

A novel model to explain dietary factors affecting hypocalcaemia in dairy cattle

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Abstract

Most dairy cows exhibit different degrees of hypocalcaemia around calving because the gestational Ca requirements shift to the disproportionately high Ca requirements of lactation. Ca homeostasis is a robust system that effectively adapts to changes in Ca demand or supply. However, these adaptations often are not rapid enough to avoid hypocalcaemia. A delay in the reconfiguration of intestinal Ca absorption and bone resorption is probably the underlying cause of this transient hypocalcaemia. Several dietary factors that affect different aspects of Ca metabolism are known to reduce the incidence of milk fever. The present review describes the interactions between nutrition and Ca homeostasis using observations from cattle and extrapolations from other species and aims to quantitatively model the effects of the nutritional approaches that are used to induce dry cows into an early adaptation of Ca metabolism. The present model suggests that reducing dietary cation–anion difference (DCAD) increases Ca clearance from the blood by dietary induction of systemic acidosis, which results in hypercalciuria due to the loss of function of the renal Ca transient receptor potential vanilloid channel TRPV5. Alternatively, reducing the gastrointestinal availability of Ca by reducing dietary Ca or its nutritional availability will also induce the activation of Ca metabolism to compensate for basal blood Ca clearance. Our model of gastrointestinal Ca availability as well as blood Ca clearance in the transition dairy cow allowed us to conclude that the most common dietary strategies for milk fever prevention may have analogous modes of action that are based on the principle of metabolic adaptation before calving.

Key words: Hypocalcaemia: Calcium homeostasis: Milk fever: Calving: Dietary cation–anion difference

Introduction

Teleological background of milk fever

Modern dairy cattle have a milk yield potential that greatly exceeds the nutritional needs of their offspring in quantities that can feed many more calves than the one or two they can bear. Under normal conditions, placental Ca transfer before calving is similar to Ca clearance into milk at the start of lactation⁽¹⁾. This is the case in beef cows but not in modern dairy cows, in which the Ca yield in milk greatly exceeds the needs of a newborn calf. Consequently, calving represents a great challenge for Ca metabolism, which requires rapid adaptations to sustain Ca homeostasis.

Cows were part of the first group of domesticated species 10 000 years ago⁽²⁾, and dairy breeds have existed for centuries. Much more recently, the intensification of dairy production brought cows into even closer symbiosis with man, which promoted the selection of certain genotypes. Consequently, diseases such as milk fever and

ketosis, which are specific to dairy breeds, have been induced by this process. The milk production capacity in dairy cows has been largely increased by genetic improvement, but the robust physiology required to sustain production may be lagging behind. Involuntary culling of high-producing cows increases as the production system intensifies^(3–5). High milk yield is positively related to disease incidence⁽⁶⁾, and negative genetic correlations have been calculated between milk production traits and disease resistance⁽⁷⁾. Nevertheless, these correlations are not necessarily causal because the intensification of dairy production includes many potential confounding factors, such as increased herd sizes and changes in farm management and feeding practices. While disease greatly increases involuntary culling, a high milk yield prevents voluntary culling⁽⁸⁾. Therefore, the limited longevity of modern dairy cows cannot be considered an unequivocal consequence of their extremely high productivity.

Darwin⁽⁹⁾ defined evolution under domestication as the cumulative result of two different means of selection,

Abbreviations: DCAD, dietary cation–anion difference; DMI, DM intake; GI, gastrointestinally; PTH, parathyroid hormone; TRPV, transient receptor potential vanilloid.

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'methodical selection' and 'unconscious selection'. 'Methodical selection' induces changes much faster than 'unconscious selection'. This principle may apply to dairy cows because modern genetic improvement has increased milk production within decades. Additionally, the high involuntary culling rates are a powerful means of 'unconscious selection' driven by insufficient disease resistance. If Darwin's intuition proves true, these means of selection will not soon provide a solution to this problem.

The mismatch between milk yield and disease resistance seems most dramatic in older cows because they reach maximum production. As cows age, they become more prone to ketosis⁽¹⁰⁾ and milk fever; specifically, there is a 9% increase in the incidence of milk fever with each lactation⁽¹¹⁾. Multiparous cows have a greater disparity in their nutrient intakes and yields at their lactation peaks compared with first parity cows. This disparity is most evident in the greater fluctuation in body condition scores during the production cycle⁽¹²⁾. Therefore, older cows experience a greater physiological adaptation of energy and mineral metabolism at the onset of lactation. The failure to adapt energy metabolism after calving results in ketosis. Similarly, the failure to adapt Ca metabolism can result in milk fever. Production diseases and infertility associated with the transition period result from an ineffective homeorhetic adaptation to the new physiological state⁽¹¹⁾. Among the many homeorhetic changes in nutrient partitioning during the transition between gestation and lactation⁽¹³⁾, adaptations in Ca metabolism seem to be especially critical. These dynamic adaptations should be coordinated without breaking the inflexible requirement of Ca homeostasis in the blood, which is the main junction of Ca partitioning.

The lifespan of dairy cows has declined in the last few decades. Only 40% of dairy cows live beyond two lactations, and very few reach the eighth lactation⁽³⁾. Currently almost all dairy cows are younger than one-third of the maximum lifespan of bovine species, which is estimated to be 30 years⁽¹⁴⁾. Therefore, the greater incidence of production diseases with increased parity is unlikely to be caused by ageing. Failure to adapt to the shifts in nutrient partitioning at calving, which becomes greater with age, may be a more plausible hypothesis to explain the increase in metabolic problems with increased parity.

Dairy nutritionists aim to facilitate the metabolic adaptations that occur around calving to prevent these health disorders. The final goal is to maintain animal welfare to consequently reduce replacement rates. This will improve the economic profitability and environmental footprint of dairy production⁽¹⁵⁾.

Definition of milk fever

Most cows have some degree of hypocalcaemia at calving; therefore, it is difficult to define milk fever as a discrete parameter. Serum Ca at calving drops below 2.0 mM in

25% of heifers, 41% in second lactation cows and up to 54% in fifth lactation cows⁽¹⁶⁾. It is generally accepted that cows requiring treatment are considered to be clinical cases of milk fever, although this definition remains subjective. Periparturient subclinical or clinical hypocalcaemia as discrete observations are imprecisely defined, but arbitrary boundaries of 2.0 and 1.4 mM have been proposed for serum Ca⁽¹⁷⁾. Serum Ca is, however, a continuous parameter that can objectively be measured.

Ca homeostasis seldom fails under natural conditions. Dairy cows around the time of calving represent an exception to this rule. Hypocalcaemia occurs within a few days of calving but does not persist despite the increased Ca demand during early lactation. The cause of hypocalcaemia is therefore a delayed response of the adaptive mechanisms of Ca metabolism that eventually restore homeostasis.

Calcium homeostasis

General definition

Evolution has provided elaborate mechanisms for the physiological control of Ca. At the cellular level, the concentration of ionic Ca in the cytoplasm remains several-fold below that of the extracellular environment. This is accomplished via regulation by membrane channels and complexation with proteins⁽¹⁸⁾. With the development of higher forms of life, an accurate control of Ca remained a high priority. Animals benefit from the precise homeostasis of blood Ca because positive or negative fluctuations can have fatal consequences. At the animal level, hormonal signals direct several Ca control mechanisms in different tissues. Transepithelial transport in the kidneys and intestine are the most important of these mechanisms⁽¹⁹⁾. Ca control is necessary to preserve vital functions, such as signalling and muscle contraction, the management of Ca reserves and the structural function of Ca in the bones.

Any change in influx or clearance of Ca in the blood challenges the homeostatic system. Positive fluctuations are naturally possible with high Ca intake, which results in increased, non-regulated, passive absorption. In this situation, the inflow would be quantitatively moderate. Ca infusions as a treatment for milk fever would be a non-physiological but extreme example of a positive fluctuation in blood Ca. Negative fluctuations are caused by increased clearance rates of Ca from the blood. This fluctuation, for example, occurs at the start of the lactation in the dairy cow or during hypercalciuria induced by metabolic acidosis.

