet and not the risk of milk fever.

Urinary pH: monitors efficacy of the DCAD; does not monitor milk fever risk. Good transition diets prevent milk fever; urinary acidification is only part of this, and urinary pH is not a good predictor of milk fever risk. We recommend sampling and testing feeds for mineral concentrations and assessing quality in preference to (but not exclusive of) testing urine.

Our recommendations for the balancing of the macromineral component of transition diets are listed in a series of recommendations at the end of this article.

Calcium

The optimum concentration of dietary Ca intake for the control of milk fever is also contentious, with Lean and colleagues[6] and Thilsing-Hansen and colleagues[5] suggesting that the precalving intake of calcium be limited to 60 on a negative DCAD diet and 20 g per day, respectively. McNeill and colleagues[2] also concluded that excessive calcium intake was an important risk factor for milk fever, but less so than potassium. However, Goff[4] concluded that calcium concentration in precalving diets had little influence on the incidence of milk fever when fed at levels higher

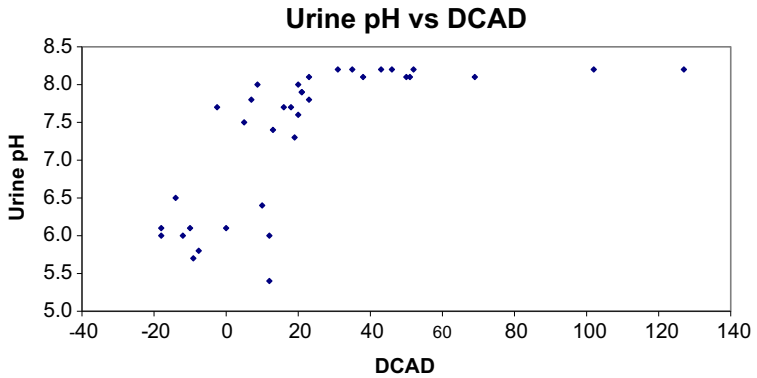

Fig. 1. Curvilinear relationship between urine pH and DCAD [$(Na^+ + K^+) - (Cl^- + S^{2-})$]. (*From* DeGaris PJ, Moss N, Lean IJ, et al. The transition period–preventing milk fever and more. In: Proceedings of the Australian Cattle Vets 2005 - Gold Coast AVA Conference. Gold Coast: Australian Association of Cattle Veterinarians. 2005. p. 66.)

than the daily requirements of the cow (approximately 30 g/d). Oetzel[29] recommended a daily intake in the precalving diet of 150 g/d, a calcium concentration of between 1.1% and 1.5% of DM, in conjunction with a dietary DCAD of approximately –15 mEq/100 g DM. However, the meta-analyses of Oetzel[10] and Lean and colleagues[12] found that a calcium concentration of 1.1% to 1.5% of DM provided near-maximal risk of milk fever (**Fig. 2**). When the effects of length of time cattle were exposed to a transition ration before calving were investigated, a quadratic interaction with calcium was found.[12] This relationship suggests that short exposures to high concentrations of calcium markedly increase milk fever risk, whereas a prolonged exposure to the same concentrations produces only a moderate risk (**Fig. 3**). These observations may explain the differences in recommended calcium concentrations of different workers.

The total exchangeable body calcium mass is only 1.5% of total body calcium in mature cows.[30] Goff and colleagues[21] estimated an even smaller pool of readily labile calcium bone stores, 6 to 10 g, based on responses of cattle to ammonium chloride-induced acidosis.[31] We have observed mild milk fever cases arising before calving with low-DCAD and low-calcium diets, possibly reflecting calciuria stimulated by the low-DCAD diets, and find that diets containing 0.4% to 0.6% calcium overcome this problem.

Magnesium

The most significant factor influencing risk of milk fever is the magnesium content of the diet.[12] Magnesium may prevent milk fever through roles in

- The release of PTH and in the synthesis of 1,25-dihydroxycholecalciferol
- In hypomagnesemic states, kidney and bone are less responsive to PTH[4,32]
- Reducing renal calcium excretion. Wang and Beede[33] found that nonpregnant, nonlactating cows fed a diet high in Mg had lower renal calcium excretion than those fed a diet low in Mg

Contreras and colleagues[34] and van de Braak and colleagues[35] reported poor calcium mobilization in hypomagnesemic cattle. Although clinical hypomagesemia is rare in dairy cattle, very low dietary Na or high dietary K concentrations may interfere with

