

Milk fever in dairy cows: A review of pathophysiology and control principles

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Abstract

The periparturient or transition period of 4 weeks before and 4 weeks after calving is characterised by a greatly increased risk of disease. Hypocalcaemia around calving is a risk factor for many of these diseases and is an indirect risk factor for increased culling. The incidence of clinical hypocalcaemia (milk fever) in the field generally ranges from 0–10%, but may exceed 25% of cows calving. In research trials conducted on milk fever the incidence has approached 80% of cows calving.

Homeostasis of calcium (Ca) is regulated by calcitonin, parathyroid hormone and 1,25(OH)₂ vitamin D₃. Age increases the risk of milk fever by approximately 9% per lactation. Control of milk fever has revolved around stimulation of homeostatic mechanisms through feeding a pre-calving diet low in Ca. More recently, the role of the dietary cation anion difference (DCAD) in the prevention of Ca disorders has been examined, both by field research and meta-analysis. The most appropriate form of the DCAD equation has been contentious, but recent meta-analyses have shown that the equation $(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{S}^{2-})$ is most effective for predicting milk fever risk. Decreased risk of milk fever is linear with DCAD, whereas the effect of DCAD on urinary pH is curvilinear. A pivotal role of providing dietary magnesium (Mg) before calving has been confirmed by meta-analysis, and a quadratic effect of Ca on milk fever risk was found with a peak occurring with dietary levels of 1.1–1.3% of dry matter.

Risks of milk fever increase with increased dietary phosphorus (P) fed pre-calving and with increasing days of exposure to a pre-calving diet. Meta-analysis has revealed that the important roles of dietary Ca, Mg and P, as well as the duration of exposure to the pre-calving diet in milk fever control strategies are independent of DCAD. Studies on the effect of exposure to well designed pre-calving diets have shown that substantial improvements in production, reproduction and animal health can be made but further examination of the influence of the period of exposure to different diets is warranted.

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Introduction

The periparturient or transition period of 4 weeks before and 4 weeks after calving is characterised by greatly increased risk of disease (Shank et al., 1981; Curtis et al., 1985; Stevenson and Lean, 1998). The period is dominated by a series of adaptations to the demands of lactation, a process described as *homeorhetic* (Bauman and Currie,

1980). Homeorhetic processes are the long term physiological adaptations to changes in state, such as from non-lactating to lactating or non-ruminant to ruminant, and involve an orchestrated series of changes in metabolism that allow an animal to adapt to the challenges of the altered state. The problems that result from disordered homeorhetic change reflect disorders in homeostasis and include hypocalcaemia, the downer cow syndrome, hypomagnesaemia, ketosis, udder oedema, abomasal displacement, metritis and poor fertility.

These conditions are often inter-related (Curtis et al., 1983, 1985; Curtis and Lean, 1998) and this, combined with

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the dramatic changes in endocrine function and metabolism associated with calving and the initiation of lactation, makes transition an inherently difficult period to study. Notwithstanding these challenges, the potential to increase subsequent production, improve health and improve reproduction has made nutritional manipulation of the periparturient cow a focus for research.

It can be broadly stated that the transition cow should be adapted to provide minimal risk of metabolic disorders of macro-mineral metabolism including absolute or conditioned calcium (Ca), magnesium (Mg) or phosphorus (P) deficiencies, or excesses of sodium (Na) and potassium (K); disorders of lipid metabolism arising from inadequate energy intake in the dry period and early lactation; disrupted rumen function associated with dietary change and impaired immune response.

The aims of this paper are to review some of the changes that occur around calving related to hypocalcaemia and to examine the potential to modify the risk of disease, reproductive failure and milk production through better nutritional management.

Incidence and background

While the focus on nutritional management of hypocalcaemia is appropriate, not all of the risks for periparturient hypocalcaemia results from ration formulation. Recumbency is often caused by hypocalcaemia, but other significant causes include hypomagnesaemia, musculo-skeletal injury predisposed by calving and hypocalcaemia, ketosis associated with twinning, hypophosphataemia and a number of less frequent problems such as peracute mastitis and other infections. Dystocia is a major cause of periparturient recumbency and Chamberlain (1987) reported that 46% of primary recumbencies in cows were due to dystocia, 38% to hypocalcaemia and 16% to other causes.

Clinical hypocalcaemia (milk fever) (total blood Ca < 1.4 mmol/L) as well as sub-clinical hypocalcaemia (total blood Ca 1.4–2.0 mmol/L) are risk factors for many of the important diseases of lactation including mastitis, ketosis, retained placenta, displaced abomasum and uterine prolapse. Hypocalcaemia is also a risk factor for reproductive disorders and is an indirect risk factor for increased culling (Erb et al., 1985; Stevenson and Call, 1988). Cows with milk fever produced less milk than non-affected cows for the first 4–6 weeks of lactation, but over a whole lactation produced more milk than non-affected cows (Rajala-Schultz et al., 1999).

The heritability of hypocalcaemia is reportedly low. Payne (1977) stated that the heritability of parturient paresis was 12.8%, but other workers (Norman and van Vleck, 1972; Dohoo et al., 1984) reported heritabilities of 0% and 4%, respectively. The higher susceptibility of Channel Island breeds compared with Holsteins to hypocalcaemia is well established (Harris, 1981). Older cows are at greater risk of hypocalcaemia. This increased risk is associated with a decreased capacity to mobilise Ca from bone (van

Mosel et al., 1993) and possibly a decreased number of 1,25 dihydroxy-cholecalciferol ($1,25(\text{OH})_2\text{D}_3$) receptors in the small intestine (Horst et al., 1990). We have found that the risk of milk fever increased by 9% per lactation in the sub-population of studies used for a meta-analysis (Lean et al., 2006) that reported age (P.J. DeGaris and I.J. Lean, unpublished data).

Examination of field studies reporting incidence of milk fever from 1977 to the present found that the incidence in 10 North American studies was 3.45% (range 0–7%), in 10 European studies it was 6.17% (range 0–10%), and for 10 Australasian studies it was 3.5% (range 0–7%). The potential to manipulate the incidence of milk fever is indicated by the differing incidence between herds. In some herds the incidence is <1% while other herds may have incidence >25% that may peak and ebb within the herd (Mullen, 1975). These observations are strongly supported by the incidence of hypocalcaemia observed in the 135 controlled trials from the meta-analysis data set used by Lean et al. (2006), for which the mean incidence was 21% with a range of 0–83%.

Over-conditioned cows (body condition score >3.5, in a scale of 1–5) are at increased risk of hypocalcaemia (Heuer et al., 1999). There is some evidence that indicates that the disorder is associated with higher producing cows and that the incidence increased with increasing production levels (Payne, 1977).

The pathophysiology of hypocalcaemia

Changes in Ca metabolism induced by lactation are more significant than parturition per se to the pathogenesis of parturient paresis, as the loss of blood Ca to milk may exceed 50 g per day. Before calving, the approximate daily requirement for Ca is only 30 g, comprising 15 g in faecal and urinary loss and 15 g to fetal growth. This demand for Ca may only be satisfied by increasing absorption from the rumen or intestines, and increasing mobilisation from tissue, especially bone reserves of Ca, as circulating blood Ca reserves are limited. Most cows have some degree of hypocalcaemia at calving.

Blood Ca is maintained within a narrow range (2.0–2.5 mmol/L). Cows can only afford to lose approximately 50% of circulating blood Ca reserves before a hypocalcaemia crisis is precipitated. Physiological controls over Ca homeostasis include calcitonin, secretion of which is stimulated in response to elevated blood Ca concentrations. Parathyroid hormone (PTH) is released from the parathyroid glands in response to lowered blood Ca. The final hydroxylation of 25-hydroxycholecalciferol (25-hydroxy- D_3) to $1,25(\text{OH})_2\text{D}_3$ in the kidney is regulated by PTH. PTH increases mobilisation of Ca from bone. The active metabolite of vitamin D, acts to increase intestinal absorption of Ca and resorption from bone. Vitamin D is provided by the photochemical conversion of 7-dehydrocholesterol to cholecalciferol (vitamin D_3) and is provided by ingestion of vitamin D_2 in plants. There are many

vitamin D₃ metabolites present in blood, but the major circulating form is 25-hydroxy D₃, which follows hydroxylation in the liver of vitamin D₃ from the diet or skin.

