Magnesium chloride in dry cow silage to prevent hypocalcaemia

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Uppsala 2011

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Keywords: Dairy cow, parturient paresis, hypomagnesaemia, metabolic alkalosis, dietary cation-anion difference

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Kurskod: EX0552

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SUMMARY

Milk fever, or parturient paresis, is the second most common disease in Swedish dairy cows. The disorder is associated with the onset of lactation when some cows are unable to meet the metabolic demands of calcium to support milk production and therefore develop a state of hypocalcaemia. Clinical hypocalcaemia (milk fever) may lead to coma and death in severe cases but subclinical hypocalcaemia has also been shown to have negative effects on e.g. feed intake and production and to increase the susceptibility of the cow to develop secondary diseases. The nutritional strategy applied precalving is of great importance in preventing milk fever and e.g. manipulation of the dietary cation-anion difference (DCAD) or the supply of an adequate dietary level of magnesium may affect the degree of hypocalcaemia at calving.

The objective of the present study was to investigate if the degree of hypocalcaemia at parturition could be affected by feeding silage preserved with an additive containing magnesium chloride ($MgCl_2$) to lower DCAD and to increase the amount of dietary magnesium in the prepartum ration. The purpose was also to investigate if $MgCl_2$ would affect feed intake and if supplying magnesium above feeding recommendations would affect plasma calcium level at calving. Twelve heifers and 24 cows (all pregnant) of the Swedish Red Breed were divided into six blocks based on age and calving date. Animals within block were randomly allocated to one of three experimental diets; a control diet (C) consisting of late harvested silage, a magnesium chloride diet (MCL) with 8 g $MgCl_2$/kg DM silage added at ensiling and a magnesium oxide diet (MO) with 22.7 g/day of magnesium oxide manually mixed in the silage at feeding. Animals began receiving the diets ~21 days before expected parturition and collection of blood and urine samples was started and continued until 7 days after parturition. Urine pH, Ca and Mg and blood Ca, Mg, parathyroid hormone (PTH) and C-terminal peptide (CTx, marker of bone resorption) were analyzed. Fresh crop and silages were analyzed for DM, energy, crude protein and minerals and DCAD was calculated using ([Na+] + [K+]) – ([Cl-] + [S-]).

The results showed that the degree of hypocalcaemia at calving was not affected by a lower DCAD or an increased dietary content of magnesium. However, $MgCl_2$ caused a significant increase in urinary excretion of calcium in the week before calving, which may imply that the feeding of $MgCl_2$ lead to an increased activation of the mechanisms regulating calcium homeostasis and/or an increased responsiveness of these mechanisms to the secretion of PTH close to parturition. Feed intake was not affected by treatment and the plasma level of CTx was only affected by age and calving. The extra amount of magnesium supplied in the MO and MCL diets caused a higher urinary excretion of magnesium prepartum compared to control. Therefore it was concluded that the amount of magnesium provided in the control diet was sufficient to meet magnesium requirements in prepartum dairy cows.

SAMMANFATTNING

Kalvningsförlamning, eller pares, är den näst vanligaste sjukdomen bland mjölkkor i Sverige och uppkommer i samband med kalvning när kons kalciummetabolism ställer om sig från dräktighet till att börja producera mjölk. En del kor är då oförmögna att möta det snabbt ökade behovet av kalcium och får problem med att upprätthålla en normal kalciumbalans i blodet. Klinisk pares kan orsaka förlamning och även dödsfall men även subklinisk hypokalcemi har visats ge negativa effekter på till exempel foderintag och mjölkproduktion samt öka risken för att kon drabbas av sekundära sjukdomar. Utfodringen innan kalvning är mycket viktig för att förebygga pares och till exempel manipulering av fodrets katjon-anjon balans (DCAD) eller tillförsel av en tillräcklig mängd magnesium kan påverka graden av hypokalcemi vid kalvning.