The main framework of the hormonal system that controls Ca homeostasis in animals is well known. This system is mainly controlled by the coordinated action of parathyroid hormone (PTH) and calcitriol. This is also applicable to cattle^(11,20,21), although not all evidence

can be extrapolated from the most extensively studied single-stomached species. The points of similarity and the divergences between homeostatic control in ruminant and single-stomached species have been extensively reviewed by Schröder & Breves⁽²²⁾. Controversy remains about the role of ruminal Ca absorption in the homeostatic system. Active ruminal absorption of Ca has been demonstrated *in vitro*⁽²³⁾; however, *in vivo* validation⁽²⁴⁾ and the quantification of the significance of this effect⁽²⁵⁾ have not consistently been confirmed in ruminants. The latest data from goats suggest that active Ca absorption can occur simultaneously in the rumen and the intestine⁽²⁶⁾. Hence, the role in Ca regulation of the postruminal gastrointestinal segments, and by extension, in the overall gastrointestinal tract is assumed to be comparable with that of single-stomached species.

The Ca²⁺ sensing receptor in the parathyroid gland monitors blood Ca⁽²⁷⁾. This receptor was first identified in bovine parathyroid tissue two decades ago⁽²⁸⁾. The parathyroid will respond to a decrease in blood Ca by releasing PTH. This signal sustains the extensive recovery of Ca in the kidney, and its absence allows for increased urinary Ca excretion to compensate for positive fluctuations in blood Ca⁽²⁹⁾. On the contrary, an increase in PTH can slightly increase renal reabsorption, which is already high, in response to the negative fluctuations in blood Ca. This reaction occurs within hours after its secretion⁽³⁰⁾, but because urinary Ca is naturally low, the value of this action against hypocalcaemia is quantitatively limited. Additionally, PTH will act against hypocalcaemia by initiating bone Ca mobilisation and by inducing the hydroxylation of 25-hydroxyvitamin D to calcitriol (1,25-dihydroxyvitamin D) in the kidney. Calcitriol activates Ca absorption in the gastrointestinal tract, which represents a large Ca resource to compensate for Ca clearance. In rats, this adaptation has been proven to take longer than 1 d to take effect⁽³¹⁾. To our knowledge, this delay has not been directly observed in cattle; however, we have observed indirect indications of this delay by monitoring urinary Ca in cows, which suggest that the inactivation of gastrointestinal Ca absorption may present a 2 d delay^(29,32). Furthermore, calcitriol sustains bone Ca mobilisation, offering an extensive pool for sustaining Ca homeostasis during lactation⁽³³⁾; however, in the short term, the readily available bone Ca is limited⁽³⁴⁾. Calcitriol only sustains bone mobilisation if the PTH signal is maintained. This happens only when gastrointestinal absorption is insufficient to compensate for the Ca deficit⁽³⁵⁾. Consequently, this mechanism prioritises dietary Ca above bone Ca in the compensation of blood Ca levels.

Dairy cows constantly need to adapt their Ca metabolism during their reproductive cycle. During early lactation, Ca from bone is mobilised because dietary Ca is insufficient for the high amounts of Ca required for milk production, regardless of very efficient active gastrointestinal Ca absorption. At a certain point in lactation, dietary Ca

should suffice for the milk yield, but high intestinal absorption is maintained for several months to replenish bone reserves. In late lactation and the dry period, passive absorption is sufficient to compensate for low levels of blood Ca clearance, consisting of small faecal and urinary losses and fetal needs. This metabolic state suddenly changes to active absorption and bone mobilisation at calving, which requires very rapid adaptation to avoid hypocalcaemia. To understand the aetiology of milk fever, it is necessary to study the nature of these adaptive mechanisms and their ability to respond to the challenge of calving.

Adaptive mechanisms of calcium homeostasis

Practical animal nutrition often considers nutrient absorption to be a constant competence; however, Ca absorption is constantly changing due to endogenous signals and external stimuli from the diet. Many examples of these adaptations are known⁽³⁶⁾. Ca homeostasis has been described by Ramberg *et al.*⁽¹⁾ as a system with 'controlled signals, disturbing signals and controlling signals'. This approach suggests that the cause of milk fever could be a delay in the adaptive mechanisms ('controlling signals') that cannot provide timely responses to the sudden changes in Ca clearance from the blood. These adaptive mechanisms involve renal reabsorption, gastrointestinal absorption and bone turnover. These mechanisms are now much better understood thanks to new molecular techniques that have become available in the last few decades⁽³⁷⁾; data specific to ruminant species are beginning to emerge^(24,38,39). Renal reabsorption and intestinal absorption are both tightly regulated processes of transepithelial Ca transport. Bone turnover is a tissue modification process that is regulated in such a way that it can respond anabolically or catabolically to sustain Ca homeostasis while maintaining the structural function of bone.

Transepithelial transport processes. Passive, non-saturable Ca diffusion occurs through the tight junctions of epithelia when Ca gradients allow for this paracellular transport. This mechanism can be regulated in the intestine of single-stomached species by modifying epithelial permeability^(40,41). Nevertheless, the transcellular, saturable Ca transport processes are subject to greater regulation and thus play a prominent role in Ca homeostasis.

Ca reabsorption in the kidney and active gastrointestinal absorption are transcellular processes that are coordinated by the hormonal Ca homeostatic system and that transport Ca against the concentration gradient into the blood. Transcellular epithelial transport of Ca consists of the following three steps: facilitated entry into epithelial cells, intracellular diffusion mediated by a binding protein and active transport from the cell into the next extracellular compartment⁽³⁷⁾. Such an elaborate system allows for efficient and accurately controlled Ca transport while maintaining free intracellular Ca at a minimum.

Ca enters the epithelial cells through two highly specific Ca channels; these transient receptor potential vanilloid (TRPV) channels are TRPV5 (formerly known as ECaC1 or CaT2) and TRPV6 (formerly known as ECaC2 or CaT1). Ca entry, although a passive transfer, is believed to represent the limiting, key regulatory step of the process and is strongly regulated by calcitriol⁽⁴²⁾ and extracellular Ca concentration⁽⁴³⁾.

Once inside the epithelial cell, Ca diffuses through the cytoplasm. Luminal Ca concentration is kept extremely low to protect normal cell function. At a low concentration, simple diffusion cannot quantitatively provide the requirements of Ca transfer in the intestine or kidney. Two cytosolic Ca-binding proteins, calbindin-D_{9k} and calbindin-D_{28k}, have been described⁽³⁷⁾. These proteins are calcitriol-dependent and are responsible for ionic Ca buffering in the cell and facilitating intracellular diffusion in transepithelial transport⁽⁴⁴⁾. This step can limit transport across the cell because the lack of calbindin proteins impedes Ca transport⁽⁴⁴⁾.

The final step in transcellular transport is Ca export out of the cell into the bloodstream. This process is mediated by ATP via the following two active Ca transporters: the Ca-ATPase protein, also called plasma membrane Ca²⁺-ATPase (PMCA), and the Na⁺/Ca²⁺ exchanger (NCX)⁽⁴²⁾. This step is also regulated by calcitriol⁽³⁷⁾. However, this step is unlikely to be rate limiting⁽⁴⁴⁾, which reduces its importance in the control of transcellular Ca transport.