Dietary levels of Ca influence the absorption of Ca from the intestine, under the control of 1,25(OH)₂D₃, and the mobilisation of Ca from bone. As dietary Ca concentration increases, total dietary intake of Ca also increases resulting in a decrease in the efficiency of intestinal absorption and a decrease in Ca mobilisation from bone (Ramberg, 1995).

The normal periparturient cow responds to decreased plasma Ca concentrations by increasing PTH and subsequently, 1,25(OH)₂D₃ concentrations. The result is increased intestinal absorption of Ca, increased resorption of Ca from bone and increased renal tubular reabsorption of Ca. The cow is, however, limited in its capacity to respond to increased metabolic demands by the rate at which increased absorption may occur from the intestine and the rate at which Ca may be mobilised from the bones. Availability of dietary Ca to the intestine gradually changes even if more dietary Ca is supplied due to the buffering action of the rumen which acts as a reservoir for Ca (Ramberg, 1972). The capacity for cows to absorb Ca through the rumen is uncertain. In vivo and in vitro absorption across the rumen can occur, but at present the quantitative importance of this is to be determined (Holler et al., 1988).

The cow depends on a constant supply of dietary Ca (Hove, 1984) and may be placed at risk by gut hypomotility and stasis that are associated with hypocalcaemia (Moodie and Robertson, 1962). Huber et al. (1981) found in artificially induced hypocalcaemia that rumen contractions ceased well before the onset of signs of clinical hypocalcaemia. Such stasis may be an important factor in the development of hypocalcaemia because even temporary alimentary stasis can induce acute hypocalcaemia through reduced intestinal absorption of Ca (Moodie and Robertson, 1962).

While 99% of body Ca reserves are in bone, mobilisation of Ca from bone sources has been shown to have variable onset. Ca mobilisation from bone is less rapid in older cows and in cows fed on pre-calving diets that are high in Ca (Ramberg et al., 1984) or probably on alkalogenic salts. Cows are more dependent on gut absorption than bone resorption to maintain Ca homeostasis. It has been suggested that hormonal insufficiencies or lack of response of PTH or 1,25(OH)₂D₃ may be important in the pathogenesis of hypocalcaemia. Diets that are deficient in Ca before calving have been associated with higher plasma levels of 1,25(OH)₂D₃ (Green et al., 1981). In contrast, cows on pre-calving diets which are high in Ca are associated with high plasma concentrations of vitamin D metabolites which could have an antagonistic action on 1,25(OH)₂D₃ (Horst et al., 1983). 24,25-Dihydroxycholecalciferol increases the incidence of parturient paresis and this metabolite was elevated in paretic cows (Barton et al., 1984).

Cows with clinical milk fever have PTH and 1,25(OH)₂D₃ in higher concentrations than normal cows

(Horst et al., 1978). Various forms of vitamin D₃ have been used in pharmacological doses before calving to prevent milk fever in cows with varying results. The two metabolites most investigated are 1-alpha-hydroxylcholecalciferol and 1,25(OH)₂D₃. The vitamin D metabolites have a hypercalcaemic effect that is mediated through increased intestinal absorption of Ca (Goff et al., 1988) and possibly increased bone resorption (Littledike and Horst, 1982). The major difficulty associated with using the vitamin D metabolites remains the accurate prediction of calving date, as treatment is generally most effective between 1 and 4 days prior to calving (Gast et al., 1979; Bar et al., 1988; Sachs et al., 1997). Low Ca concentrations in the pre- and post-calving diet also can adversely impact on the success of vitamin D₃ supplementation (Allsop and Pauli, 1985; Goff et al., 1988). Another concern with the vitamin D₃ metabolites is the risk of toxicity resulting in persistent hypercalcaemia and metastatic calcification of body tissues (Petrie and Breeze, 1977; Littledike and Horst, 1982).

Milk fever prevention: Dietary Ca, Mg and P

One of the areas of continuing contention is the role of pre-calving dietary Ca intake as a risk factor for milk fever. Beede et al. (1992) stated that high Ca intake pre-calving is not the primary cause of subclinical hypocalcaemia and milk fever. However, it is important to note that the improved understanding of the role of the monovalent cations in hypocalcaemia does not refute the research on Ca and vitamin D metabolism over the previous 40 years (Ramberg et al., 1996).

Early studies (Boda and Cole, 1954; Goings et al., 1974) found that feeding diets low in Ca reduced the risk of milk fever. However, recent reviews have highlighted the fact that the perceived central role of Ca in the pathogenesis of milk fever is contentious. Literature reviews conducted by Lean et al. (2003) and Thilising-Hansen et al. (2002) suggest limiting pre-calving intake of Ca to 60 and 20 g per day, respectively. Lean et al. (2003) based this suggestion on the seminal studies of Boda and Cole (1954), Ramberg et al. (1976) and Ramberg et al. (1984). A qualitative literature review (McNeill et al., 2002) also concluded that excessive Ca intake was an important risk factor for milk fever, but less so than K. However, Goff (2000) concluded that Ca concentration in pre-calving diets had little influence on the incidence of milk fever when fed at levels above the daily requirements of the cow (approximately 30 g/day).

Oetzel (2000) recommended a daily intake in the pre-calving diet of 150 g/day, a Ca concentration of between 1.1% and 1.5% of dry matter (DM), in conjunction with a dietary DCAD of approximately –15 mEq/100 g DM. However, this recommendation was not supported by his meta-analysis because Oetzel (1991) found that the highest milk fever risk occurred with a dietary Ca concentration of 1.16%. Further examination with logistic regression using the data generated by Horst et al. (1997), shows that lower

concentrations of Ca were of less risk of causing milk fever than higher concentrations, although this was not statistically significant.

Mg and P also play an important role in Ca homeostasis. There are sound physiological bases for a protective role of Mg in the pathogenesis of milk fever. Mg is critical for the release of parathyroid hormone and in the synthesis of 1,25(OH)₂D₃. In hypomagnesaemic states, kidney and bone are less responsive to PTH (Sampson et al., 1983; Goff, 2000). Wang and Beede (1992) found that non-pregnant, non-lactating cows fed a diet high in Mg had lower renal Ca excretion than those fed a diet low in Mg. Contreas et al. (1982) and van de Braak et al. (1987) both demonstrated poor Ca mobilisation in hypomagnesaemic cattle.

P may also play an important role in the pathogenesis of milk fever, with increasing P concentrations increasing milk fever risk. Although P concentrations are not as tightly regulated as Ca, both are closely related with plasma PO₄ concentrations regulated directly by 1,25(OH)₂D₃ and indirectly by the PTH/Ca negative feedback loop (Goff, 1999). In cattle, there is evidence that a pre-calving diet high in P can have a negative impact on Ca homeostasis (Julien et al., 1977; Kichura et al., 1982; Barton et al., 1987).

Milk fever prevention: Dietary cation anion difference (DCAD)

Early studies by Norwegian workers found that diets high in Na and K and low in chlorine (Cl) and sulfur (S) tended to increase the incidence of milk fever, while those high in Cl and S and low in Na and K or containing added anionic salts (AS), decreased the occurrence of milk fever (Ender et al., 1962; Dishington, 1975; Dishington and Bjornstad, 1982). Block (1984) found a significant increase in the incidence of milk fever for cattle fed on diets that differed only in their quantities of Cl, S and Na. Further studies (Oetzel et al., 1988; Gaynor et al., 1989; Leclerc and Block, 1989; Goff et al., 1991; Beede et al., 1992; Phillipou et al., 1994) supported the earlier findings that feeding diets containing higher concentrations of Cl and S can reduce the risk of parturient paresis. Increasing K in the diet causes hypocalcaemia (Horst et al., 1997).

The dietary cation anion difference (DCAD) theory of milk fever prevention has its basis in the strong ion model of acid/base balance (Singer and Hastings, 1948), modified by Stewart (1981) and simplified by Constable (1997). The basic principle of the simplified strong ion model is that plasma pH is determined by four independent factors; the partial pressure of CO₂ (p_{CO_2}); solubility of CO₂ in plasma (S) which is temperature dependent; the net strong ion charge or strong ion difference ($[\text{SID}^+]$) and the total plasma concentration of non-volatile weak buffers, principally albumin, globulin and phosphate ($[A_{\text{TOT}}]$). The major strong ions consist of cations (Na^+ , K^+ , Mg^{2+} , Ca^{2+} and NH_4^+) and anions (Cl^- and SO_4^{2-}). The simplified

strong ion model (Constable, 1999) to predict plasma pH is:

$$\text{pH} = \text{p}K'_1 + \log \frac{[\text{SID}^+] - K_a[A_{\text{TOT}}]/(K_a + 10^{-\text{pH}})}{S * p_{\text{CO}_2}}$$

where $\text{p}K'_1$ is the ion product of water, K_a is the effective equilibrium disassociation constant for plasma non-volatile weak acids, $[\text{SID}^+]$ is the strong ion difference, $[A_{\text{TOT}}]$ is the plasma non-volatile weak acid concentration, S is the solubility of CO₂ in plasma and p_{CO_2} is the partial pressure of CO₂ in plasma.