Syftet med denna studie var att undersöka om graden av hypokalcemi vid kalvning kunde reduceras genom utfodring med ett ensilage som tillsatts magnesiumklorid ($MgCl_2$) vid ensileringen för att sänka DCAD och öka innehållet av magnesium i foderstaten. Syftet var också att undersöka om $MgCl_2$ hade någon effekt på foderintaget och om högre utfodring med magnesium än rekommenderat skulle påverka plasmakoncentrationen av kalcium vid kalvning. Tolv kvigor och 24 kor (alla dräktiga) av rasen SRB delades upp i sex block efter ålder och kalvningsdatum. Djur inom block randomiserades till en av tre behandlingar; en kontrollbehandling (C) bestående av sent skördat gräsensilage, en $MgCl_2$-behandling (MCL) med 8 g $MgCl_2$/kg ts tillsatt vid ensileringen och en magnesiumoxidbehandling (MO) där 22,7 g magnesiumoxid per dag blandades in i ensilaget för hand innan utfodring. Djuren startades på behandlingarna ~21 dagar före förväntad kalvning. Då började även blod- och urinprover samlas in och detta gjordes fram till och med sju dagar efter kalvning. Urin analyserades för pH, Ca och Mg och blod för Ca, Mg, parathormon (PTH) och C-terminal peptid (CTx, en markör för bennedbrytning). Grönmassa och ensilage analyserades för ts, energi, råprotein och mineraler och DCAD beräknades med formeln ([Na +] + [K +]) - ([Cl-] + [S-]).

Resultaten visade att graden av hypokalcemi vid kalvning inte påverkades av ett lägre DCAD eller ett högre foderinnehåll av magnesium. Däremot orsakade $MgCl_2$ en signifikant ökning av kalcium i urinen veckan före kalvning. Detta kan betyda att utfodring av $MgCl_2$ prepartum ledde till en ökad aktivering av de mekanismer som reglerar kalciumbalansen och/eller att dessa mekanismer blev mer känsliga för utsöndringen PTH nära kalvning. Foderintaget påverkades inte av behandling och plasmanivån av CTx påverkades endast av ålder och kalvning. Det extra tillskottet av magnesium på MO- och MCL-behandlingarna resulterade i en ökad utsöndring av magnesium i urinen innan kalvning jämfört med kontrollgruppen. Därför kan den mängd magnesium som kontrollbehandlingen innehöll betraktas som tillräckligt för att uppfylla magnesiumbehovet hos sinkor nära kalvning.

INTRODUCTION

Milk fever, or parturient paresis, is a hypocalcaemic disorder associated with the onset of lactation in dairy cows. In Sweden this is the second most common disease with about 10 000 treated cases per year. In addition, it is believed that many cows develop a state of subclinical hypocalcaemia at parturition. This condition is not as severe as milk fever, but has negative effects on feed intake, digestion, milk production, reproduction and increases the cow's susceptibility for developing secondary diseases.

An adequate blood level of calcium and magnesium is required for proper function of several physiological processes and of major importance to the periparturient cow. The loss of calcium in milk is replaced through increased resorption of calcium in the kidneys, increased mobilization of calcium from bone and increased calcium absorption from the intestinal tract (Goff *et al*., 1991). These calcium homeostatic mechanisms are governed by hormones where parathyroid hormone (PTH) is the most important regulator. The cow also experiences a loss of magnesium through milk, but because magnesium metabolism is not regulated by specific hormones the cow is solely dependent on a dietary intake of the mineral (Martens & Schweigel, 2000).

Several nutritional strategies have been applied to dry cows in the periparturient period to improve calcium homeostasis at calving. Manipulation of the dietary cation-anion difference (DCAD) is one method that has been shown to successfully reduce the degree of hypocalcaemia at parturition in a number of studies. DCAD affects the acid-bases status of the cow where a high DCAD induces a mild form of metabolic alkalosis and a low DCAD a mild metabolic acidosis. Metabolic alkalosis has been shown to blunt the response of PTH on its target tissues (Goff *et al*., 1991). The intention is therefore usually to reduce DCAD of the prepartum ration to a level close to zero to make the cow acidotic and more able to respond to the increased demand of calcium at calving (NRC, 2001). A reduction in DCAD can be achieved for example by the addition of anionic salts to the diet. Extra anions in prepartum diets have however been associated with a decreased dry matter intake (Charbonneau *et al*., 2006). A probable explanation for this is a lack of palatability of the anion sources and anionic salts can therefore not be fed separately but must be blended into feed in a way that does not negatively affect feed consumption. The action of PTH is also affected by magnesium status and hypomagnesaemia has been shown to decrease the secretion and effects of PTH on its target tissues, increasing the cow's susceptibility to hypocalcaemia (Goff, 2007). Several researchers have investigated the effects of a low DCAD ration prepartum on calcium status at calving, but the effects of a low DCAD diet in combination with an extra addition of magnesium on calcium and magnesium status at calving has not been explored to the same extent.