Despite overall similarities, there are specific differences between intestine and kidney in the transport of Ca across the epithelium. These differences enable their distinct roles in Ca homeostasis while other differences are derived from the distinct nature of these tissues. There are molecular differences between renal and intestinal transcellular Ca transport. The main Ca entry channel in the intestine is TRPV6⁽²⁷⁾, whereas TRPV5 is the only known entry channel in the kidney⁽⁴⁵⁾. In mammals, the predominant intracellular Ca-binding protein is calbindin-D_{28k} in the kidney and calbindin-D_{9k} in the intestine⁽⁴²⁾. It is understood that PTH predominantly controls renal Ca reabsorption and that calcitriol plays a similar role in the intestinal system⁽²²⁾. Vitamin D receptors are present in both tissues⁽³⁸⁾. Additionally, PTH can directly affect renal and intestinal transport processes⁽³⁷⁾.

These tissues differ in the specific regulation of the three steps of transepithelial transport because they respond differently to Ca hormones and Ca concentration in the compartments. In rats, the response of calbindin-D_{28k} in the kidney and calbindin-D_{9k} in the intestine to calcitriol differs in speed and intensity⁽³¹⁾. This same study described the age-dependent relationship of calbindin-D_{9k} expression. The responsiveness of calbindin-D_{9k} to calcitriol was not confirmed in the bovine intestine⁽³⁹⁾ or bovine rumen⁽⁴⁶⁾, but more recent data report calbindin-D_{9k} responses to calcitriol in the duodenum and rumen of goats⁽²⁶⁾. Ca channels also present differences in regulation. The structural

differences between TRPV5 and TRPV6 result in different down-regulation kinetics induced by Ca⁽⁴³⁾, which translates into further differences between renal and intestinal Ca transport.

The functions and tissue specificity of the two mentioned Ca-binding proteins are not universal across the animal kingdom. Calbindin-D_{9k} is the Ca-binding protein in the intestine, and calbindin-D_{28k} is the renal Ca-binding protein in all mammals, including bovines. In contrast, calbindin-D_{28k} is the intestinal and renal Ca-binding protein in birds⁽⁴⁷⁾. The function of transepithelial Ca channels is well preserved across animal species. Therefore, bovine species appear to share the main framework of transepithelial Ca transport with mice and man in intestinal and renal tissue, thus allowing careful extrapolation from the data generated within these species.

A specific characteristic of TRPV5 is its pH sensitivity. TRPV5 has approximately half of its normal activity during metabolic acidosis⁽²⁷⁾. This inhibition results in hypercalciuria, a common condition in cattle that are fed low-dietary cation–anion difference (DCAD) diets^(48,49). TRPV5 failure has been studied in TRPV5 gene knock-out mice. These animals combine the expected hypercalciuria with the hyperactivation of intestinal Ca absorption and present high circulating calcitriol levels and increased TRPV6 expression⁽⁵⁰⁾.

Prevention of milk fever with low-DCAD diets has been clearly demonstrated experimentally, although its mode of action is still unclear. It has been suggested that the bone mobilisation caused by metabolic acidosis would increase urinary Ca, causing increased intestinal absorption due to increased calcitriol levels⁽¹¹⁾. However, high calcitriol levels would not correspond with high urinary Ca because this should correspond with a depressed PTH signal. It has been proposed that lowering systemic pH would increase the functionality of calcitriol receptors that would have otherwise lost receptivity during metabolic alkalosis⁽⁵¹⁾; however, PTH responsiveness is adequate under physiological conditions with a high systemic pH, as in early lactation. TRPV5 inactivity under acidic conditions combined with the counter-reaction of intestinal TRPV6 represents a plausible mode of action for the prevention of milk fever by lowering the DCAD.

A major difference between renal and intestinal epithelial tissues is the lifespan of the epithelial cells. The intestine is characterised by a cell differentiation process of enterocyte maturation through migration from the crypts to the villi tips (Fig. 1). Intestinal epithelium cells have a short lifespan of approximately 4 d in poultry⁽⁵²⁾ and between 1 and 3 d in mice⁽⁵³⁾; however, the lifespan of renal cells is approximately 160 d in poultry⁽⁵²⁾ and many weeks in rats⁽⁵⁴⁾. This constant enterocyte turnover makes it biologically less essential for intestinal cells to have reversible regulatory mechanisms because they will soon be replaced by other enterocytes. Conversely, the lifespan of renal cells is too long to maintain a fixed

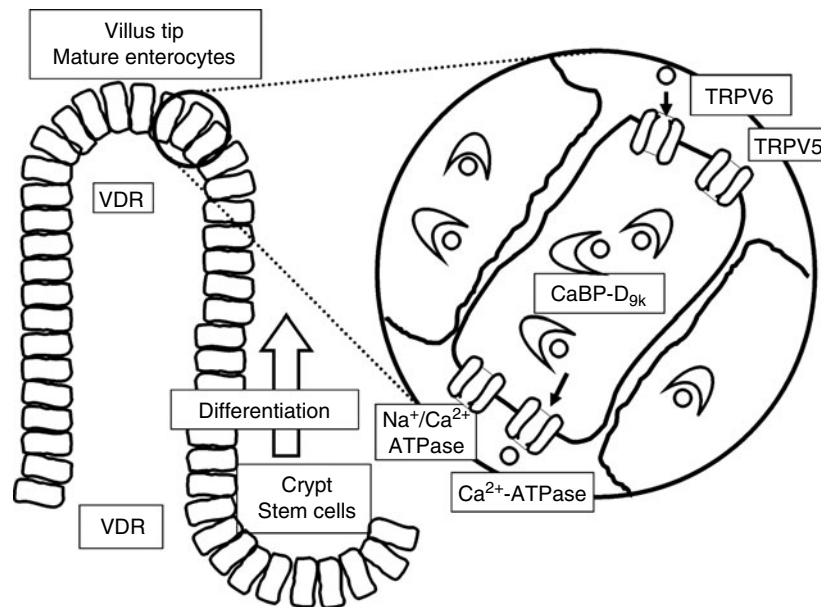


Fig. 1. Regulation of gastrointestinal calcium absorption. Schematic representation of the gastrointestinal transepithelial transport mechanism and its regulation by calcitriol as affected by enterocyte differentiation (based on rat studies). VDR, vitamin D receptor; TRPV5/6, calcium entry channels; CaBP-D_{9k}, intestinal calbindin; Ca²⁺-ATPase, Na⁺/Ca²⁺, calcium transporters.

regulatory configuration. It has been suggested that enterocytes of rodents acquire Ca transport competence in the early stages of differentiation and express that ability when they mature and reach the villi tips⁽⁵⁵⁾. This suggestion is supported by the fact that maximum Ca transport in rats is reached 24–48 h after the first calcitriol signal⁽⁵⁶⁾. This lag time coincides with the time required for the migration of crypt cells to the villi tips⁽⁵⁷⁾. The tips of the villi respond to calcitriol by increasing Ca uptake, whereas villus base cells do not respond in this way. The calbindin response is more evident in the base cells than in the cells of the villi tips⁽⁵⁸⁾. Additionally, vitamin D receptor presence in chickens is greater in crypt cells than in tip cells, which suggests that calcitriol induces the production of cells that are more capable of expressing calcitriol-dependent genes for active Ca absorption at the mature stage⁽⁵⁹⁾. The main consequence of the different adaptation to the calcitriol signal between the renal and intestinal tissues is the time delay before acquiring Ca transport competence. This delay also exists when the competence is resumed after the calcitriol signal ceases.

Avian intestinal calbindin synthesis peaks 10 h after calcitriol induction, and its presence in intestinal tissue reaches its maximum at 20 h. This level is maintained up to 48 h after induction⁽⁵²⁾. A similar pattern of the calbindin response to calcitriol in chickens has been explained by changes in the calbindin synthesis control mechanisms during enterocyte differentiation because calbindin mRNA is expressed maximally in the basal villus enterocytes⁽⁶⁰⁾. In contrast, rat studies show that TRPV6 is only expressed in villi tips⁽²⁷⁾, which further supports the hypothesis that the delay in positive or negative adaptation is a result of enterocyte differentiation (Fig. 1).