This equation predicts that plasma pH can be lowered by: increasing the temperature thereby increasing solubility of CO₂ in plasma (S); increasing p_{CO_2} (respiratory acidosis); decreasing $[A_{\text{TOT}}]$ and decreasing $[\text{SID}^+]$.

Application of the DCAD theory to prevent milk fever aims to reduce the $[\text{SID}^+]$, consequently lowering plasma pH, resulting in strong ion metabolic acidosis. This can be achieved by feeding salts of the strong cations (CaCl_2 , CaSO_4 , MgCl_2 , MgSO_4 , NH_4Cl and $(\text{NH}_4)_2\text{SO}_4$) 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 GIT than are the strong anions (Cl^- and SO_4^{2-}). This results in a relative excess of absorbed anions compared to absorbed cations lowering the $[\text{SID}^+]$ and subsequently plasma pH. Salt (NaCl) and KCl have a net effect of zero on the $[\text{SID}^+]$, because Na^+ and K^+ are absorbed with near 100% efficiency in the intestine.

A number of possible means by which risk of milk fever may be influenced by feeding greater concentration of anionic salts have been identified. (1) Diets high in AS cause metabolic acidosis in goats (Fredeen et al., 1988a,b) and cattle (Gaynor et al., 1989), these observations being consistent with the simplified strong ion model. (2) Diets high in AS stimulate a calciuria (Lomba et al., 1978; Gaynor et al., 1989; Oetzel et al., 1991; Phillipou et al., 1994). (3) Elevated hydroxyproline concentrations have been observed in cows fed AS (Block, 1984; Gaynor et al., 1989), probably indicating bone mobilisation. (4) Plasma ionised Ca concentrations increase with feeding of AS (Oetzel et al., 1991; Phillipou et al., 1994). (5) Diets high in AS stimulate higher plasma levels of 1,25(OH)₂D₃ before calving (Gaynor et al., 1989; Phillipou et al., 1994). Calciuria can be induced by acute acidosis in a number of species. Metabolic acidosis increases mobilisation of Ca from rat liver mitochondria (Akerman, 1978) and mobilisation of Ca from bone, independent of, and in conjunction with, PTH (Beck and Webster, 1976).

It appears that the feeding of AS acts to increase mobilisation of Ca from bone, allows loss of urinary Ca and increased absorption of dietary Ca through an increase in pre-calving plasma 1,25(OH)₂D₃ concentrations. Ramberg et al. (1996) calculated that this loss was of the order of 3 g per cow per day. The acidotic state allows a higher concentration of ionised Ca. Acidotic cows are in a state where both bone mobilisation and dietary absorption of Ca are more active. The efficacy of acidification can be monitored

by evaluating the pH of urine (Jardon, 1995). Jardon (1995), based on personal experience and communication with other researchers, suggested that a urinary pH of 6–7 was optimal for Holstein cattle and a pH of 5.5–6.5 was optimal for Jersey cattle to indicate metabolic acidosis. Charbonneau et al. (2006) concluded that a group urinary pH of 7.0, regardless of breed, may be more appropriate.

The potency of different salts to induce metabolic acidosis has been evaluated (Beck and Webster, 1976; Akerman, 1978; Oetzel et al., 1991; Wang and Beede, 1992; Jardon, 1995; Ramberg et al., 1996). Ramberg et al. (1996) suggested that MgSO_4 is not an effective acidifier and concluded that the alkalotic state may be considered to be a relative deficiency of chloride. Hydrochloric and sulphuric acids are effective acidifying agents (Dishington, 1975) and are used in commercial anionic feeds such as BioChlor and SoyChlor, which may be more palatable than AS.

Many formulae have been proposed for calculating the DCAD of rations. The variations are generally broken into short equations that contain only Na^+ , K^+ , Cl^- and S^{2-} and longer equations that contain also Ca^{2+} , Mg^{2+} and P in varying combinations and with differing coefficients. The equation cited by Ender et al. (1962) and used by Block (1984) of $\text{DCAD} = (\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{S}^{2-})$ is the most commonly used form of the equation. Horst et al. (1997) recommended that other anions and cations be included in the equation and proposed $\text{DCAD (mEq)} = (0.38 \text{ Ca}^{2+} + 0.3 \text{ Mg}^{2+} + \text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{S}^{2-})$. Goff (2000) proposed a variation of this equation based on the capacity of different salts to acidify urine and recommended $\text{DCAD} = (0.15 \text{ Ca}^{2+} + 0.15 \text{ Mg}^{2+} + \text{Na}^+ + \text{K}^+) - (\text{Cl}^- + 0.25 \text{ S}^{2-} + 0.5 \text{ P}^{3-})$. Following the research of Spears et al. (1985) who estimated the absorption of sulfur from the gastrointestinal tract was 60% of dietary intake, Tucker et al. (1991) suggested that $\text{DCAD} = (0.38 \text{ Ca}^{2+} + 0.3 \text{ Mg}^{2+} + \text{Na}^+ + \text{K}^+) - (\text{Cl}^- + 0.6 \text{ S}^{2-} + 0.5 \text{ P}^{3-})$. Goff et al. (2004) recently proposed $\text{DCAD} = (\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + 0.6 \text{ S}^{2-})$ based on the relative acidifying effects of various anionic salts. The appropriate form of equation to use in the context of predicting milk fever has been a matter unresolved until recently.

Given, the apparently disparate observations on means of controlling milk fever risk using either manipulation of macro-mineral concentrations (either directly or via vitamin D_3 administration) or DCAD management, there was a need to re-examine the information on dietary control of milk fever. Areas of contention identified from the reviews of Goff (2000), Oetzel (2000), McNeill et al. (2002), Thilising-Hansen et al. (2002) and Lean et al. (2003) include the most appropriate equation to use to predict DCAD of the pre-calving diet and the roles of dietary Mg, P and Ca concentrations in the pathogenesis of hypocalcaemia.

Integrating milk fever prevention: A meta-analysis

Meta-analysis is a systematic and rigorous method of pooling data from previous studies and re-analysing them.

It is a particularly powerful tool for examining rare events such as disease studies conducted in relatively small populations, such as is evident in the milk fever data. Two significant meta-analyses of milk fever risk were conducted by Oetzel (1991) and Enevoldsen (1993) who both used fixed effects models for predicting outcomes, although it is now widely accepted that these models are vulnerable to over-dispersion associated with clustering of effects in trials. Random effects models are preferred for such analyses.

There are a large number of new studies that could be added to the original data of Oetzel (1991) to increase the statistical power of a new meta-analysis. The review and meta-analysis conducted by Lean et al. (2006) developed two statistically significant and biologically plausible equations for predicting milk fever risk based on pre-calving dietary constituents. The logit transformations (LT) of the logistic regression equations for predicting the incidence of milk fever for the Holstein-Friesian breed from pre-calving dietary nutrients (% DM), DCAD calculated as $(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{S}^{2-})$ in meq/100 g DM, and days exposed to the pre-calving diet calculated using the random effect model are:

$$\begin{aligned} \text{LT1} = & -5.76 + 5.48 (\text{Ca})5.05 (\text{Mg}) + 1.85 (\text{P}) \\ & + 0.02 (\text{DCAD}) - 2.03 (\text{Ca}^2) \\ & + 0.03 (\text{days exposed to diet}) \end{aligned}$$

$$\begin{aligned} \text{LT2} = & -5.17 + 5.74 (\text{Ca}) - 8.66 (\text{Mg}) + 2.30 (\text{P}) \\ & + 0.78 (\text{K}) - 3.48 (\text{S}) - 2.16 (\text{Ca}^2) \\ & + 0.04 (\text{days exposed to diet}) \end{aligned}$$

The predicted incidence of milk fever can be calculated from each of the logit transformations using the following equation:

$$\text{Milk fever \%} = e^{\text{LT}} / (1 + e^{\text{LT}}) * 100$$

These equations were developed from a database of 137 published trials detailing 2545 calvings. The authors preferred equation LT1 whereby the risk of milk fever could be predicated from the dietary levels of Ca, Mg, P, DCAD as calculated by $(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{S}^{2-})$, breed and duration of exposure to the diet. The effects of manipulating the predictor variables in equation LT1 on the risk of milk fever are shown in Table 1.