Aim and Hypothesis

The aim of this experiment was to study the effects on hypocalcaemia at calving by feeding silage ensiled with an additive containing magnesium chloride ($MgCl_2$) to periparturient dry cows and first calf heifers. We also wanted to study the effects of this silage on feed intake and if providing magnesium above feeding recommendations would affect the extent of hypocalcaemia at calving.

The hypothesis were that dry cows consuming the $MgCl_2$-silage would be more acidic and have a lower degree of hypocalcaemia at calving, that feed consumption would not decrease because of the addition of $MgCl_2$ and that extra addition of dietary magnesium would have a positive effect on hypocalcaemia at calving.

LITTERATURE REVIEW

Calcium

Function and requirement

Calcium (Ca) is an important mineral required for normal function of a number of tissues and physiological processes in the body. Calcium is needed for proper bone formation, muscular contraction, nerve transmission, enzyme activity, blood clotting, and functions as a second messenger regulating the actions of many hormones (Horst, 1986; NRC, 2001). Bone is the main reservoir for body calcium (99%) and approximately 1% exists in a soluble form in the cells and in the extracellular fluid (NRC, 2001; Sjaastad *et al.*, 2003). The extracellular calcium pool contains between 8 to 10 g in total out of which 2.5 to 3 g is located in the plasma calcium pool (see Fig 1). The blood level of calcium in an adult cow is maintained within a narrow range of 2.1-2.5 mmol/l (Horst *et al.*, 1994). Around 50% of the total calcium in plasma is bound to proteins or to negatively charged ions. The remaining fraction exists as soluble ionized calcium (Ca^{2+}) and it is essential that this fraction is maintained in the range of 1.0-1.25 mmol/l to assure accurate neural, muscular and endocrine function (NRC, 2001; Sjaastad *et al.*, 2003). Cows can only afford to lose around 50% of the circulating calcium reserves in blood before a severe hypocalcaemia is induced (DeGaris & Lean, 2008).

The daily maintenance requirement of dietary calcium for a 500 kg dairy cow is 28 g. The calcium demand of the fetus is negligible until the last trimester of pregnancy and an extra addition of 15 g calcium per day is required during the last month of pregnancy (Spörndly, 2003). According to NRC (2001) calcium requirement for lactation is 1.22 g absorbed calcium/kg milk produced for Holsteins, 1.45 g/kg for Jerseys and 1.37 g/kg for other breeds. Spörndly (2003) recommend that 2.6 g calcium/kg energy corrected milk should be added to the diet for all breeds.

Regulation of calcium homeostasis

Parathyroid hormone

The release of parathyroid hormone (PTH) from the parathyroid glands is continually regulated by the extracellular concentration of calcium. Secretion of PTH is increased when the calcium concentration in plasma declines, reaching a maximum at calcium levels of 0.5-0.7 mmol/l. At Ca^{2+} levels above 1.25 mmol/l secretion of PTH is reduced to a minimum. Rapid actions of PTH take place within minutes and hours in response to changes in plasma level of the hormone. First an elevated secretion of PTH stimulates an increased release of calcium from bone through the activation of osteoclasts and osteoblasts that quickly transfers calcium to the extracellular fluid (see Fig 1). Secondly, the excretion of calcium in urine is reduced through an improved reabsorption of calcium in the renal tubuli. The capacity of renal absorption is however limited and contributes less than 1 g calcium to the extracellular pool. For a maximum response to an elevated level of PTH the secretion has to continue for several days or weeks. The long term effects of PTH include increased synthesis of $1,25(OH)_2D_3$ in the kidneys and it also leads to a further increase in bone mobilization

through the recruitment of new osteoclasts and enhanced bone resorption by individual cells (Sjaastad *et al.,* 2003).