The rapid adaptation of renal reabsorption and delayed adaptation of intestinal absorption can explain the increases in urinary Ca excretion observed by our group in cows for 2 d after the withdrawal of a restriction in dietary Ca availability^(29,32). Urinary excretion of Ca is an effective means to correct positive fluctuations in blood Ca⁽¹⁾. We observed that when the dietary Ca restriction was withdrawn, the negative signal for Ca regulation disappeared, but gastrointestinal absorption remained up-regulated for about 2 d. This surplus of Ca is corrected by the down-regulation of renal reabsorption, and this down-regulation ceases when intestinal absorption is inactivated again after a 2 d delay.

Tissue remodelling. Bone remodelling is a very important adaptive mechanism of Ca homeostatic control. Osseous tissue requires Ca to maintain its structural function; therefore, this requirement is a factor of blood Ca clearance. Ramberg *et al.*⁽¹⁾ described the role of bone as a controlled, disturbing and controlling signal within the Ca control system. Bone Ca represents a quantitatively large source that sustains blood Ca levels in periods of Ca deficit during early lactation. Nevertheless, this Ca is not readily available to be brought into the bloodstream. Only a small fraction (less than 10 g) is in solution in bone fluids; the remainder is a part of the bone structure⁽³⁴⁾ and requires bone catabolism to be released. Bone remodelling is a continuous homeorhetic process that results from the anabolic actions of osteoblasts and the catabolic actions of osteoclasts. The prevalence of the activity of either of these cell types results in a net bone calcification or resorption. The control of bone tissue anabolism and catabolism presents great complexity because it involves direct hormonal regulation of the activity of these cells,

and the differentiation and maturation of these two cell lineages from stem cells is also regulated⁽⁶¹⁾.

Bone remodelling is controlled by local and systemic regulation⁽⁶²⁾, and it is through systemic hormonal control that it interacts with Ca homeostasis. The most important hormone affecting bone remodelling is PTH, but its action is tightly coordinated with calcitriol⁽⁶³⁾. PTH induces bone mobilisation when its signal is maintained over time. However, pulsatory administration of PTH has anabolic effects on bone⁽⁶⁴⁾. Calcitriol will cause opposite effects on bone Ca depending on its time of exposure. In contrast to PTH, calcitriol is catabolic in acute applications but suppresses resorption during continuous administration⁽³⁵⁾; thus, it can be a direct or indirect stimulator of bone formation.

The actions of these hormones on bone metabolism can only be understood as positive and negative signals. The PTH initiates bone resorption to compensate for decreases in blood Ca, but at the same time, it induces the synthesis of calcitriol, which, in turn, activates intestinal Ca absorption. If blood Ca is normalised by intestinal input, PTH ceases and consequently induces renal excretion of the surplus. In a situation of sufficient dietary Ca, the half-life of PTH is approximately 4 min⁽⁶⁵⁾, compared with a half-life of several hours for calcitriol. This difference would create a pulsatory PTH release and a continuous action of calcitriol to induce bone formation. If intestinal absorption does not suffice, the PTH signal is sustained and bone resorption continues. Through this coupling, bone resorption only occurs when intestinal absorption is insufficient for maintaining Ca homeostasis.

The transient regulatory effects of PTH and calcitriol are explained by their effects on the activity of bone remodelling cells and by the recruitment of these cells by the differentiation process. Osteoclasts are inhibited through the direct hormonal action of calcitonin⁽⁶⁶⁾, but their activation is mediated indirectly through osteoblasts⁽⁶⁷⁾. Osteoblasts have specific receptors for both PTH and calcitriol, and they act on the pre-osteoclasts by inducing a transformation into active osteoclasts. The activation of existing osteoclasts occurs within 6 h, but an increase in osteoclasts may take 2 d. In the case of sufficient Ca, this process is reversed by a decrease in osteoclasts by day 7, although bone mobilisation is sustained by a lack of sufficient dietary Ca⁽³⁵⁾.

The nature of the regulation of bone remodelling has several practical implications for the transition cow. First, adaptations do not occur fast enough after a sudden change in blood Ca clearance. As with intestinal adaptation, the involvement of a cell differentiation process delays any adaptation for approximately 2 d. Pregnant cows required 48 h of PTH stimulation to effectively mobilise bone⁽⁶⁸⁾. This lag time coincides with the time around calving when the cow suffers from hypocalcaemia. A second implication is that bone remodelling occurs only if the adaptation of intestinal absorption is not enough to

compensate for Ca clearance. These implications were clearly illustrated in an experiment where cows that had been fed a low-Ca diet for weeks were administered an EDTA infusion. The bone resorption marker hydroxyproline remained low during dietary restriction and peaked 2 d after the EDTA infusion⁽³⁴⁾. Finally, the pharmacological induction of adaptation by injections of vitamin D metabolites can yield effects that are the opposite of those intended. If bone adaptation is reversed 1 week after the application of sufficient dietary Ca, the calving challenge may occur when bone resorption is inhibited. This observation provides an explanation for some of the problems that were observed with the use of vitamin D to prevent milk fever^(69,70). With the use of vitamin D, the timing of the application was critical to prevent the disease.

Conclusion on adaptive mechanisms. Intestinal absorption and bone resorption present a delay of approximately 1 or 2 d when adapting to an increased Ca clearance from the blood. This time-frame coincides with the period around calving when cows suffer from hypocalcaemia. Renal adaptation is much faster, but reabsorption of Ca in the kidney is quantitatively too small to enable the cow to cope with the challenge of homeostasis around calving.

Induction of Ca homeostasis adaptations before calving should trigger gastrointestinal absorption, which is the first limiting mechanism in the reactions against hypocalcaemia. If the challenge is beyond the capacity of intestinal Ca absorption, bone remodelling will contribute to compensate for the Ca clearance from the blood.

Dietary-induced modulation of calcium metabolism

For many decades, dairy nutritionists have searched for a dietary strategy to prevent milk fever. The preference for dietary prevention is related to the ease of application. Moreover, nutrient imbalances tend to be understood as nutritional problems, although, as already discussed, milk fever should be considered to be a failure of physiological adaptation rather than a case of inadequate nutrient supply. In the middle of the last century, dietary strategies, such as vitamin D supplementation and changes in dietary Ca:P ratios, were proposed for the prevention of milk fever⁽⁷¹⁾. There is also a long history of studying low-Ca diets and the reduction of the DCAD to prevent milk fever. Presently, hypocalcaemia is managed in practice by integrated approaches that act on all known nutritional risk factors. Dietary preventive strategies and nutritional risk factors have been properly reviewed elsewhere^(11,72); therefore, only the effect of nutritional strategies as inducers of the adaptation of Ca metabolism will be discussed here.

Non-nutritional supply of vitamin D metabolites

Vitamin D, its hydroxylated forms (hydroxylation in position 1 or 25), and calcitriol in oral or injected applications

have been extensively tested for milk fever prevention in the past several decades^(73–76) as well as more recently⁽³³⁾. These experiences have been reviewed elsewhere⁽⁷⁷⁾. As expected, these acute applications induce hypercalcaemia by acting on gastrointestinal transport but not by activating bone resorption⁽⁷⁵⁾. These treatments reflect the above-described actions of calcitriol on the gastrointestinal tract, where serum Ca is increased but reaches a maximum only after 24 h⁽⁷⁶⁾. The protection that these applications may provide appears to be highly dependent on the timing of the application in relation to calving⁽⁷³⁾. These products received a large amount of attention in the past few decades. Although some injected applications remain in use, nutritional applications are difficult because the effective doses are too close to the toxic doses⁽⁶⁹⁾ and exceed some legal restrictions of vitamin D feeding (for example, that of the European Union). Furthermore, if the effectiveness is dependent on the timing of the application before calving, the implementation of these strategies is difficult in practice, as the exact calving date cannot be predicted days in advance.