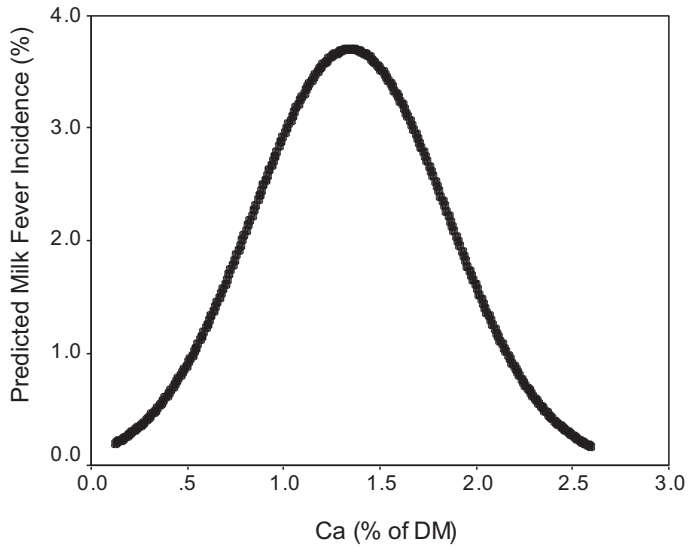

Fig. 2. Milk fever incidence in response to varying dietary Ca concentrations. (*From* Lean IJ, DeGaris PJ, McNeil DM, et al. Hypocalcemia in dairy cows: meta analysis and dietary cation anion difference theory revisited. J Dairy Sci 2006;89:674.)

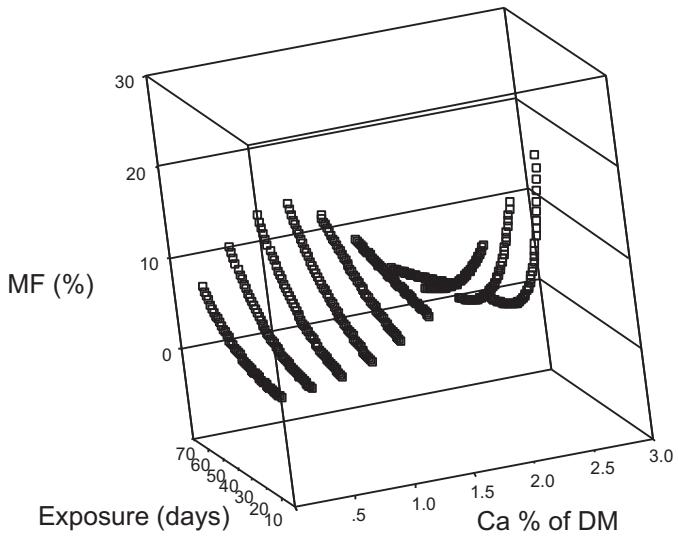

Fig. 3. Relationship between calcium %, days exposure to the transition diet, and milk fever incidence (MF %). (*Adapted from* DeGaris PJ, Lean IJ. Milk fever in dairy cows - A review of pathophysiology and control principles. Vet J 2008;176:64; with permission.)

Mg transport across the rumen wall and result in clinical disease. Magnesium is best supplied as magnesium sulfate, magnesium chloride, and magnesium oxide; caution needs to be applied to supply in the chloride form, because this is unpalatable (we have observed successful application in water). As with any other mineral, it should not be assumed that more is better; we have observed problems associated with supply of 0.8% magnesium in diets.

Phosphorus

Phosphorus also plays an important role in milk fever, with increasing phosphorus concentrations increasing milk fever risk. Although phosphorus concentrations are not as tightly regulated as calcium, both are closely related, with plasma PO_4 concentrations regulated directly by 1,25(OH) vitamin D_3 and indirectly by the PTH/calcium negative feedback loop.[36] However, in rats, hyperphosphatemia can inhibit the renal production of 1,25(OH) D_3 sufficiently to cause hypocalcemia.[37–39] In cattle, there is evidence that a prepartum diet high in phosphorus can have a negative impact on calcium homeostasis, possibly by the same pathways.[40–42] Hypophosphatemia may contribute to the alert downer cow syndrome and long-term dietary P deficiency has been implicated in the development of postparturient hemoglobinuria; however, it is likely that the latter disease is multifactorial, with copper, selenium, and antioxidant status playing an important role in the development of the disease.

Optimum Duration of Exposure

The duration of exposure to the transition diet was included in the models to predict milk fever risk developed by Lean and colleagues,[12] but had not been validated in trial work. Subsequent studies tend to support an optimal period of exposure to transition diets of 25 days before calving.[9] Increased urinary calcium loss on low-DCAD diets has been reported,[43,44] and depletion of calcium stores over time may explain part of this effect.