Vitamin D

Vitamin D exists in two chemical forms called vitamin D_3 and D_2. Vitamin D_3 is produced by vertebrates and vitamin D_2 is the major naturally occurring form of the vitamin in plants (Horst *et al.,* 1994). Both forms are precursors for the biologically active form of vitamin D named 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), also called calcitriol, which is important in calcium metabolism and plays an active role in the regulation of calcium absorption. Vitamin D_2 is provided to the animal through the diet and vitamin D_3 through the conversion of 7-dehydrocholesterol to vitamin D_3 by ultraviolet light in the epithelial cells of the skin. Both forms are transported to the liver where they are hydroxylated to 25(OH)-D_3. This compound is more active in the body than vitamin D_2 and D_3, but it is still considered to be a precursor for $1,25(OH)_2D_3$. Excess production of 25(OH)-D_3 is stored in the body and utilized when sun exposure is inadequate (Sjaastad *et al.,* 2003). The last step of activation occurs in the kidneys where another hydroxyl-group is added to 25(OH)-D_3 producing the biologically active form $1,25(OH)_2D_3$. This step is catalyzed by the enzyme 1α-hydroxylase and PTH is the most important stimulator of its activity (Horst *et al.,* 1978). $1,25(OH)_2D_3$ stimulates the formation of calcium binding proteins in a number of cell types for example the intestinal epithelial cells, renal tubuli cells, nerve cells and bone cells and it is important for the normal function of osteoblasts and osteoclasts. Thus the active metabolite of vitamin D acts to increase the absorption of calcium from the intestines and to enhance the resorption of bone. The production of $1,25(OH)_2D_3$ is inhibited by hypercalcemia and hyperphosphatemia (Sjaastad *et al.,* 2003).

Calcitonin

Calcitonin is a peptide hormone produced in the thyroid gland. It is secreted when blood concentrations of calcium are elevated to prevent hypercalcaemia. Calcitonin reduces the resorption of bone through reduction of the surface population of active osteoclasts and causes increased urinary excretion of calcium (Sjaastad *et al.,* 2003).

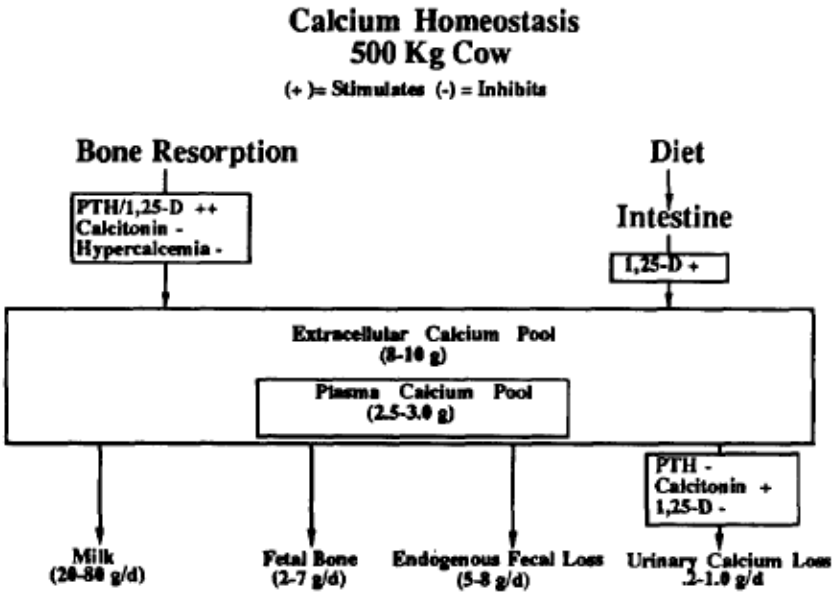

Figure 1. Calcium metabolism in the dairy cow. PTH = Parathyroid hormone; 1,25-D = 1,25-dihydroxyvitamin D (adopted from Goff *et al.* 1994).