Acute administration of vitamin D metabolites has been proven to modify Ca metabolism. The effects observed can be generally recognised as being analogous to the natural action of calcitriol. Nevertheless, it is our understanding that these applications do not constitute a feasible nutritional strategy to anticipate the adaptation of Ca metabolism to the upcoming lactation state. If the artificially induced up-regulation of Ca absorption coincides with the increased Ca clearance associated with calving, hypocalcaemia may be prevented. Nevertheless, if this artificial calcitriol signal is introduced too early and creates a hypercalcaemic state, the homeostatic system will be down-regulated to counteract the artificial calcitriol signal. PTH depression associated with the acute applications of vitamin D has been shown to induce hypercalciuria⁽⁷⁸⁾, as well as induce bone anabolism when combined with the sustained calcitriol signal, and ultimately cause soft tissue calcification⁽⁶⁹⁾. In our opinion, this would be an undesirable configuration of the homeostatic system of Ca during calving.

Low-calcium diets

Once milk fever was understood as a failure of Ca homeostasis, low-Ca diets were proposed to challenge Ca homeostasis and anticipate the necessary adaptation to calving. This strategy was proven effective in numerous studies^(79–84). The efficacy of this strategy has been suggested to be close to 100% when daily Ca intake is kept below 20 g/d⁽⁷²⁾. Creating an extreme dietary Ca shortage represents a preventive strategy that induces the adaptation of Ca metabolism. At the same time, dietary Ca within the common range is one of the multiple dietary factors that define milk fever risk. The effect of dietary Ca on the incidence of milk fever has been

characterised as quadratic in two meta-analyses^(85,86). These models identify a lower risk at low and high Ca intakes within the normal range.

Dietary Ca affects the incidence of milk fever by quadratic means with a maximum incidence at 11.6 g dietary Ca per kg DM intake (DMI)⁽⁸⁵⁾ or 13.5 g dietary Ca per kg DM⁽⁸⁶⁾. Apparently, a low level of dietary Ca initiates active Ca absorption before calving, thus preventing milk fever^(82–84). However, a high level of Ca intake around and after parturition allows for high passive Ca absorption, and this may compensate for the drain of Ca into the milk around calving.

Exploration of low-Ca diets has increased our understanding of the aetiology of the disorder, but reproducing the high preventive effectiveness of synthetic low-Ca diets described in the literature remains a challenge in practice. Reducing the Ca content of the diet is used as a risk factor to manage milk fever in a multifactorial approach. Nevertheless, formulating a dry cow ration below 1.5 g dietary Ca per kg DM is difficult to achieve with the main nutritional targets of these rations. Green forages exceed that Ca level by several-fold, leaving cereal straws as the only suitable source of effective fibre. These practical difficulties have restricted the implementation of low-Ca diets as a specific strategy for milk fever prevention.

Reduction of the dietary cation–anion difference

In commercial nutritional practice, the most widespread and successful dietary method for the prevention of milk fever has been the modification of the DCAD, which induces a moderate state of metabolic acidosis. The prophylactic value of acidifying blood and urine through the dietary modulation of the DCAD has been extensively documented^(49,87–90). The dietary cation–anion balance is calculated as the sum of the cation equivalents of Na and K content in the diet minus the anion equivalents of Cl and S⁽⁹⁰⁾ and is expressed in meq/kg DM in feed. The reduction of the DCAD is achieved in practice with the use of mineral salts containing S or Cl without Na or K, the so-called anionic salts.

Supplying anionic salts is a preventive dietary intervention, and the DCAD is also a dietary risk factor. The effect of the DCAD level on milk fever incidence has been modelled by linear regressions^(86,91). These models describe a curvilinear relationship between the DCAD and milk fever risk when the non-linear transformations of milk fever incidence data are considered. Increasingly, negative DCAD values approach minimal milk fever incidences asymptotically for a fixed set of other dietary factors⁽¹¹⁾. The urinary pH and urinary Ca respond to the DCAD in a similar fashion to the reduction of milk fever with a decreasing DCAD. Urinary pH^(49,86,91) and urinary Ca excretion⁽⁴⁹⁾ respond to DCAD with greater slopes as DCAD becomes negative.

The mode of action of the preventive effect of a low DCAD is still under discussion. A high DCAD has been explained as a negative factor for Ca metabolism^(89,92,93) rather than a negative DCAD as a positive factor against milk fever. It has been proposed that metabolic alkalosis reduces renal responsiveness to PTH⁽⁵¹⁾ and that this may be reversed by metabolic acidification. Furthermore, improved responsiveness to calcitriol at a lower DCAD has been suggested⁽⁹²⁾. However, a positive DCAD is not unique to the periparturient period. In fact, a positive DCAD is common in lactation diets, and its increase is advised for highly fermentable diets⁽⁹⁴⁾. Under these conditions, Ca homeostasis is adequately maintained during lactation.

Alternatively, the preventive effect of a low dietary DCAD can be explained as an inducer of the adaptation of Ca metabolism in the prevention of milk fever. When the DCAD is low enough, it can increase apparent Ca absorption in cows⁽⁴⁸⁾ as a response to the effect of the DCAD on urinary Ca excretion. Urinary Ca clearance from the blood must therefore be compensated at the first instance it occurs by increased intestinal absorption. This is because the reduction of endogenous intestinal Ca secretion is not among the control mechanisms of Ca homeostasis⁽¹⁾. As discussed in the Transepithelial transport processes section, the molecular mechanisms of hypercalciuria that is induced by anionic salts are now better understood from mouse studies as a result of a pH-related failure of renal TRPV5. It is plausible to suggest that lowering the DCAD prevents milk fever in a similar way to lowering dietary Ca. Both create a Ca deficit before calving and induce the adaptation of Ca metabolism by activating gastrointestinal absorption. If this is insufficient to compensate for urinary Ca losses, bone Ca resorption will also be activated.

Dietary inclusion of calcium antagonists

In the last decade, the principle of low Ca to prevent milk fever has been reinvented with dietary interventions to reduce Ca availability without modifying Ca intake. Zeolite clays have consistently shown a reduced milk fever incidence^(95–102). This effectiveness is similar to that of the synthetic low-Ca diets. The mode of action of zeolites is still up for discussion. The initial hypothesis was that intestinal binding of Ca would challenge Ca absorption; however, it has been recently proposed that the induction of hypophosphataemia by the product may play an active role in milk fever prevention⁽¹⁰³⁾.

Zeolites have demonstrated great potential for the dietary prophylaxis of milk fever. However, a major drawback of this application is a depression of DMI. Initial data do not report the large effects on DMI that were later observed^(101,102). Those initial studies indicate that leftover feed was minimal^(95,103). This may suggest restricted feeding that would not allow for observing differences in the voluntary feed intake. However, the effect on

DMI depends on the product dose, and a dose with a compromise between effectiveness and DMI depression was determined to be 23 g/kg DM in another study⁽¹⁰²⁾.

A different approach for reducing Ca in the diet was proposed by Wilson^(104,105). In his case, unsaturated fat was used to decrease Ca absorption and induce the adaptation of Ca homeostasis before parturition with apparent success. Dietary fat has been considered an antagonist of Ca⁽¹⁰⁶⁾, but the magnitude of this effect is questionable in ruminants because the Ca complexation with the fat in the rumen can dissociate in the duodenum⁽¹⁰⁷⁾. An additional drawback to this approach is that feeding fat before calving also has a negative effect on DMI⁽¹⁰⁸⁾.

It would be desirable to lower the dietary availability of Ca without inducing DMI depression. Among the natural components known to reduce Ca availability, phytic acid is abundantly available in rice bran; therefore, this feed may be a potential alternative. This feed has no known negative effects on DMI and has an adequate feed value for ruminants. Phytic acid from rice bran has been used to reduce dietary Ca availability to prevent renal calculi in humans^(109,110). Among common cereal brans, rice bran contains the highest phytic acid levels and presents the greatest *in vitro* binding potential (20 g Ca/kg bran)⁽¹¹¹⁾.