Age and Protein

There is good evidence that increasing age increases the risk of milk fever as a result of decreased intestinal calcium absorption and responsiveness to hypocalcemia,[45–47]

reduced bone turnover,[48] and decreased bone responsiveness to PTH and vitamin D.[21] We found that the risk of milk fever increased by 9% per lactation in the subpopulation of studies used for a meta-analysis[12] that reported age (unpublished data).

Increased protein concentration in the diet increased the risk of milk fever in some models and approached significance in many models tested by Lean and colleagues[12] (unpublished data). This effect was not large compared with magnesium and calcium concentrations. However, too few studies provided data on this finding to include in final published meta-analytical models.

Importance of Bone in Energy Metabolism

Important homeostatic links between bone and energy metabolism have been established. This relationship was first postulated when obesity was discovered to reduce the risk of osteoporosis in humans.[49] Ducy and colleagues[50] then proposed that the bone and energy metabolism may be regulated by the same hormones. Mouse models show that osteocalcin, produced by mature osteoblasts, completes the negative feedback loop between bone and energy metabolism, which is the hallmark of homeostatic regulation.[51] The uncarboxylated form of osteocalcin promotes

- β-cell proliferation
- Insulin secretion
- Independently increases peripheral tissue insulin sensitivity
- Adiponectin secretion by adipose cells

Although there is no specific research in cattle linking bone and energy metabolism, there are findings that support the hypothesis that these may be linked, and this interaction seems to be a vital aspect of the homeorhetic adaptations to lactation. Many studies support this conceptual framework at the physiologic level, and clinically, Heuer and colleagues[52] found that obese cows (body condition scoring [BCS] >4.5/5) were at greater risk of milk fever. Binger and colleagues[53] found an increase in insulin resistance in cows fed low-DCAD rations before calving. DeGaris and colleagues[8] found a positive relationship between BCS and area under the curve of blood Ca after calving, and reanalysis of the data used in that study using time series techniques found significant, positive correlations between blood calcium and glucose both before and after calving (DeGaris, and Lean, unpublished data). Associations between ketosis and hypocalcemia are well recognized and have been recently reconfirmed in prospective, randomized trials with anionic diets[54] and cohort studies examining the effects of hypocalcemia.[55]

Critically, links between calcium metabolism and health and reproduction are also evident. Hypocalcemia is a risk factor for many of the important diseases of lactation, including mastitis, ketosis, retained placenta, displaced abomasums, and uterine prolapse. Hypocalcaemia is also a risk factor for reproductive disorders and is an indirect risk factor for increased culling.[25,56,57] Curtis and colleagues[58] observed an increased odds of mastitis of 8-fold for cows with milk fever. Although such increases have been ascribed to recumbency and failure of teat sphincter closure, these mechanisms are speculative. Cows with subclinical hypocalcemia (defined as <8.59 mg/dL) were at greater risk of developing fever, metritis, and puerperal metritis compared with normocalcemic cows.[55] There are differences in peripheral mononuclear cells function, which indicate impaired function in hypocalcemic cows.[59] Borsberry and Dobson[60] in the United Kingdom found that cows with clinical milk fever had 13 more days from calving to conception, a finding supported by Martinez and colleagues[55] in hypocalcemic cows. These investigators also found lower conception rates in hypocalcemic cows and that 66.6% of metritis and 91.3% of puerperal metritis in this population

was attributable to hypocalcemia. New Zealand studies[61,62] found a tendency to improved interval to conception in cows treated with calcium-containing products after calving. Although further targeted research is needed to explore this potentially important aspect of energy and bone metabolism in dairy cattle, those evaluating or formulating diets should work on the premise that these links between bone health and energy metabolism are substantial.

Specific Interventions

Vitamin D and calcium

One of the emerging areas of understanding is the important role of vitamin D in immune function.[63] Vitamin D has roles in both innate and adaptive immune responses. Although cattle exposed to sunlight may obtain sufficient vitamin D, it is clear that housed cattle do not unless supplemented. The target levels for optimal performance are being identified, as are the optimal forms of supplementation. Supplying 40,000 IU of vitamin D_3 may be appropriate; however, there should be careful consideration of the optimal timing of such supplementation before calving.

The potential benefits of the use of vitamin D metabolites have been recognized for many years. Problems in finding satisfactory therapies have been encountered as a result of needs to predict calving dates, variation in responses associated with different prepartum calcium intakes,[22,64] and the potential for toxic reactions to arise from the administration of the vitamin D metabolites as a result of persistent hypercalcemia, such as the deposition of calcium in tissues, particularly the cardiovascular system.[65,66] Notwithstanding these limitations, several studies have reported positive responses to 25-OH cholecalciferol on milk fever risk and metabolism. As better understandings of vitamin D, calcium, and energy metabolism emerge, more detailed recommendations on vitamin D use can be anticipated.