Another meta-analysis examining DCAD only (Charbonneau et al., 2006) determined the most appropriate form of the DCAD equation was $(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + 0.6 \text{ S}^{2-})$ on the basis that this equation could be used to predict both milk fever risk and urine pH. However, the equation $(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{S}^{2-})$ was equivalent in predicting milk fever risk (Charbonneau et al., 2006). Consequently, based on the simplified strong ion model and the meta-analyses of Lean et al. (2006) and Charbonneau et al. (2006), we conclude that the equation $\text{DCAD} = (\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{S}^{2-})$ should be used to predict the effect of a diet on the risk of milk fever.

Table 1
Effect of predictor variables on risk of milk fever as calculated from equation LT1 (Lean et al., 2006)

Variable	Effect of increasing variable on risk of milk fever	Odds ratio	P value
Breed (Holstein or Jersey)	↑	2.321	0.02
Calcium	↑	293.362	0.01
Calcium * calcium	↓ and ↑	0.131	0.01
Magnesium	↓	0.006	0.002
Phosphorus	↑	6.373	0.01
DCAD ($\text{Na}^+ + \text{K}^+$) – ($\text{Cl}^- + \text{S}^{2-}$) meq/100 g DM	↓	1.105	0.04
Days exposed to transition diet (12–60 days)	↑	1.030	0.03

Importantly, this linear relationship between DCAD and milk fever risk (Lean et al., 2006; Charbonneau et al., 2006) predicts that any reduction in the DCAD will decrease the risk of milk fever (Fig. 1). 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 DM (Fig. 2). The curvilinear relationship between urinary pH and DCAD reflects renal buffering systems that maintain an alkaline urinary pH until overwhelmed. The aim of DCAD manipulation of pre-calving diets must be to reduce milk fever risk and not necessarily manipulate blood or urine pH.

The second order effect of Ca present in both the models developed by Oetzel (1991) and Lean et al. (2006) supports a hypothesis that either low dietary Ca percentage (Boda and Cole, 1954; Goings et al., 1974; Wiggers et al., 1975) or high dietary Ca percentage (Lomba et al., 1978; Oetzel

et al., 1988) fed pre-calving reduces the risk of milk fever. The effect of low Ca diets on Ca homeostasis is well established, although the process by which very high dietary Ca concentrations pre-calving may reduce milk fever risk is unclear.

A protective effect of high dietary Ca was first proposed in the 1930s (Gould, 1933) but has been substantially refuted (Boda and Cole, 1954; Boda, 1956; Goings et al., 1974; Wiggers et al., 1975). However, the hypercalciuric effect of low DCAD diets (van Mosel et al., 1993; Vagnoni and Oetzel, 1998) may lower readily available bone Ca and so bone Ca reserves available for mobilisation after calving. It has been suggested that feeding higher dietary Ca concentrations pre-calving may reduce this effect (Lean et al., 2003). This hypercalciuric effect may be exacerbated with increased duration of exposure to a low DCAD diet pre-calving. Longer exposure to a pre-calving transition diet would, therefore, increase the incidence of milk fever as predicted in both models. Although increased urinary Ca loss on low DCAD diets has been demonstrated (van Mosel et al., 1993; Vagnoni and Oetzel, 1998), an effect of duration of exposure to the low DCAD diet on milk fever risk has not been established in trial work.

Equations for predicting DCAD that included Ca on the cationic side of the equation appear flawed as the effect predicted by the meta-analysis (Lean et al., 2006) is a linear increase in milk fever risk with increasing dietary Ca, whereas the effect of Ca on milk fever risk is quadratic (Oetzel, 1991; Lean et al., 2006). Similarly, Mg, if included on the cationic side of a DCAD equation, would have a linear effect of increasing the risk of milk fever. The opposite effect is observed (Contreras et al., 1982; Sampson et al., 1983; Wang and Beede, 1992; Goff, 2000) and is predicted using the equation LT1. Similarly to Ca, Mg is not included in the calculation of $[\text{SID}^+]$ (Constable, 1999). The equation LT1 predicts that the effect of increasing

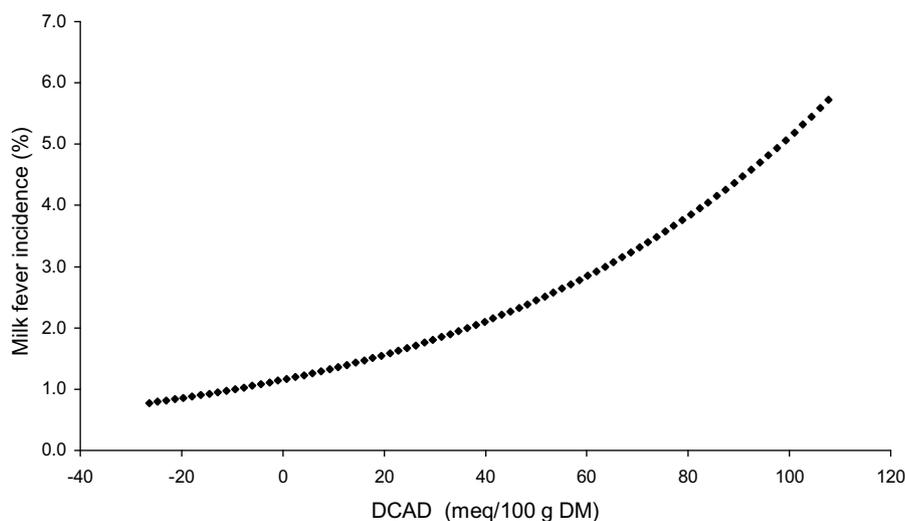


Fig. 1. Linear relationship between DCAD ($[\text{Na}^+ + \text{K}^+] - [\text{Cl}^- + \text{S}^{2-}]$) and risk of milk fever as calculated from the logit transformation of the linear regression developed by Lean et al. (2006) with dietary Ca, Mg and P set at 0.35%, 0.40% and 0.40% of DM, respectively, and duration of exposure to the pre-calving diet set at 14 days.

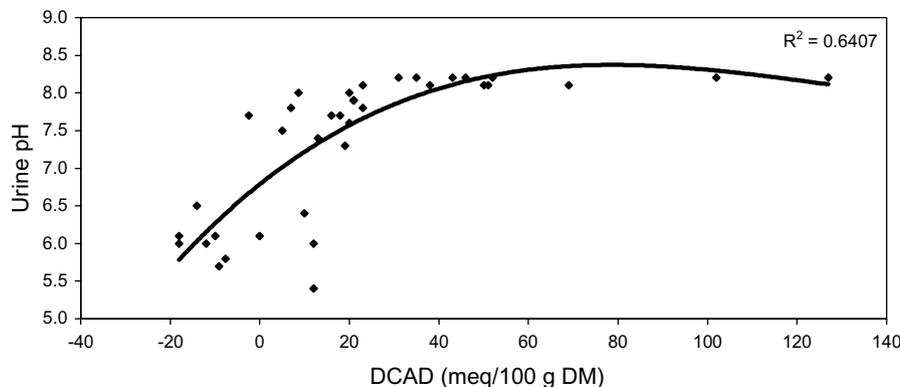


Fig. 2. Relationship between DCAD ($[\text{Na}^+ + \text{K}^+] - [\text{Cl}^- + \text{S}^{2-}]$) and urine pH. Modified from McNeill et al. (2002).

Mg concentration in the pre-calving diet is a very substantial decrease in the risk of milk fever (Lean et al., 2006).

P was thought to contribute to the anionic side of the DCAD equation, but high P concentrations have been associated with increased risk of milk fever (Julien et al., 1977; Kichura et al., 1982; Barton et al., 1987; Lean et al., 2003,2006). This is consistent with the simplified strong ion model of Constable (1999) that predicts increasing plasma phosphate will increase $[A_{\text{TOT}}]$ and increase plasma pH.

Length of exposure to the transition diet was a consistently significant variable in models developed with this variable and acted to substantially modify coefficients for Ca and Mg. Increasing exposure to the diet before calving from 20 to 30 days increased risk of milk fever by 42%. Therefore, it was necessary to include this variable in models, in contrast to those previously developed by Oetzler (1991) and Enevoldsen (1993).