It should be noted that phytic acid is rumen degradable⁽¹¹²⁾; therefore, dietary inclusion of rice bran would not represent a viable alternative to zeolites. Ruminal degradation of phytic acid has been studied extensively in recent decades to determine the need for supplemental P in ruminants and minimise its environmental impact. The results of these studies demonstrated that ruminal degradation of phytic acid is associated with the degradation of protein and that feed treatments used to promote a higher rumen protein escape fraction also result in lowered digestibility values for P^(113–115). Phytic acid in rice bran can be protected from ruminal degradations by different treatments^(29,116).

Rumen-protected rice bran can induce the adaptation of Ca metabolism in cows⁽²⁹⁾. This effect is caused by very low Ca content and the action of lowering the dietary availability of Ca⁽³²⁾. The prophylactic value of this feed against milk fever is currently under evaluation by our group, and the preliminary results indicate that rumen-protected rice bran can improve calcaemia recovery after calving⁽¹¹⁷⁾.

Mechanistic analysis of the adaptation of calcium homeostasis at calving

In the dry period, dairy cows do not produce milk and thus have a low metabolic expenditure of Ca compared with the gastrointestinally (GI) available Ca. Active absorption becomes dormant, and passive paracellular absorption from the gut is sufficient to cover basal Ca requirements. Any surplus is excreted into the urine.

Adaptation can be stimulated either by increasing Ca clearance from the blood or by reducing intestinally available

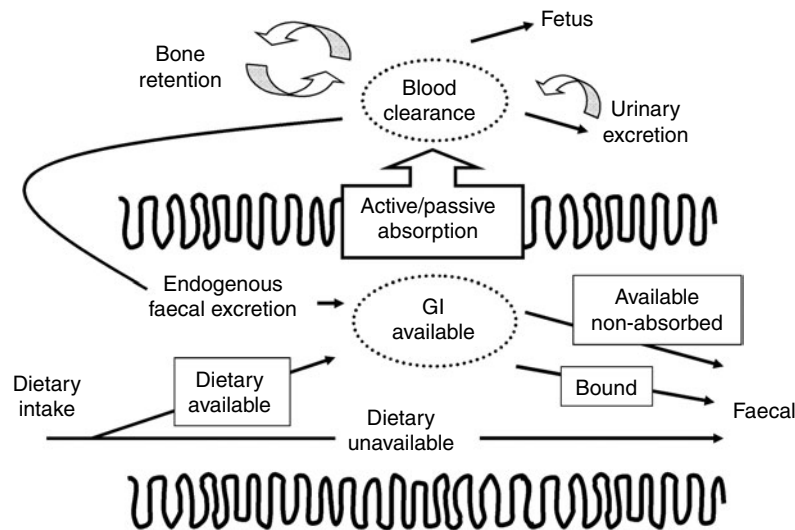


Fig. 2. Diagram of the digestive and physiological fate of calcium in pre-calving dairy cows. GI, gastrointestinally.

Ca. Either of these two actions can cause passive Ca absorption to be insufficient to compensate for metabolic needs, which would trigger the adaptation of Ca homeostasis. In the Dietary-induced modulation of calcium metabolism section, we have discussed several dietary strategies to modulate Ca metabolism. Nevertheless, to understand the effectiveness of these strategies for the prevention of milk fever, it is important to quantify the effects of these dietary strategies on Ca clearance and GI available Ca. A schematic of the factors affecting these effects is described in Fig. 2.

Estimation of blood calcium clearance

The factors defining blood Ca clearance are as follows: endogenous faecal output, urinary excretion, net bone deposition, fetal requirements and lactation requirements.

The endogenous faecal losses are the sum of the Ca excreted into the gastrointestinal tract in the saliva, bile, gastric juices, pancreatic juices and other intestinal secretions. Endogenous faecal losses are a function of body weight and feed intake⁽¹¹⁸⁾ and represent most of the maintenance requirements of Ca. Endogenous faecal clearance is largely independent of Ca intake^(118,119).

In the analysis presented here, it will be assumed that these levels are 6 g/d in multiparous dry cows and 5.5 g/d in dry heifers based on values from the literature^(120–123), which are displayed in Table 1.

Urinary excretion is a small fraction of the maintenance Ca requirements. As already described, urinary Ca can be increased or minimised during the time lag for intestinal and bone adaptation, but reabsorption is as high as 99%⁽¹⁾. Therefore, urinary losses are as low as 0.4 g/d⁽⁴⁸⁾ and 0.8 g/d⁽⁸⁷⁾. An exception to this rule is when dietary-induced metabolic acidosis causes hypercalciuria. The urinary Ca excretion increases in a curvilinear fashion as the systemic pH decreases and can be as high as 6⁽⁴⁸⁾, 5.3⁽⁸⁷⁾ or 7.9 g/d⁽¹²⁴⁾.

The relationship between the DCAD and urinary Ca has been described by Roche *et al.*⁽⁴⁹⁾. To translate urinary Ca excretion values expressed as a fraction of creatinine excretion into daily Ca excretion, it is necessary to assume a constant daily creatinine excretion for the dry cow. In this model, daily creatinine excretion is considered to be 17.5 g/d based on estimates from literature references^(125,126). In this mechanistic analysis, urinary Ca excretion is estimated as a function of the DCAD with an adapted version of the equation from Roche *et al.*⁽⁴⁹⁾.

Table 1. Endogenous faecal calcium in cattle measured by the isotope dilution technique (Mean values and standard deviations)

Bovine animal type	Body weight (kg)		Endogenous faecal Ca (g/d)		Reference
	Mean	SD	Mean	SD	
Lactating Jersey/Guernsey cows	327	37	6.8	1.2	Comar <i>et al.</i> (1953) ⁽¹²¹⁾ ; Vissek <i>et al.</i> (1953) ⁽¹²²⁾
Lactating heifers	368	44	5.5	2.1	
Steers	340	74	4.6	0.7	
Mature steers	485	87	7.6	1.7	Hansard <i>et al.</i> (1957) ⁽¹²⁰⁾
Young steers	181	39	2.8	0.5	
Non-lactating dairy cows	678	–	5.6	1.1	Martz <i>et al.</i> (1999) ⁽¹²³⁾

This adaptation assumes that the non-reported units for the Ca:creatinine ratio were mg/100 ml per mmol/l and the above-mentioned daily creatinine excretion (Table 2). This equation predicts an excretion of approximately 0.5 g Ca/d for a high DCAD and approximately 7 g/d for a low DCAD. The adapted equation was validated by ten dietary treatments that were described in four studies from the literature. Observed and predicted daily urinary Ca excretions produced an R^2 of 0.76 (Table 2).

During the Ca deficit, the homeostatic system can reduce the urinary excretion to urinary Ca losses that are as low as 0.1 mmol Ca per g creatinine⁽²⁹⁾ (approximately 0.07 g/d). The aim of this simulation is to predict whether a Ca imbalance can induce this and other adaptive reactions. Therefore, this situation is not included in the model.

The net bone deposition of Ca depends on the long-term Ca balance of the cow. At the end of the dry period, multiparous cows should have replenished the bone reserves that were mobilised during early lactation. However, when feeding Ca levels are as low as 5.2 g/kg DM in the lactation diet, a positive Ca balance is regained within the first half of the lactation period⁽¹²⁷⁾, which allows enough time to replenish the Ca reserves before the dry period. Heifers instead are still growing, and their increase in body mass is a relevant factor for Ca clearance from the blood. The US National Research Council proposes a factorial approach to derive net Ca requirements, in which growth needs are a function of daily weight gain, body weight and mature weight gain⁽¹²⁸⁾. According to the National Research Council, a heifer weighing 700 kg, aiming for a mature weight of 750 kg and growing at 800 g/d, would have a net Ca requirement for growth of 8 g/d.