Acidifying feeds

Lowering the DCAD of the prepartum diet using mineral salts has produced a significant increase or a trend toward increased milk production in lactation.[23,67–70] These responses are approximately 1 to 2 L per cow per day. The most researched of the interventions is the acidifying protein meal BioChlor (Church and Dwight, NJ), produced using sulfuric and hydrochloric acids. Soychlor (West Central Soy, IA) is another acidifying protein meal that is based on hydrochloric acid and soya-proteins. Corbett[71] retrospectively examined 13,000 DairyComp 305 records and found an increase in daily milk yields of between 2.0 and 3.0 L for cows exposed to a transition diet containing BioChlor for 15 to 21 days compared with 0 to 7 days' exposure. DeGroot[72] in a randomized controlled trial found an average 2.0 L/d production response in cows exposed to a prepartum diet containing BioChlor for 21 days over cows fed control diet with a similar DCAD.

After calving

Recommendations for the target DCAD for lactating cows range from +35 to +40 mEq/100 g DM and are based on the meta-analysis of Hu and Murphy.[73] Increasing the lactating diet DCAD to these levels has been shown to increase DM intake (DMI), milk components, and milk yield and possibly improve amino acid balance.[73–77]

Zeolites and calcium binding

Thilsing-Hansen and colleagues[5] concluded that limiting the precalving calcium intake to 20 g/d or less is 100% effective at preventing milk fever, but may be too low to incorporate with a negative DCAD diet. However, it is often difficult to limit daily calcium intake to these levels. Calcium-binding agents (eg, zeolite A) have been shown to

bind calcium and reduce Ca availability in precalving diets. However, some binders have been associated with reduced DMI before calving and because they are nonspecific, the potential exists for reduced availability of other divalent cations such as magnesium, an action that would increase the risk of milk fever.[5,78,79]

Calcium drenches

Calcium drenches and gels are available and have been widely used to prevent and treat hypocalcemia. Preventive gels are given as an oral drench during the 24-hour period around calving. Most calcium gels are based on calcium chloride, which supplies a soluble form of calcium and acidifies. Goff and Horst[80] compared the effectiveness of various calcium salts, including calcium propionate, calcium chloride, and calcium carbonate. Although calcium chloride increased plasma calcium concentrations higher than those of the other products, it could cause a severe acidosis[80] and may irritate the oral and ruminal mucosa. Calcium propionate has the advantage of being glucogenic and may reduce the risk of ketosis.[81]

Micromineral metabolism: free radicals and antioxidants in transition The homeorhetic and homeostatic responses to lactation can be exaggerated or perturbed by release of inflammatory mediators from lipid mobilization, environmental stressors, or subclinical disease conditions that increase postparturient disease risks[82–87] (see also the article by Sordillo and colleagues elsewhere in this issue). The magnitude of effects of these responses are most clearly shown by studies in which the antiinflammatory agent acetylsalicylate (aspirin), either fed or injected, markedly reduced the risks of disorder after calving, increased milk production, and improved reproduction.[88]

Clinical situations in which inflammation is increased and free radicals may be generated include:

- Challenge from infectious agents (novel agents, highly pathogenic, substantial exposure)
- Deficiency states of antioxidants, either single antioxidants or several antioxidants
- Parturition when cows are exposed to bacterial contamination of the reproductive tract, increased metabolic demands, and depletion of antioxidants associated with lactation and production of colostrum
- Higher-producing animals have higher metabolic activity rates and greater loss of antioxidants in the milk
- Excessive intakes of prooxidants (eg, polyunsaturated fatty acids or catalysts such as iron, copper or zinc)
- Estrus activity; there is a considerable capacity for free radical generation and challenge during steroidogenesis and in the period of growth and atresia of ovarian structures[89]; reproduction is not a sterile process, and consequently, there is considerable potential for bacteria to create free radical challenge during conception and early embryonic development

The processes of calving and lactation are proinflammatory. Inflammation is a critical part of innate immune responses and is an adverse response only when uncontrolled. Control of inflammation is exerted by ensuring that there is a good balance between exposure to pathogens and that cows are able to mount effective innate and humoral immune responses. When inflammatory effects are uncontrolled, these effects are often mediated through propagating reactions that involve the generation of free radicals.