Calcium absorption

Calcium is only absorbed to a very small extent from the fore-stomachs of ruminants and the main site of absorption is the small intestine (Sjaastad *et al.*, 2003). Here calcium is absorbed by passive diffusion between the intestinal epithelial cells (paracellular transport) or through active transport across the epithelial cells (transcellular transport). The paracellular transport is strictly dependent on the concentration of ionized calcium in the lumen of the intestine. It is uncertain how much passive diffusion that occurs from diets typically fed to dairy cattle and the diluting effect of the rumen will probably reduce the extent of this route of absorption. Active transport of calcium instead appears to be the major mechanism for calcium absorption. This transport requires $1,25(OH)_2D_3$, which facilitates the diffusion of calcium into the epithelial cells. Calcium is then bound to a calcium-binding protein that takes it to the basal lateral side of the cell, where it is extruded against a concentration gradient by calcium pumps dependent on Ca-Mg-ATPase. The amount of $1,25(OH)_2D_3$ produced is carefully regulated to assure that the amount of calcium absorbed from the intestine is sufficient to maintain a constant concentration of calcium in the extracellular fluid (Horst *et al.*, 1994).

Bone metabolism

Mobilization of calcium from bone is one mechanism involved in the process of maintaining calcium homeostasis. The dynamic changes in bone metabolism can be studied in vivo by measuring the concentration of specific bone biochemical markers in serum, plasma and urine (Holtenius & Ekelund, 2005). One marker of the bone resorption process is C-terminal peptide fragments (CTx), which are released into circulation as a result of degradation of type-1-collagen by osteoclasts (Christenson, 1997). In 2005, Holtenius & Ekelund found that the plasma concentration of CTx in dairy cows was highest in the first week postpartum,

indicating that bone resorption was induced at parturition. CTx concentration then steadily declined over the next 33 weeks and remained low until next parturition. A similar result was demonstrated by Liesegang (2008), where CTx was significantly increased in dairy sheep and dairy goats on the day of parturition. After the peak the concentration continuously declined until the end of the trial (16 w). An interesting observation from the study by Holtenius & Ekelund (2005) was that the concentration of CTx did not seem to be directly related to the lactation curve. This suggests that the calcium demand of lactation is not the only factor regulating bone metabolism in the dairy cow and that other factors, for example variations in estrogen level, may be of greater importance. Staric *et al.* (2008) found a significant difference in CTx level at peak lactation between primiparous (1.0 ng/ml) and fourth lactation cows (0.78 ng/ml), indicating a more intensive rate of bone metabolism in the younger cows. Another common marker used to study bone resorption in dairy cows is hydroxyproline. This marker does however have some limitations because it is not specific to bone and can be derived from other tissues (Liesegang *et al*., 2003).

Factors affecting calcium homeostasis at parturition

Onset of lactation

During the dry period approximately 30 g calcium/day is lost from the body in urine and feces and for the support of fetal growth (Riond, 2001). At parturition and the subsequent onset of lactation there is a sudden increase in demand for calcium because of its secretion into colostrum. Colostrum contains approximately 2.3 g calcium/l and a cow producing 10 l colostrum may therefore lose around 23 g calcium in her first single milking. This is about three times more calcium than is contained in the extracellular calcium pool (Horst, 1997). At peak production of colostrum the loss of extracellular calcium through milk may exceed 50 g/day for a high yielding cow (Sjaastad *et al.*, 2003). During the dry period the mechanisms regulating calcium homeostasis are relatively inactive due to the low need for calcium. At the beginning of lactation the cow is therefore momentarily unable to replace the amount of calcium lost through milk. As a result all cows therefore generally experience some degree of hypocalcaemia at parturition. In response to this low-calcium stress most cows rapidly begin to increase the mobilization of calcium from bone, increase the efficiency of absorption of calcium from the intestinal tract and to enhance the renal tubular reabsorption of calcium (Goff *et al*., 1991). In those cases where calcium homeostatic mechanisms fail to maintain a proper calcium concentration in blood clinical hypocalcaemia, total blood calcium <1,4 mmol/l, or subclinical hypocalcaemia, total blood calcium 1.4-2.0 mmol/l, may occur (Goff *et al.*, 1991; DeGaris & Lean, 2008). The nadir in blood calcium usually appears between 12-24 h after parturition and blood sampling during this time period can give a good estimation of the degree of hypocalcaemia in a herd (Goff, 2007).