During the process of developing the meta-analysis of the milk fever data (Lean et al., 2006), an interesting interaction between the quadratic effect of Ca and duration of exposure to the pre-calving diet was revealed (Fig. 3). The term had statistical significance in some of the fixed effects models and although this term was not included in the final random effects models ($P = 0.09$), its implications are worthy of further exploration. While recommendations have suggested that high dietary Ca concentrations are needed with low DCAD, the analysis of Lean et al. (2006) provides evidence that this approach may not be appropriate, because the effect of Ca, and the interaction between Ca and length of exposure to diet before calving, are independent of DCAD. Fig. 3 shows that with short exposure to the pre-calving diet (12–18 days), high concentrations of dietary Ca greatly increase the risk of milk fever. It is likely that this effect takes place after approximately 4 days' exposure based on the time taken to re-establish normal blood Ca when cows are exposed to a Ca deficient diet (Goings et al., 1974).

With increasing exposure to diets with excess Ca, it is possible that Ca stores are increased via passive absorption and, when combined with a high Ca diet post-calving, is

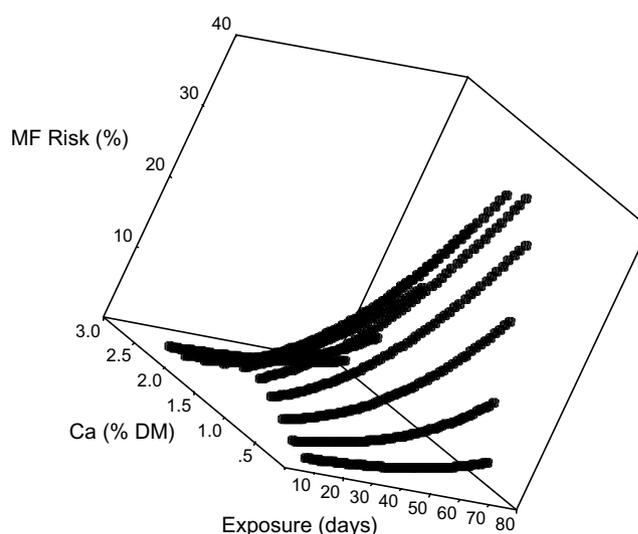


Fig. 3. Relationship between milk fever risk, days exposed to the pre-calving diet and dietary calcium (% of DM) at a high DCAD (+25 meq/100 g DM). Modified from Lean et al. (2006).

protective against milk fever. There are however, no experimental data to support this assumption at a physiological level. The increased risk over time for cows exposed to a pre-calving diet deficient in Ca may be explained by a gradual loss of bone Ca stores resulting in increased risk of milk fever post-calving. The peak risk of milk fever with long exposures to pre-calving diets occurs at approximately 1.1–1.3% Ca. The lack of data and a demonstrated physiological effect of pre-calving diets high in Ca are topics in need of urgent research.

From the variables included in the equation used to predict milk fever, developed by Lean et al. (2006), it is obvious that both the DCAD as calculated by $(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{S}^{2-})$ and macro-mineral concentrations, in particular Ca and Mg, of the diet are of great importance in determining the risk of milk fever, as is the breed of cattle and the time spent on a pre-calving transition diet.

While there were no trials included in the meta-analysis of Lean et al. (2006) that examined the effect of spending

between 1 and 11 days on transition diets, there is anecdotal evidence that effective prevention of milk fever can be achieved with exposure to a low DCAD, low Ca diet for as few as 3–5 days, and this is supported both by data demonstrating the activation of Ca homeostatic mechanisms with 4 days of exposure to a low Ca diet (Goings et al., 1974), and by data showing effective urinary acidification within 7 days of exposure to a low DCAD transition diet (P.J. DeGaris – unpublished data).

In summary, the mineral nutritional management needed to effectively prevent milk fever requires attention to both macro-mineral content of the diet as well as to the DCAD. Manipulation of macro-mineral nutrition is needed to stimulate Ca homeostatic mechanisms, and manipulation of DCAD to induce a strong ion metabolic acidosis is needed to quickly stimulate Ca turnover. Time spent on a low DCAD diet pre-calving is also important with the potential for long exposures, particularly if the diet is also low in Ca, to increase risk of milk fever.

The equations developed by Lean et al. (2006) have allowed the prediction of optimal dietary concentration of the major minerals for the prevention of milk fever. The practical power of the predictive model is that mineral concentrations can be manipulated to account for concentrations of other minerals in the diet. For example, if forage K concentrations are high and the forage is cheap, then the inclusion of higher concentrations of Mg, lower concentrations of Ca and DCAD become more critical. Broadly, intakes of Ca in the range of 50–70 g/day, Mg intake of 40–50 g/day, P intake of <35 g/day and a DCAD between +15 and –15 meq/100 g DM are consistent with good control of milk fever risk. However, we stress that careful evaluation of diets using the predictive model from equation LT1 is preferable to application of broad recommendations.

Effects of exposure to pre-calving diet: Production, reproduction and health

Grummer (1995) stated that: “If transition feeding is important, then perturbations in nutrition during this period should affect lactation, health and reproductive performance.” While physiological research on the impact of manipulating various fractions of the pre-calving diet is substantial, studies on the effects of integrating dietary strategies and providing an ‘optimal’ pre-calving diet are rare. A prospective cohort study was used to examine the effect of increasing days of exposure to ‘optimally’ formulated pre-calving diets on subsequent production (DeGaris et al., 2004a); reproduction (DeGaris et al., 2004b) and health (DeGaris et al., 2004c).

The pre-calving diets contained on a DM basis, 16.0% CP, 4.2% rumen undegradable protein, and 10.7 MJ of metabolisable energy. The diet provided an average metabolisable protein balance of 286 g/day of metabolisable energy balance of 18.9 MJ and a dietary cation anion difference of –15.0 meq/100 g DM. The macro-mineral concentrations of the diet were consistent with those required for milk fever prevention as evidenced by the extremely low rate of milk fever across the study period (<0.5%). The diets consisted of ryegrass pasture, ryegrass silage or cereal hay, grain or grain by-product, canola or cottonseed meals, BioChlor, Na monensin, virginiamycin or tylosin, MgSO₄, trace elements and vitamins.

Increasing exposure to the pre-calving diet significantly increased 4.0% fat and 3.2% protein corrected milk (FPCM) yield and milk protein yield as a linear and quadratic effect (Figs. 4 and 5).

The increase in production found between minimal exposure (3 days or less) and optimal exposure (22 days for FPCM and 25 days for milk protein yield) was approx-

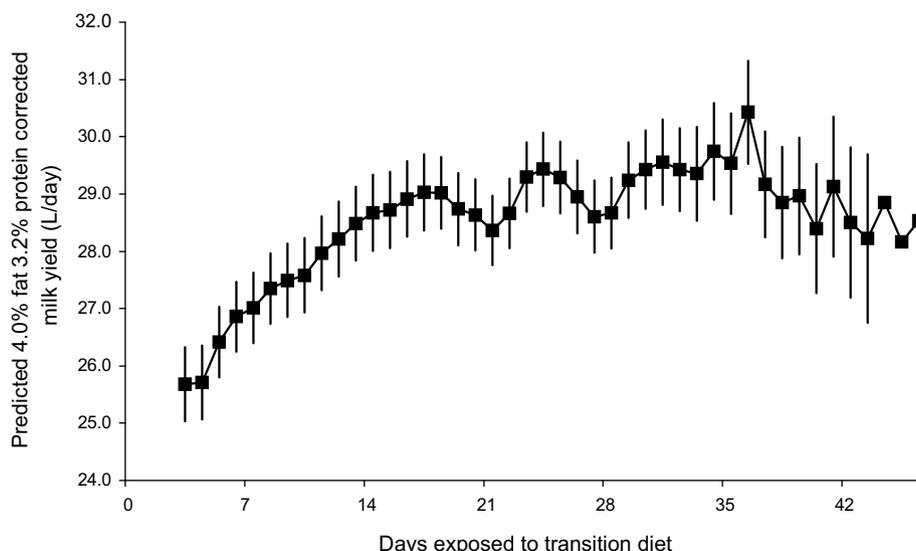


Fig. 4. Four-day moving average and 95% confidence interval of predicted 4.0% fat and 3.2% protein corrected milk yield with increasing days exposed to the pre-calving transition diet.