Free radicals are generated as a normal part of metabolism in cellular respiration, electron transport via cytochrome P450, enzymatic reactions, and significantly in the

killing systems used by macrophages, neutrophils, and other phagocytic leucocytes. This controlled release of free radicals is part of the immune response through the respiratory burst of phagocytic leucocytes. Granulocytes, mononuclear macrophages, and lymphocytes use free radicals such as H_2O_2, myeloperoxides, and superoxides as a means of destroying invading organisms and damaged tissue. The oxidative agents released extracellularly or within phagosomes are a controlled response to defined activated pathways.[90] This process involves the production of high levels of superoxide, which can cause significant damage to biological molecules in an iron catalyzed reaction, in which $OH^{\cdot}$ is an intermediate. This reaction is called the Fenton reaction and allows the formation of free radicals that are destructive to tissues.[91] Free radicals are unstable, react with the environment, and create toxic lipids, reactive proteins, and other free radicals and further damaging tissues, DNA and RNA.[92] Damage from free radicals is more severe when systems that quench propagating reactions are impaired, often through deficiencies in the antioxidant vitamins and minerals.

The balance of radical generation and antioxidant control is complex, because the processes involved are highly interrelated, and excesses of trace elements can be as damaging as deficiencies. Iron and copper are needed in key protective enzymes such as transferrin, catalase (Fe), and Cu/Zn superoxide dismutase (Cu) that bind these; however, excessive supplementation with copper or iron saturates potential binding sites and increases the level of these metals in their free states. Free iron and copper may catalyze oxidative reactions, as shown in the spectacular sudden death syndromes associated with acute and chronic copper toxicity.

Serum concentrations of the fat-soluble vitamins retinol (vitamins A) and α-tocopherol (vitamin E) decline around the time of calving,[93] a decline that cannot be completely accounted for by losses through the mammary gland.[94] Curtis[83] serially sampled Holstein cows from 1 month before calving until 1 month after calving and identified the likely transfer of many antioxidants to the calf, in utero and through colostrum, a finding supported by many other studies. Plasma retinol, α-tocopherol and β-carotene concentrations were depleted to nadirs at a mean of 4.5 days after calving. Subsequently, plasma retinol and α-tocopherol concentrations increased.[83] At the time that plasma retinol, α-tocopherol, and β-carotene concentrations were lowest, plasma ceruloplasmin activities were highest, but these decreased at the end of the sampling period. Whole blood glutathione peroxidase activities increased and peaked 3.6 days before calving. Plasma ascorbate concentrations and erythrocyte Cu/Zn superoxide dismutase activities did not display consistent patterns of change over the sampling period. There were significant correlations between the changes in plasma retinol, α-tocopherol, and β-carotene concentrations and also between plasma ceruloplasmin and whole blood glutathione peroxidase activities. Initial increases in malondialdehyde (an indicator of free radical damage) concentrations were associated with decreases in concentrations of the fat soluble vitamins and the decrease in malondialdehyde over calving was associated with increases in plasma ceruloplasmin and whole blood glutathione. The findings of Curtis[83] show strong interactions among antioxidants.

At calving, cows with plasma α-tocopherol concentrations less than 3.0 μg/mL were at 9.4 times greater risk of having mastitis within the first 7 days of lactation compared with cows with higher concentrations.[95] LeBlanc and colleagues[96] did not find a protective effect of prepartum serum α-tocopherol concentration on mastitis, but for every 1 μg/mL increase, retained placenta incidence was reduced 21%. Because serum retinol concentration increased 100 ng/mL during the last week of gestation, risk of clinical mastitis in early lactation was decreased 60%.[96] Serum vitamin concentrations can be augmented with appropriate dietary supplementation.[97–100] However,

caution should be exercised in use of transition metal and vitamins antioxidants, because these can have adverse or no effects when fed greater than requirements. Responses to additional vitamin E in dairy cattle have been variable, and a meta-analysis of use of vitamin E and selenium in beef feedlot cattle indicated that feeding vitamin E at concentrations greater than the National Research Council (NRC) recommendation, or the administration of vitamin E as an injection, did not improve average daily gain, efficiency of gain, or morbidity in feedlot cattle.[101]

Although trace mineral nutrition of dairy cattle is of great importance and many trace minerals improve immune function,[102] the capacity for interactions with other dietary inputs and variation in individual animal requirements means that despite extensive study, there are still many areas that require clarification. Although the inflammatory pathways that influence health, productivity, and reproduction are complex, the clinician need not understand all of the pathways to be aware and enact preventive strategies. Ensuring that mineral and vitamin intakes meet or moderately exceed NRC requirements is essential. The source of such minerals may be important because responses have been noted in both production and reproduction to organic sources of minerals.[103]

Table 2 highlights the dietary sources, active forms, sites of action, and types of action of antioxidants in cattle.