The intrinsic cause of milk fever is the discontinuity of the Ca yields to the calf between fetal and lactation requirements. The factorial model of the National Research Council⁽¹²⁸⁾ estimates fetal Ca requirements in the last days of pregnancy to be approximately 7 g/d. This contrasts with the Ca content of the first colostrums that represents approximately 23 g in the dairy cow⁽¹²⁹⁾.

Gastrointestinally available pool

The GI available pool of Ca is the amount of Ca present in the lumen of the complete gastrointestinal tract that can potentially be absorbed. Ca absorption is a regulated process that differentiates between availability and absorption. Although Ca availability influences absorption by allowing passive inflow, Ca absorption only reflects the dietary Ca availability under conditions of Ca shortage. The endogenous Ca secretions and the fraction of dietary Ca that becomes available during the digestion process constitute the GI available pool. In this pool, we exclude the fraction that may become unavailable during digestion (for example, via precipitation with other dietary components).

Dietary Ca intake is defined by the total feed intake and the Ca content of the feeds, plus any supplemental Ca. Voluntary feed intake is a very important factor that affects the Ca intake of the transition cow because it is greatly affected by the diet characteristics, the health status of the animal and calving. Ca content in feeds is highly variable in feed value tables⁽¹²⁸⁾. Ca content can range from 12 to 15 g/kg DM in legume forages, grasses are in the range of 4 to 8 g/kg DM, and maize silages and cereal straws are mostly within 2 to 4 g/kg DM. Among the concentrates, most grains are poor sources of Ca (mostly under 1 g/kg DM), and the protein concentrates contain Ca in a range of 3 to 6 g/kg DM. Depending on the ration formulation, the final Ca content is variable, but there is a clear association between the effective fibre and Ca content.

A negative correlation exists between the dietary Ca content and Ca availability. The model presented by the US National Research Council in 2001⁽¹²⁸⁾ assigns an availability coefficient of 0.6 for Ca in concentrates and maize silages (low Ca content) and 0.3 for Ca in other forages (high Ca content). This negative correlation greatly reduces the variability of the available Ca in the rations. In the present model, the availability of Ca in the rations is assumed to be inversely proportional to their total Ca content. Rations below 2 g Ca/kg DM are calculated with

Table 2. Urinary calcium excretion in cattle as affected by the dietary cation–anion difference (DCAD)

Reference	DCAD (meq/kg DM)	Urinary Ca (g/d)	Urinary Ca (g/d) model prediction*
Schonewille <i>et al.</i> (1994) ⁽⁴⁸⁾	–170	6.1	7.61
	276	0.4	0.91
Vagnoni & Oetzel (1998) ⁽¹²⁴⁾	–63	6.87	5.44
	–40	7.87	5.02
	–51	6.70	5.22
	203	0.92	1.59
Schonewille <i>et al.</i> (1999) ⁽³⁰⁾	–230	11.44†	8.98
	332	1.06†	0.51
Roche <i>et al.</i> (2007) ⁽⁸⁷⁾	–200	5.3	8.28
	180	0.8	1.83

* Prediction of daily urinary Ca excretion from the DCAD (meq/kg DM) using the equation from Roche *et al.* (2003)⁽⁴⁹⁾ adjusted in units and assuming a daily creatinine excretion of 17.5 g. Urinary Ca (g/d) = 1.5470(0.001 (0.1 DCAD)² – 0.1075(0.1 DCAD) + 4.794). Linear fit between the literature observations and the predicted values: R^2 0.76.

† Calculated from the creatinine ratio, assuming a daily creatinine excretion of 17.5 g.

Table 3. Estimated daily blood calcium clearance and the available gastrointestinal calcium pool in different pre-calving scenarios in multiparous dairy cows fed two levels of calcium, heifers, multiparous cows fed a low-dietary cation–anion difference (DCAD) diet and multiparous cows fed a calcium antagonist

	Mid–low Ca	Mid–high Ca	Heifer	Low-Ca diet	Low-DCAD diet	Ca binder
Endogenous faecal excretion (g)	6.0	6.0	5.5	6.0	6.0	6.0
Urinary excretion (g)	0.7	0.7	0.7	0.7	7.2	0.7
Bone deposition (g)	–	–	8.0	–	–	–
Fetal needs (g)	7.0	7.0	7.0	7.0	7.0	7.0
Total BCC (g)	13.7	13.7	21.2	13.7	20.2	13.7
DM intake (kg)	14.0	14.0	12.0	14.0	13.0	14.0
Dietary Ca (g/kg DM)	2.5	5.5	4.0	1.5	4.0	4.0
DCAD (meq/kg DM)	300	300	300	300	–150	300
Ca intake (g)	35.0	77.0	48.0	21.0	52.0	56.0
Availability	0.58	0.43	0.50	0.60	0.50	0.50
Available Ca intake (g)	20.1	32.7	24.0	12.6	26.0	28.0
Gastrointestinal precipitation (g)	–	–	–	–	–	15.0
Total GIAP (g)	26.1	38.7	29.5	18.6	32.0	19.0
BCC:GIAP ratio	0.53	0.35	0.72	0.74	0.63	0.72

BCC, blood Ca clearance; GIAP, gastrointestinally available pool.

an availability coefficient of 0.6, and rations above 8 g Ca/kg DM are calculated with an availability coefficient of 0.3. Rations with a Ca content between 2 and 8 g/kg DM are calculated with intermediate values between 0.6 and 0.3, which is determined by a simple linear function of their Ca content (availability = $0.7 - 0.05 \times \text{Ca}$).

Ca availability is not simply an intrinsic property of the feeds. Digestive processes can result in the formation of chemical compounds containing Ca that are not susceptible to intestinal absorption. Endogenous faecal Ca can also be subject to availability loss. Precipitation with zeolites or phytate would be examples of this fraction, which is subtracted from the GI available pool.

Prediction of metabolic adaptation from the ratio between calcium clearance and gastrointestinally available calcium

It is not easy to predict the situations in which dietary regulatory adaptation will take effect. Both Ca clearance and intestinal availability do not present constant rates in time. Therefore, choosing days as a time unit for assessment may be inadequate. The clearance rate of Ca into colostrum may be greater at a given moment than as a daily average. Additionally, the gastrointestinal availability will depend on the transit speed through the tract and the residence times of chyme in the different compartments⁽⁴⁴⁾. Despite this, the degree of Ca adaptation before calving should be directly related to Ca clearance before calving and inversely related to GI available Ca. The ratio of these factors represents the fraction of the GI available Ca that is absorbed to compensate for the Ca clearance during homeostasis. This ratio should be indicative of the point beyond which passive absorption would not suffice and active absorption would be required for Ca homeostasis (Fig. 2).

The above-described model has been used to evaluate a representative set of dietary and physiological pre-calving scenarios (Table 3). These include the high and low

range of dietary Ca contents in diets fed to multiparous cows and an average heifer diet. Furthermore, the following three milk fever prevention strategies are presented: a Ca diet low enough for milk fever prevention, a low-DCAD diet, and a hypothetical Ca diet in which the available Ca is reduced by 15 g using a dietary antagonist. At first glance, this model shows that multiparous cows absorb a relatively small fraction of the GI available Ca throughout a typical range of dietary Ca. Heifers instead absorb a fraction of the available Ca with the estimates for milk fever prevention strategies. This finding explains why heifers are considered to be less susceptible to hypocalcaemia. The ratio between the Ca clearance and GI available Ca seems to be indicative of the adaptation of Ca metabolism before calving. Within the assumptions of the proposed model, it seems that before calving, when nearly 70% of the intestinally available Ca is absorbed, the incidence of hypocalcaemia is reduced.