Milk fever

Milk fever, or parturient paresis, is a hypocalcaemic disorder associated with the onset of lactation in dairy cows where the loss of calcium through milk results in decreased muscle and nerve function, (NRC, 2001). Clinical hypocalcaemia occurs when total blood calcium level falls below 1.4 mmol/l and leads to paresis, lateral recumbency and in severe cases to

death. If untreated, milk fever leads to death in 60 to 70 % of the cases (Riond, 2001). A decrease in blood calcium concentration to a level between 1.4-2.0 mmol/l causes subclinical hypocalcaemia and leads to reduced ruminal function, reduced feed intake and reduced rate of defecation (Hansen *et al.*, 2003). Clinical and subclinical hypocalcaemia are important risk factors for many of the diseases related to lactation such as mastitis, ketosis, displaced abomasum, retained placenta and uterine prolapse (DeGaris & Lean, 2008). Clinical hypocalcaemia also increases the risk for culling due to reproductive disorders in multiparous cows (Erb *et al.*, 1985). It has been found that the development of hypocalcaemia contributes to periparturient immune suppression (Kimura *et al.*, 2006). The incidence rate of clinical hypocalcaemia can be very variable and field studies performed from 1977 to the present showed an incidence of 6.17% in ten European studies, 3.45% in ten North American studies and 3.5% in ten Australian studies (DeGaris & Lean, 2008). Mullen *et al.* (1975) found that incidence rate for some herds were less than 1% and for other herds more than 25%. In a meta-analysis of 135 controlled trials by Lean *et al.* (2006) the mean incidence rate of milk fever was 21% but with a range of 0-83%. The differences in incidence rate indicate that the occurrence of milk fever is possible to manipulate (DeGaris & Lean, 2008).

Animal factors

Age and parity

Most dairy cows experience some degree of hypocalcaemia in the periparturient period. The incidence of milk fever increases greatly with age from the second parturition and onwards (Sjaastad *et al.*, 2003). In a study by Horst *et al.* (2003), blood samples collected from 1 446 cows within 48 hours of parturition showed that the incidence of subclinical hypocalcaemia increased with parity and was >50% for cows in or beyond their third lactation. Even first lactation cows develop hypocalcaemia to some extent the first days of lactation, but clinical hypocalcaemia is rare in young cows. This may in part be explained by their greater rate of bone turnover and that their intestines are more rapid to adapt to the greater demand of calcium for lactation (Horst *et al.*, 1994). In older cows the skeleton is fully developed and the readily exchangeable pool of bone mineral is thereby reduced because of the much slower rate of bone turnover. Osteoblasts are the only bone cells that express receptors for $1,25(OH)_2D_3$ and it has been demonstrated in rats that bone in older animals contain less numbers of receptors than bone in young animals (Horst *et al.*, 1990). The number of osteoblasts is also reduced as the animal becomes older, further decreasing the number of receptors for $1,25(OH)_2D_3$. Thus, with advancing age less osteoblasts exists to be stimulated by PTH and $1,25(OH)_2D_3$ and fewer osteoclasts will therefore respond to the resorption signal from the osteoblasts. Therefore there is a delay in the ability of the bone of older cows to contribute calcium to the plasma calcium pool (Horst *et al.*, 1994).

In the bovine species the efficiency of intestinal absorption of calcium also decreases with age. The number of receptors for $1,25(OH)_2D_3$ in the intestine has been shown to decline in both rat and cow as the animal becomes older. An old cow will therefore be less able to respond to$1,25(OH)_2D_3$ and increase the absorption of calcium from the diet as compared to a young cow (Horst *et al.*, 1990).