Free radical management and controlling inflammation: the major implication of Curtis' work is that vitamin and mineral status of cattle should not be considered in isolation of other antioxidants, nor of the level of oxidative challenge; adequacy is a function of these interactions, not just a single vitamin or mineral; increased concentrations of 1 of these may also not be better.

Putting it all Together: a Cohort Study of Integrated Interventions

In a large, prospective observational study examining the effect of increasing days exposure to a BioChlor-based transition diet that was formulated to deliver on a DM basis, 16.0% CP (crude protein), 4.2% rumen undegradable protein, and 6.9 MJ/kg (0.65 Mcal/#) NE_L.[7–9] The diets provided an average metabolizable protein balance of 286 g/d based on the Cornell Net Protein and Carbohydrate Model and a dietary cation anion difference of –15.0 mEq/100 g, provided micronutrients to meet or exceed NRC requirements, and rumen modification to control risk of acidosis. Increasing exposure to the prepartum transition diet had positive effects on milk and milk protein yield. The increase in production reported between minimal exposure (3 days or less) and optimal exposure (22 days for milk yield and 25 days for milk protein yield) was approximately 3.75 L of 4.0% fat and 3.2% protein corrected milk per day and 100 g of milk protein per day (**Figs. 4** and **5**). DeGaris and colleagues[7] also found that exposure to the transition diet increased risk of conception by 1.2% per day on the transition diet. This effect is large and is shown in **Fig. 6**, showing the cumulative pregnancy rate for cows exposed to the diet for less than 10 days, those exposed for 10 to 20 days, and those cows exposed for more than 20 days. Numerically, more cows were in calf at the end of the mating periods for cows with greater exposure to the transition diet.

Putting it all Together: Troubleshooting/Formulating: a Checklist

The following guidelines are useful when formulating or troubleshooting transition diets and management. Given the multivariable nature of the disorder and other benefits

Table 2
Dietary sources, active forms, sites of action and type of action of antioxidants in cattle

Dietary Input[a]	Biologically Active Antioxidant	Site of Action	Type of Action
Selenium	GSHPx	IC/membrane	ROOH, H_2O_2
Copper	Cu/Zn superoxide dismutase	IC	O_2^-
	Caeruloplasmin	EC	Binds Cu/oxidizes Fe, weak O_2^- scavenger
	EC superoxide dismutase	EC	O_2^-
Zinc	Cu/Zn superoxide dismutase	IC	O_2^-
	EC superoxide dismutase	EC	O_2^-
	Metallothionine	EC	Binds metal ions
Manganese	Mn superoxide dismutase	IC	O_2^-
Iron	Catalase	IC	H_2O_2
	Transferrin	EC	Binds Fe
	Lactoferrin	EC-milk/sweat	Binds Fe
Cobalt	Vitamin B_{12}		
Vitamin E	α-Tocopherol	Membrane	Blocks peroxidation in lipids especially
Vitamin A	Retinol	EC	Maintains cell integrity
β-Carotene	β-Carotene	Membrane	Scavenges singlet O_2
	Retinol	EC	Cell integrity
Glucose	Ascorbate	EC	Vitamin E, GSSG reduction, radical scavenger
Sulfur amino acids	GSSG	IC	Replenishes GSHPx
Protein	Albumin	EC	Binds Fe and Cu
	Hemopexin	EC	Binds Fe
	Haptopexin	EC	Binds Fe
	Histidine-rich glycoproteins	EC	Binds other metal ions
	Erythrocytes	EC	Transport radicals IC
	Mucins	EC	$OH^·$ Scavenging

Abbreviations: EC, extracellular; GSHPx, glutathione peroxidase; GSSG, glutathione; H_2O_2, controls hydrogen peroxide; IC, intracellular; NIR, near infrared spectroscopy; O_2^-, controls superoxides; $OH^·$, controls hydroxyl radicals; ROOH, controls lipid peroxides.

[a] Limiting dietary component.

Data from Lean IJ, Westwood CT, Rabiee AR, et al. Recent advances in nutrition and reproduction in temperate dairy management. In: Webber W, ed. Proceedings of the Society of Dairy Cattle Veterinarians of the NZVA Annual Conference. Palmerston North, New Zealand: VetLearn Foundation, 1998. p. 87–118.

of correct transition diets, care should be taken not to crudely apply rules of thumb, but to evaluate the diets in total.