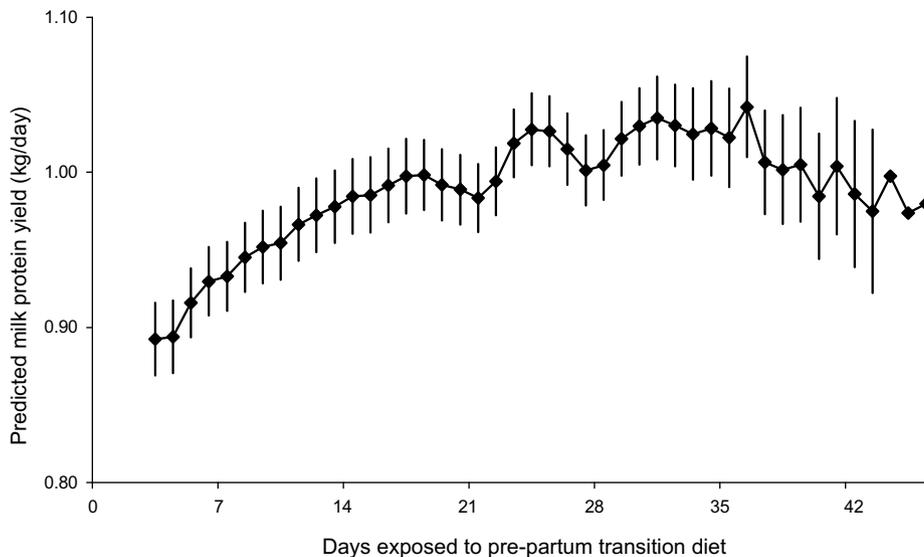


Fig. 5. Four-day moving average and 95% confidence interval of predicted milk protein yield with increasing days exposed to the pre-calving transition diet.

imately 3.75 L of FCPM per day and 100 g of milk protein per day. The magnitude of this effect is similar to those seen by Corbett (2002) and DeGroot (2004). Increasing exposure to the pre-calving diet also significantly improved reproductive measures and lowered the risk of removal from the herd. The risk of breeding per day exposed to the pre-calving diet significantly increased the risk of breeding by 1.015 (95% CI = 1.004–1.027). The risk of conception per day of exposure also significantly increased by 1.019 (95% CI = 1.008–1.030). This effect is large and is well demonstrated in Fig. 6, showing the cumulative pregnancy rate for cows exposed to the diet for <10 days, those

exposed for 10–20 days and those cows exposed for >20 days. The risk of being removed from the herd by day 150 of lactation due to voluntary culling or death per day decreased significantly by 4.7% per day (Hazard ratio 0.953; 95% CI = 0.913–0.981) with each day exposed to the transition diets.

Conclusions

While further studies are required on a number of aspects of milk fever, particularly in relation to the length of exposure to diets and Ca concentrations, many tools are now present to control the risk of milk fever. The exposure studies conducted by DeGaris et al. (2004a,b,c) suggest that positive dietary changes in the pre-calving diet will result in substantial production, reproduction and health benefits.

Conflict of interest statement

The two authors of the paper entitled *Milk fever in dairy cows: A review of pathophysiology and control principles* are Peter Degaris and Ian Lean. Peter Degaris advises on feeds that control milk fever risk and sells products to treat and prevent milk fever. Ian Lean has conducted research and consulted to companies producing products to control milk fever; he is involved with importation to Australia and sale of products that control milk fever risk.

References

- Akerman, K.E.O., 1978. Effect of pH and Ca^{2+} on retention of Ca^{2+} by rat liver mitochondria. *Archive of Biochemistry and Biophysics* 189, 156–262.
- Allsop, T.F., Pauli, J.V., 1985. Failure of 25-hydroxycholecalciferol to prevent milk fever in dairy cows. *New Zealand Veterinary Journal* 13, 19–22.

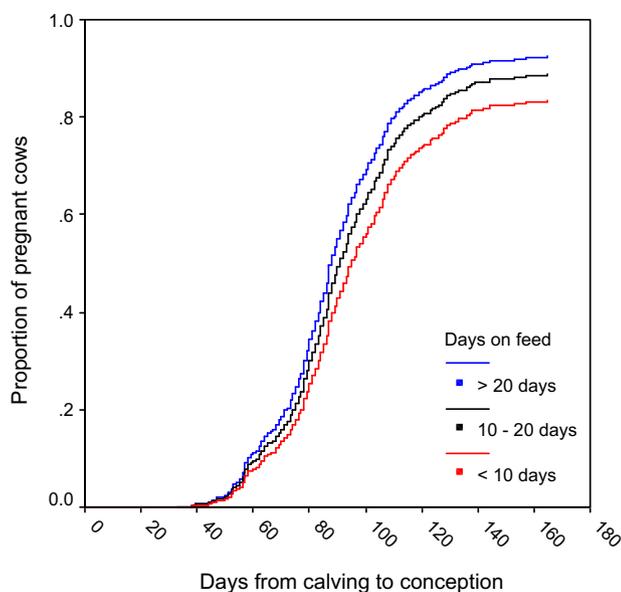


Fig. 6. Survival graph for calving to conception and cumulative pregnancy for cows exposed to the pre-calving transition diet for <10, 10–20 and >20 days.