The effect of parity and dietary Ca on the calculated ratio of Ca clearance:GI available Ca is further explored in Fig. 3. The model clearly explains the different susceptibilities of heifers and cows to milk fever in terms of the induction

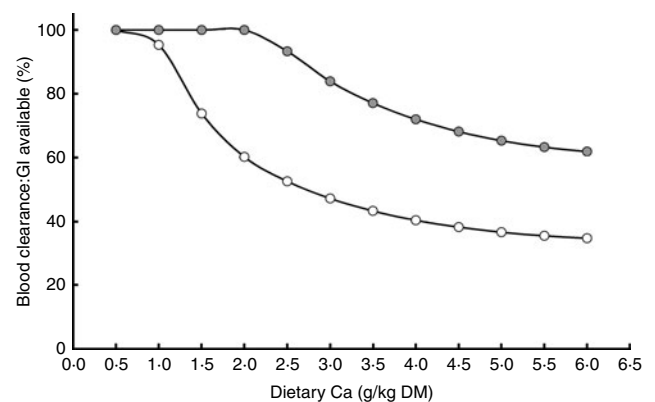


Fig. 3. Predicted effect of dietary calcium (g/kg DM) on the fraction of gastrointestinally (GI) available calcium required for physiological purposes in heifers (—●—) and multiparous cows (---○---).

of metabolic adaptation. It predicts that heifers will always need to absorb more than 60% of the available intestinal Ca even at high dietary Ca; however, older cows will absorb a much smaller fraction of the available Ca. It is also remarkable that the curve increases its slope as the dietary level decreases to 1.5 g/kg DM (about 20 g/d). This value is the dietary Ca level that is considered to effectively prevent milk fever⁽⁷²⁾. These estimates explain the absence of changes in Ca homeostasis in heifers observed by our group⁽¹³⁰⁾. The Ca regulation of heifers is adapted because their Ca clearance that is driven by growth is high in relation to their GI available Ca.

The model is also used to study the effect of the DCAD on urinary Ca excretion and the fraction of GI available Ca that is absorbed at different dietary levels (Fig. 4). Reduction of the DCAD increases the fraction absorbed. At the recommended DCAD levels of -200 and -100 meq/kg DM, the blood Ca clearance:GI available Ca ratios are in the range of those calculated for heifers with common dietary Ca levels. This finding supports the milk fever prevention value of this strategy and explains its mode of action through the induction of metabolic adaptation.

The reports on the effect of dietary Ca levels on the DCAD have been controversial. While some authors proposed that increasing dietary Ca may have a preventive value in low-DCAD diets⁽¹²⁹⁾, others suggest that the quadratic effect of dietary Ca on milk fever incidence is independent from the DCAD level⁽⁸⁶⁾. The present mechanistic simulation (Fig. 4) predicts the preventive value of lowering Ca, even at a low DCAD. This effect would not be linear but curvilinear, suggesting a smaller stimulation of the adaptation to changes in dietary Ca in the higher range than in the lower range. This relationship would correspond with the left slope of the quadratic relationship that was empirically determined by Lean *et al.*⁽⁸⁶⁾ and earlier by Oetzel⁽⁸⁵⁾. The present model is limited to milk fever prevention through metabolic adaptation. Therefore, it is not suitable to describe the

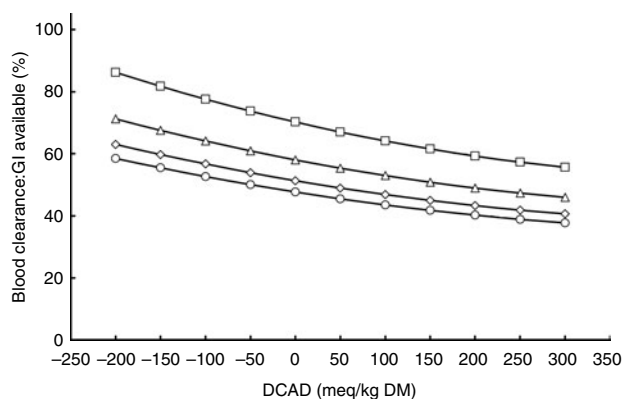


Fig. 4. Predicted effect of the dietary cation-anion difference (DCAD) on the fraction of gastrointestinally (GI) available calcium required for physiological purposes at different levels of dietary calcium: (—○—), 5.5 g Ca/kg DM; (—◇—), 4.5 g Ca/kg DM; (—△—), 3.5 g Ca/kg DM; (—□—), 2.5 g Ca/kg DM.

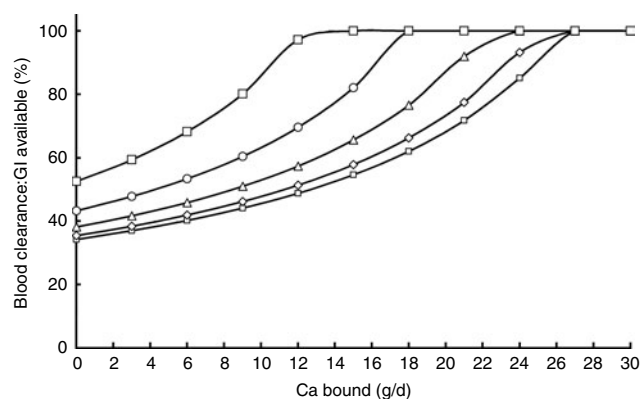


Fig. 5. Predicted effect of calcium binding on the fraction of gastrointestinally (GI) available calcium required for physiological purposes at different levels of dietary calcium: (—□—), 6.5 g Ca/kg DM; (—◇—), 5.5 g Ca/kg DM; (—△—), 4.5 g Ca/kg DM; (—○—), 3.5 g Ca/kg DM; (—□—), 2.5 g Ca/kg DM.

preventive effect of high levels of dietary Ca; however, the mode of action is most probably explained by the sufficient paracellular intestinal absorption that compensates for Ca clearance at calving.

The model proposed here can be used to quantify the amount of Ca that must be made unavailable with a dietary binding agent, such as a zeolite clay or phytic acid, to induce the adaptation of Ca metabolism (Fig. 5). Obviously, higher dietary Ca levels require greater gastrointestinal precipitation to induce this adaptation. The model shows that the preventive efficiency of precipitation decreases at high dietary Ca intake. At intakes of 2.5 g/kg DM, precipitating 15% of the intake could be sufficient, while at intakes of 5.5 g/kg DM, as much as 25% of dietary Ca would have to be precipitated.

Conclusions

Milk fever is a disease specific to dairy cattle that is caused by the disparity between the great milk yield potential after calving and the small Ca requirements in late gestation. Milk fever has a clear and direct impact on animal welfare, but its importance is largely found in its indirect effects on early lactation health and its high economic impact on dairy production.

Ca homeostasis is a robust system controlled by metabolic adaptations that take place in the kidney, the gastrointestinal epithelium and bone. Modulation of renal reabsorption occurs rapidly, controlling small negative or large positive Ca fluctuations. The adaptation of gastrointestinal absorption represents an effective mechanism to compensate for hypocalcaemia. When this mechanism is insufficient, bone mobilisation is utilised. These last two adaptations involve processes of cell differentiation that involve a delay of 1 or 2 d. Our model shows that the delay in gastrointestinal adaptation might be the underlying cause of the failure of dairy cows to cope with the sudden change in the rate of Ca clearance at calving, which results in transient hypocalcaemia.

The model quantitatively illustrates the effects of different diets and metabolic states before calving on the early activation of gastrointestinal absorption. Heifers present an up-regulated Ca metabolism before calving because of the Ca requirements for growth, causing them to be less susceptible to milk fever. Limiting the dietary Ca availability quantitatively or qualitatively induces a similar metabolic adaptation. Inducing metabolic acidosis prevents the renal reabsorption of Ca, thereby inducing hypercalciuria, which challenges the homeostatic system and up-regulates Ca metabolism. The induction of a metabolic adaptation before calving seems to be the mode of action of the currently proposed strategies for the dietary prevention of periparturient hypocalcaemia.

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