1. Analyze available feeds for macromineral content using wet chemistry methods. NIR can be unreliable for determination of mineral composition of forages in particular. Analyze feeds to allow macronutrient balancing. Comprehensive and cost-effective feed testing can be performed (eg, using an accredited laboratory from http://www.foragetesting.org [National Forage Testing Association]).
2. Select feed ingredients that have a low DCAD (<20 mEq/100 g DM). Of particular importance are forages that are low in K (<2.0%) and possibly Ca Select forages, which allow adaptation of the cow's rumen to the early lactation diet. Forages (hays/silages) or paddocks may need to be specifically grown or prepared for transition cows and receive minimal potassium-based fertilizers or manure applications.

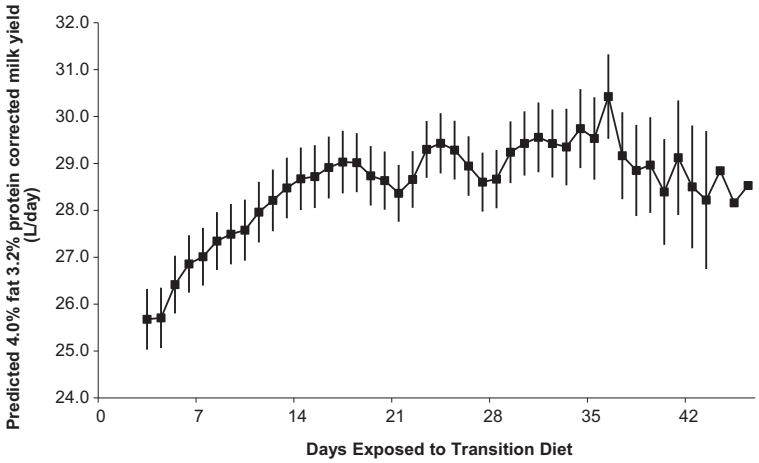

Fig. 4. Four-day moving average and 95% confidence interval of predicted 4.0% fat and 3.2% protein corrected milk yield with increasing days exposed to the prepartum transition diet. (*From* DeGaris PJ, Lean IJ, Rabiee AR, et al. Effects of increasing days of exposure to pre-partum transition diets on milk production and milk composition in dairy cows. Aust Vet J 2008;86:346.)

3. Formulate ration to deliver a positive energy and protein balance without placing cows at risk of acidosis; consider strategies that minimize this risk (eg, limiting NFC [non fibre carbohydrate] to 36% and ensuring the NDF [neutral detergent fibre] is at least 30% of DM, and physically effective fiber is at least 24% of DM). Do not use straw or poor-quality hays as a significant source of forage, because energy density is insufficient for maintenance.
4. Include 500 to 1000 g of a commercial mineral acid-treated feed (eg, BioChlor) to provide chloride and sulfur and to increase microbial protein production.
5. Calculate DCAD; aim for less than 0 mEq/100 g DM.
6. Balance Na for requirements; target 0.12% DM.
7. Balance S (to ensure substrate for microbial protein synthesis); target 0.4% DM, but be cautious with higher concentrations, because it is possible that high dietary S concentrations place cows at risk of polioencephalomalacia. Use $MgSO_4$ up to 80 g/cow.

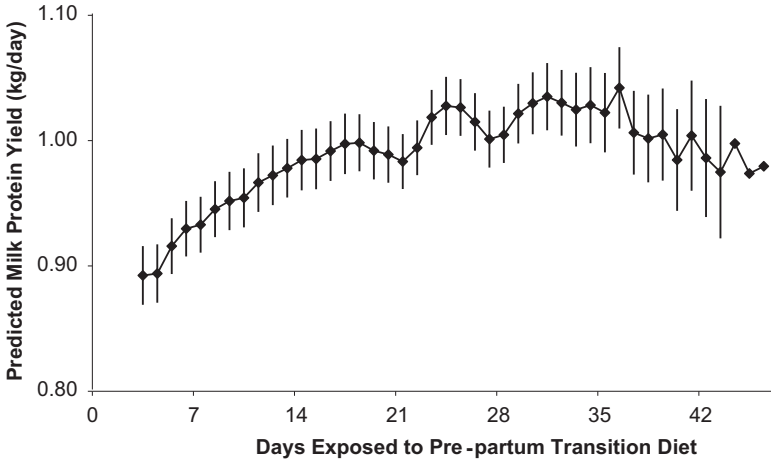

Fig. 5. Four-day moving average and 95% confidence interval of predicted milk protein yield with increasing days exposed to the prepartum transition diet. (*From* DeGaris PJ, Lean IJ, Rabiee AR, et al. Effects of increasing days of exposure to pre-partum transition diets on milk production and milk composition in dairy cows. Aust Vet J 2008;86:347.)