- Bar, A., Striem, S., Perlman, R., Sachs, M., 1988. Use of 1 alpha-hydroxyvitamin D3 in prevention of bovine parturient paresis. 8. Maternal and neonatal plasma calcium, parathyroid hormone and vitamin D metabolite concentrations. *Journal of Dairy Science* 71, 2723–2729.
- Barton, B.A., Hodnett, D.W., Jorgensen, N.A., DeLuca, H.G., 1984. 24,25-Dihydroxyvitamin D3 administration increases incidence of parturient paresis. *Journal of Dairy Science* 67, 1236–1239.
- Barton, B.A., Jorgensen, N.A., DeLuca, H.F., 1987. Impact of prepartum dietary phosphorus intake on calcium homeostasis at parturition. *Journal of Dairy Science* 70, 1186–1191.
- Bauman, D.E., Currie, W.B., 1980. Partitioning of nutrients during pregnancy and lactation: a review of mechanism involving homeostasis and homeorhesis. *Journal of Dairy Science* 63, 1514–1529.
- Beck, N., Webster, S.K., 1976. Effects of acute metabolic acidosis on parathyroid hormone action and calcium mobilisation. *American Journal of Physiology* 230, 127–131.
- Beede, D.K., Sanchez, W.K., Wang, C., 1992. Macrominerals. In: Van Horn, H.H., Wilcox, C.J. (Eds.), *Large Dairy Herd Management*. University of Florida, Gainesville, USA, pp. 272–286.
- Block, E., 1984. Manipulating dietary anions and cations for prepartum dairy cows to reduce incidence of milk fever. *Journal of Dairy Science* 67, 2939–2948.
- Boda, J.M., 1956. Further studies on the influence of dietary calcium and phosphorus on the incidence of milk fever. *Journal of Dairy Science* 39, 66–72.
- Boda, J.M., Cole, H.H., 1954. The influence of dietary calcium and phosphorus on the incidence of milk fever. *Journal of Dairy Science* 37, 360–372.
- Chamberlain, A.T., 1987. The management and prevention of the downer cow syndrome. In: *Proceedings of the British Cattle Veterinarians Association*. Nottingham, England, pp. 20–30.
- Charbonneau, E., Pellerin, D., Oetzel, G.R., 2006. Impact of lowering dietary cation–anion difference in nonlactating dairy cows: a meta-analysis. *Journal of Dairy Science* 89, 537–548.
- Constable, P.D., 1997. A simplified strong ion model for acid–base equilibria: application to horse plasma. *Journal of Applied Physiology* 83, 297–311.
- Constable, P.D., 1999. Clinical assessment of acid–base status: strong ion difference theory. *The Veterinary Clinics of North America: Food Animal Practice* 15, 447–471.
- Contreras, P.A., Manston, R., Samson, B.F., 1982. Calcium homeostasis in hypomagnesaemic cattle. *Research in Veterinary Science* 33, 10–16.
- Corbett, R.B., 2002. Influence of days fed a close-up dry cow ration and heat stress on subsequent milk production in western dairy herds. *Journal of Dairy Science* 85 (Suppl. 1), 191.
- Curtis, C.R., Erb, E.H., Sniffen, C.J., 1983. Association of parturient hypocalcemia with eight periparturient disorders in Holstein cows. *Journal of the American Veterinary Medical Association* 183, 559.
- Curtis, C.R., Erb, E.H., Sniffen, C.J., Smith, R.D., Kronfeld, D.S., 1985. Path analysis of dry period nutrition, postpartum metabolic and reproductive disorders, and mastitis in Holstein cows. *Journal of Dairy Science* 68, 2347–2360.
- Curtis, M.A., Lean, I.J., 1998. Path analysis of metabolic and antioxidant risk factors for periparturient and postparturient conditions and reproductive performance in dairy cows. In: *Proceedings of the XXth World Buiatrics Conference*. Sydney, Australia, pp. 809–818.
- DeGaris, P.J., Lean, I.J., McNeill, D.M., Rabiee, A.R., 2004a. Effects of increased exposure to pre-calving diets containing Biochlor: reproductive performance. *Journal of Dairy Science* 82 (Suppl. 1), 439.
- DeGaris, P.J., Lean, I.J., McNeill, D.M., Rabiee, A.R., 2004b. Effects of increased exposure to pre-calving diets containing Biochlor: milk production. *Journal of Dairy Science* 82 (Suppl. 1), 439.
- DeGaris, P.J., Lean, I.J., McNeill, D.M., Rabiee, A.R., 2004c. Effects of increased exposure to pre-calving diets containing Biochlor: cow health. *Journal of Dairy Science* 82 (Suppl. 1), 440.
- DeGroot, M.A., 2004. The effect of prepartum anionic supplementation on periparturient feed intake and behaviour, health and milk production. Doctor of Philosophy in Animal Sciences, Oregon State University, USA.
- Dishington, I.W., 1975. Prevention of milk fever (hypocalcemia paresis puerperalis) by dietary salt supplements. *Acta Veterinaria Scandinavica* 16, 503–512.
- Dishington, I.W., Bjornstad, J., 1982. Prevention of milk fever by dietary means. *Acta Veterinaria Scandinavica* 23, 336–343.
- Dohoo, I.R., Martin, S.W., McMillan, I., Kennedy, B.W., 1984. Disease, production and culling in Holstein-Friesian cows: Part ii, Age, season and sire effects. *Preventative Veterinary Medicine* 2, 655–670.
- Ender, F., Dishington, I.W., Helgebostad, A., 1962. Parturient paresis and related forms of hypocalcaemic disorders induced experimentally in dairy cows. *Acta Veterinaria Scandinavica* 3, 5–52.
- Enevoldsen, C., 1993. Nutritional risk factors for milk fever in dairy cattle: meta-analysis revisited. *Acta Veterinaria Scandinavica* (Suppl. 89), 131–134.
- Erb, H.N., Smith, R.D., Oltenacu, P.A., Guard, C.L., Hillman, R.B., Powers, P.A., Smith, M.C., White, M.E., 1985. Path model of reproductive disorders and performance, milk fever, mastitis, milk yield and culling in Holstein cows. *Journal of Dairy Science* 68, 3337–3349.
- Fredeen, A.H., DePeeters, E.J., Baldwin, R.L., 1988a. Characterisation of acid–base disturbances on calcium and phosphorus balances of dietary fixed ions in pregnant or lactating does. *Journal of Animal Science* 66, 157–173.
- Fredeen, A.H., DePeeters, E.J., Baldwin, R.L., 1988b. Effects of acid–base disturbances caused by differences in dietary fixed ion balance on kinetics of calcium metabolism in ruminants with high calcium demand. *Journal of Animal Science* 66, 174–184.
- Gast, D.R., Horst, R.L., Jorgensen, N.A., DeLuca, H.F., 1979. Potential use of 1,25-dihydroxycholecalciferol for prevention of parturient paresis. *Journal of Dairy Science* 62, 1009–1013.
- Gaynor, P.J., Mueller, F.J., Miller, J.K., Ramsey, N., Goff, J.P., Horst, R.L., 1989. Parturient hypocalcemia in Jersey cows fed alfalfa haylage-based diets with different cation to anion ratios. *Journal of Dairy Science* 72, 2525–2531.
- Goff, J.P., 1999. Treatment of calcium, phosphorus and magnesium balance disorders. *The Veterinary Clinics of North America: Food Animal Practice* 15, 619–639.
- Goff, J.P., 2000. Pathophysiology of calcium and phosphorus disorders. *The Veterinary Clinics of North America: Food Animal Practice* 16, 319–337.
- Goff, J.P., Horst, R.L., Beitz, D.C., Littledike, E.T., 1988. Use of 24-F-1, 25-dihydroxyvitamin D3 to prevent parturient paresis in dairy cows. *Journal of Dairy Science* 71, 1211–1219.
- Goff, J.P., Horst, R.L., Mueller, F.J., Miller, J.K., Kiess, G.A., Dowlen, H.H., 1991. Addition of chloride to a prepartal diet high in cations increases 1,25-dihydroxyvitamin D response to hypocalcemia preventing milk fever. *Journal of Dairy Science* 74, 3863–3871.
- Goff, J.P., Ruiz, R., Horst, R.L., 2004. Relative acidifying activity of anionic salts commonly used to prevent milk fever. *Journal of Dairy Science* 87, 1245–1255.
- Goings, R.L., Jacobson, N.L., Beitz, D.C., Littledike, E.T., Wiggers, K.D., 1974. Prevention of parturient paresis by a prepartum calcium deficient diet. *Journal of Dairy Science* 57, 1184–1188.
- Gould, G.N.J., 1933. The provision of minerals for milking cows by feeding and fertilizing. *Veterinary Record* 13, 7–13.
- Green, H.B., Horst, R.L., Beitz, D.C., Littledike, E.T., 1981. Vitamin D metabolites in plasma of cows fed a prepartum low-calcium diet for prevention of parturient hypocalcemia. *Journal of Dairy Science* 64, 217–226.
- Grummer, R.R., 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. *Journal of Animal Science* 73, 2820–2833.
- Harris, D.J., 1981. Factors predisposing to parturient paresis. *Australian Veterinary Journal* 57, 357–361.
- Heuer, C., Schukken, Y.H., Dobbelaar, P., 1999. Postpartum body condition score and results from the first test day milk as predictors of