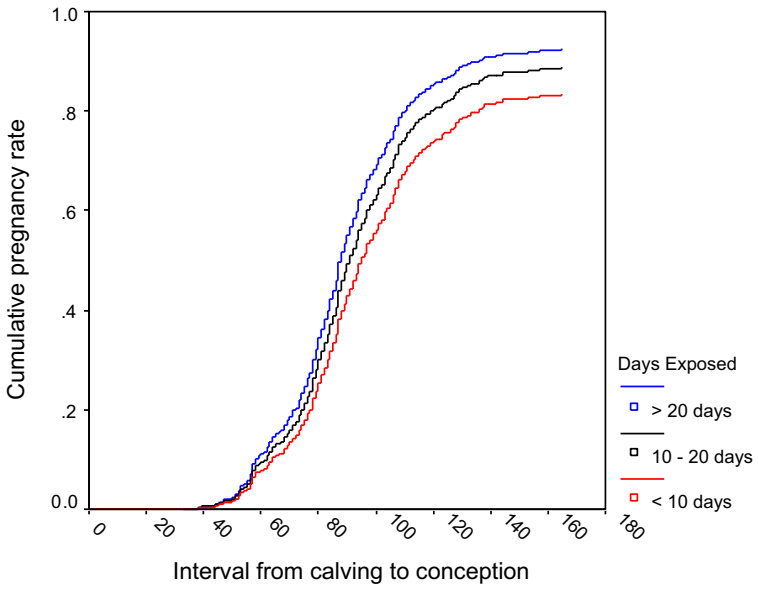

Fig. 6. Cumulative pregnancy rate for cows exposed to a BioChlor based prepartum transition diet for <10 days, 10–20 days, and >20 days. (*Adapted from* DeGaris PJ, Lean IJ. Milk fever in dairy cows - A review of pathophysiology and control principles. Vet J 2008;176:66; with permission.)

8. Balance Mg; target is 0.4%. DM: if Mg concentration is still low, add $MgCl_2$ (up to a combined inclusion rate with $MgSO_4$ of 100 g/cow). If Mg is still needed, balance with MgO.
9. Balance Ca concentration if necessary using $CaSO_4$, $CaCl_2$, or limestone. This strategy is rarely necessary because Ca concentration needs to be kept around 0.5 to 0.6% DM. Beware: Ca can occasionally be high in cereal hays. Consider increasing the Ca concentration of the diet only if it is extremely low (<0.25%) because these diets may result in significant depletion of bone stores, particularly if cows are spending an extended time on an acidifying transition diet.
10. Check P concentration; target is around 0.3 to 0.35% P. Additional sources of P are rarely required. High P feeds may need to be reduced in diet.
11. Check K concentration; this should be at least 1.1% to allow for daily requirements of the cow. As an approximate guideline, the K concentration should be about 4 times the Mg concentration. If this ratio is higher (ie, K > 1.6%) consider reducing the high K feeds in the diet.
12. Check Cl concentration; the Cl concentration of the diet is ideally about 0.5% lower than the K concentration.
13. Check DCAD; this should now be around –10 mEq/100 g DM. If DCAD is greater than 0 mEq/100 g DM, go back to step 2 and start again.
14. Balance ration for trace minerals and vitamins. Meet or moderately exceed NRC requirements for micromineral needs and vitamins.
15. Ensure
    a. Adequate access to concentrates and hay/silage sources to minimize competition; a minimum of 75 cm of linear trough space per cow is required
    b. Thorough mixing of acidogenic salts or acidifying feeds
    c. Pasture intakes are estimated as accurately as possible taking into account daily growth
    d. Heifers are integrated into transition herd at this stage to minimize competition after calving or are kept in a separate string

e. As many as possible of the postcalving feed ingredients are included in the precalving diet
f. Avoid feeding transition diets through dairy parlors; this can result in unsatisfactory intake in some cows and increases risk of mastitis in some herds by stimulating mammary letdown; cows fed in dairies should have teat spray applied.

16. Monitor the effectiveness of the DCAD aspects of the transition diet by measuring urine pH targets should be 6.2 to 6.8 for Holsteins and 5.8 to 6.3 for Jerseys. Urine pHs less than 5.8 suggest excessive metabolic acidosis that compromise cow health. The aim is to prevent milk fever and not necessarily reduce urine pH. Measure and analyze feeds in preference to testing urine (if you can choose to do only one or the other, getting the diet correct is more critical).
17. Aim to have cows on a transition diet for 21 days.

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