- disease, fertility, yield and culling in commercial dairy herds. *Journal of Dairy Science* 82, 295–304.
- Holler, H., Breeves, G., Kocabatmax, M., Gredes, H., 1988. Mineral absorption across isolated rumen mucosa. *Queensland Journal of Experimental Physiology* 73, 609–611.
- Horst, R.L., DeLuca, H.F., Jorgensen, N.A., 1978. The effect of age on calcium absorption and accumulation of 1,25-dihydroxyvitamin D₃ in intestinal mucosa of rats. *Metabolic Bone Disease and Related Research* 1, 29–33.
- Horst, R.L., Goff, J.P., Reinhardt, T.A., 1990. Advancing age results in reduction of intestinal and bone 1,25-dihydroxyvitamin D receptor. *Endocrinology* 126, 1053–1057.
- Horst, R.L., Goff, J.P., Reinhardt, T.A., Buxton, D.R., 1997. Strategies for preventing milk fever in dairy cattle. *Journal of Dairy Science* 80, 1269–1280.
- Horst, R.L., Littledike, E.T., Partridge, J.J., Reinhardt, T.A., 1983. Plasma concentrations of 1,25-dihydroxyvitamin D, 1,24R,25-trihydroxyvitamin D₃ and 1,25,26-trihydroxyvitamin D₃ after administration to dairy cows. *Journal of Dairy Science* 66, 1455–1460.
- Hove, K., 1984. Effects of 1 hydroxylated metabolites of cholecalciferol on intestinal radiocalcium absorption in goats. *British Journal of Nutrition* 51, 157–164.
- Huber, T.L., Wilson, R.C., Stattleman, A.J., Goetsch, D.D., 1981. Effect of hypocalcaemia on motility of the ruminant stomach. *American Journal of Veterinary Research* 42, 1488–1490.
- Jardon, P.W., 1995. Using urine pH to monitor anionic salt programs. *Compendium of Continuing Education for the Practicing Veterinarian* 17, 860–862.
- Julien, W.E., Conrad, H.R., Hibbs, J.W., Crist, W.L., 1977. Milk fever in dairy cows. viii. Effect of injected vitamin D₃ and calcium and phosphorus intake on incidence. *Journal of Dairy Science* 60, 431–436.
- Kichura, T.S., Horst, R.L., Beitz, D.C., Littledike, E.T., 1982. Relationships between prepartal dietary calcium and phosphorus, vitamin D metabolism, and parturient paresis in dairy cows. *Journal of Nutrition* 112, 480–487.
- Lean, I.J., DeGaris, P.J., McNeil, D.M., Block, E., 2006. Hypocalcaemia in dairy cows: meta analysis and dietary cation anion difference theory revisited. *Journal of Dairy Science* 89, 669–684.
- Lean, I.J., DeGaris, P.J., Wade, L.K., Rajczyk, Z.K., 2003. Transition management of dairy cows. In: *Proceedings of the Australian & New Zealand Combined Dairy Veterinarians' Conference*. Taupo, New Zealand, pp. 221–248.
- Leclerc, H., Block, E., 1989. Effects of reducing dietary cation–anion balance for prepartum dairy cows with specific reference to hypocalcaemic parturient paresis. *Canadian Journal of Animal Science* 69, 411–423.
- Littledike, E.T., Horst, R.L., 1982. Vitamin D₃ toxicity in dairy cows. *Journal of Dairy Science* 65, 749–759.
- Lomba, F., Chauvaux, G., Teller, E., Lengeis, L., Bienfet, V., 1978. Calcium digestibility in cows as influenced by the excess of alkaline ions over stable acid ions in their diets. *British Journal of Nutrition* 39, 425–429.
- McNeill, D.M., Roche, J.R., McLachlan, B.P., Stockdale, C.R., 2002. Nutritional strategies for the prevention of hypocalcaemia at calving for dairy cows in pasture-based systems. *Australian Journal of Agricultural Research* 53, 755–770.
- Moodie, E.W., Robertson, A., 1962. Some aspects of calcium metabolism in the dairy cow. *Research in Veterinary Science* 3, 470–484.
- Mullen, P.A., 1975. Clinical and biochemical responses to the treatment of milk fever. *Veterinary Record* 97, 87–92.
- Norman, H.D., van Vleck, L.D., 1972. Type appraisal: Part ii. variation in type traits due to sires, herds and years. *Journal of Dairy Science* 55, 1717–1725.
- Oetzel, G.R., 1991. Meta-analysis of nutritional risk factors for milk fever in dairy cattle. *Journal of Dairy Science* 74, 3900–3912.
- Oetzel, G.R., 2000. Management of dry cows for the prevention of milk fever and other mineral disorders. *The Veterinary Clinics of North America: Food Animal Practice* 16, 369–386.
- Oetzel, G.R., Fettefn, M.J., Hamar, D.W., 1991. Screening of anionic salts for palatability, effects on acid–base status, and urinary calcium excretion in dairy cows. *Journal of Dairy Science* 74, 965–971.
- Oetzel, G.R., Olson, J.D., Curtis, D.R., Fettefn, M.J., 1988. Ammonium chloride and ammonium sulphate for prevention of parturient paresis in dairy cows. *Journal of Dairy Science* 71, 3302–3309.
- Payne, J.M., 1977. Parturient paresis. In: *Metabolic diseases in farm animals*. Heinemann Medical Books, London, UK, pp. 61–83.
- Petrie, L., Breeze, R.G., 1977. Hypervitaminosis D and metastatic pulmonary calcification in a cow. *Veterinary Record* 101, 480–482.
- Phillippo, M., Reid, G.W., Nevison, I.M., 1994. Parturient hypocalcaemia in dairy cows: effects of dietary acidity on plasma mineral and calciotropic hormones. *Research in Veterinary Science* 56, 303–309.
- Rajala-Schultz, P.J., Grohn, Y.T., McCulloch, C.E., 1999. Effects of milk fever, ketosis and lameness on milk yield in dairy cows. *Journal of Dairy Science* 82, 288–294.
- Ramberg, C.F., 1972. Computer assisted modelling of the calcium system: kinetics of gastrointestinal calcium transport. In: Payne, J.M., Hibbitt, K.G., Sansom, B.F. (Eds.), *Production Diseases in Farm Animals*. Baillière Tindall, London, UK, pp. 195–200.
- Ramberg Jr., C.F., 1995. Kinetic overview: modeling calcium metabolism in pregnant and lactating cows. In: Siva Subramanian, K.N., Wastney, M.E. (Eds.), *Kinetic Models of Trace Element and Mineral Metabolism During Development*. CRC Press, Boca Raton, Florida, USA, pp. 11–28.
- Ramberg, C.F., Ferguson, J.D., Galligan, D.T., 1996. Feeding and managing the transition cow: Metabolic basis of the cation anion difference concept. In: *Proceedings of the Pennsylvania State Annual Nutrition Conference*. Pennsylvania, USA, pp. 19–30.
- Ramberg, C.F., Johnson, E.K., Fargo, R.D., Kronfeld, D.S., 1984. Calcium homeostasis in cows with special reference to parturient hypocalcaemia. *American Journal of Physiology* 246, 689–704.
- Ramberg, C.F., Mayer, G.P., Kronfeld, D.S., Potts, J.T., 1976. Dietary calcium, calcium kinetics and plasma parathyroid hormone concentration in cows. *Journal of Nutrition* 106, 671–679.
- Sachs, M., Bar, A., Cohen, R., Mazur, Y., Mayer, E., Hurwitz, S., 1997. Use of 1 alpha-hydroxycholecalciferol in the prevention of bovine parturient paresis. *American Journal of Veterinary Research* 38, 2039–2041.
- Sampson, B.F., Manston, R., Vagg, M.J., 1983. Magnesium and milk fever. *Veterinary Record* 112, 447–449.
- Shank, R.D., Freeman, A.E., Dickinson, F.N., 1981. Postpartum distribution of costs and disorders of health. *Journal of Dairy Science* 64, 683–688.
- Singer, R.B., Hastings, A.B., 1948. An improved clinical method for the estimation of disturbances of the acid–base balance of human blood. *Medicine* 27, 223–242.
- Spears, J.W., Burns, J.C., Hatch, P.A., 1985. Sulphur fertilization of cool season grasses and effect on utilization of minerals, nitrogen and fibre by steers. *Journal of Dairy Science* 68, 347–355.
- Stevenson, J.S., Call, E.P., 1988. Reproductive disorders in the periparturient dairy cow. *Journal of Dairy Science* 71, 2572–2583.
- Stevenson, M.A., Lean, I.J., 1998. Descriptive epidemiological study on culling and deaths in eight dairy herds. *Australian Veterinary Journal* 76, 482–488.
- Stewart, P.A., 1981. Strong ions, plus carbon dioxide, plus weak acid, isolated blood plasma and isolated intracellular fluid. In: *How to Understand Acid–base: A Quantitative Acid base Primer for Biology and Medicine*. Elsevier, New York, USA, pp. 110–144.
- Thilsing-Hansen, T., Jorgensen, R.J., Ostergaard, S., 2002. Milk fever control principles: a review. *Acta Veterinaria Scandinavica* 43, 1–19.
- Tucker, W.B., Hogue, J.F., Waterman, D.F., Swenson, T.S., Xin, A., Hemken, R.W., Jackson, J.A., Adams, G.D., Spicer, L.J., 1991. Role of sulphur and chloride in the dietary cation–anion balance equation for lactating dairy cattle. *Journal of Animal Science* 69, 1205–1213.

- Vagnoni, D.B., Oetzel, G.R., 1998. Effects of dietary cation–anion difference on the acid–base status of dry cows. *Journal of Dairy Science* 81, 1643–1652.
- van de Braak, A.E., Van't Klooster, A.T., Malestein, A., 1987. Influence of a deficient supply of magnesium during the dry period on the rate of calcium mobilization by dairy cows at parturition. *Research in Veterinary Science* 42, 101–108.
- van Mosel, M., Van't Klooster, A.T., van Mosel, F., Kuilen, J.V.D., 1993. Effects of reducing dietary $[(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{SO}_4^-)]$ on the rate of calcium mobilisation by dairy cows at parturition. *Research in Veterinary Science* 54, 1–9.
- Wang, C., Beede, D.K., 1992. Effects of diet magnesium on acid–base status and calcium metabolism of dry cows fed acidogenic salts. *Journal of Dairy Science* 75, 829–836.
- Wiggers, K.D., Nelson, D.K., Jacobson, N.L., 1975. Prevention of parturient paresis by a low-calcium diet prepartum: a field study. *Journal of Dairy Science* 58, 430–431.