Breed

Several investigations have showed that the incidence of milk fever is influenced by breed. An epidemiological study from 1987 on parturient paresis in Swedish dairy cows revealed that the Swedish red and white breed had a 1.4 times higher incidence rate than the Swedish Friesian breed (Bendixen *et al.*, 1987). According to statistics from the Swedish board of agriculture, the occurrence of treated milk fever cases in cows of Swedish red and white (SRB) and Swedish Holstein are now on the same level of about 3%. The occurrence rate for Swedish Jersey cattle is higher, approximately 6% (SJV, 2010). The reason behind why some breeds are more susceptible for developing milk fever than others is unclear. It has been demonstrated that Jersey cattle have a lower number of intestinal receptors for $1,25(OH)D_3$ than Holstein cattle of the same age. Since $1,25(OH)_2D_3$ stimulates increased calcium absorption from the intestinal tract a lower number of receptors would make the target tissue less sensitive to the increased concentration of $1,25(OH)_2D_3$ produced in response to hypocalcaemia. This would in turn make the cow less adaptable to the increased demand of calcium for lactation and more susceptible for developing hypocalcaemia and milk fever (Horst *et al.*, 1997).

Metabolic alkalosis

The acid-base status of the cow during the weeks before parturition has been shown to affect her susceptibility to hypocalcaemia, and metabolic alkalosis is believed to be a predisposing factor (Goff *et al.*, 2007). Goff *et al.* demonstrated in 1991 that a high blood pH blunts the response of PTH on its target tissues. They found that secretion of PTH in response to hypocalcaemia around calving was similar in cows fed either a cationic (alkalizing) or anionic (acidifying) diet. It could then have been expected that PTH secretion would stimulate renal production of $1,25(OH)_2D_3$ to the same extent in both groups of cows. However, the amount of $1,25(OH)_2D_3$ produced per unit increase in PTH was greatly reduced in cows fed the high cation diet. This result is supported by a study conducted by Gaynor *et al.* (1989) who found that the $1,25(OH)_2D_3$ concentration was significantly lower in cows fed a cationic diet. Cows fed diets high in cations had a significantly lower level of calcium in plasma on the day of parturition, compared to cows fed the anionic diets, and the incidence rate of milk fever was 26 % for the cationic cows compared to 4 % for the anionic. These data suggest that there was a temporary decreased ability of the renal tissue to respond to PTH and thereby produce $1,25(OH)_2D_3$ to restore the blood level of calcium in cows fed high cation diets. It has also been suggested that the sensitivity of bone tissue to PTH is decreased in cows fed cations in excess, which then also contributes to the lower calcium level around parturition experienced by these cows (Goff *et al.*, 1991; Goff *et al.*, 1997). This idea is supported by Bushinsky (1996) who demonstrated that metabolic alkalosis reduces the response of bone calcium resorption to PTH *in vitro.*

The current hypothesis is that metabolic alkalosis induces changes in the conformation of the PTH receptor (see Fig. 2), making it less able to recognize and bind to the hormone and thereby rendering the target tissues less sensitive to PTH (Bushinsky, 1996). A reduced response to PTH by the kidneys leads to a decrease in renal reabsorption of calcium from the glomerular filtrate and most important it prevents the 1-α-hydroxylation of 25-OH-D_3 to the

active metabolite $1,25(OH)_2D_3$. Failure of the bone tissue to act in response to PTH secretion prevents an effective utilization of calcium from the bone canalculi fluid, as well as decreasing the activity of osteoclasts. The increase in intestinal absorption of calcium and enhanced bone resorption that normally would have been the result of PTH stimulation is thereby inhibited (Goff, 2006).

Dry cows are often offered a forage based diet that contains high levels of easily absorbable cations, especially potassium (K). This places the cow in a state of mild metabolic alkalosis because a greater number of positively charged ions enter the blood than negatively charged ions (Goff, 2007). To restore the electroneutrality of the blood a positive charge in the form of a hydrogen ion must be removed from the blood compartment. A decrease in the concentration of hydrogen ions leads to an increase in the pH of the blood (Stewart, 1983). The opposite, metabolic acidosis, can in turn be induced by a diet high in easily absorbable anions and low in easily absorbable cations. This increases the total of negative charges in the blood and allows for more hydrogen ions to circulate, reducing the pH of the blood.

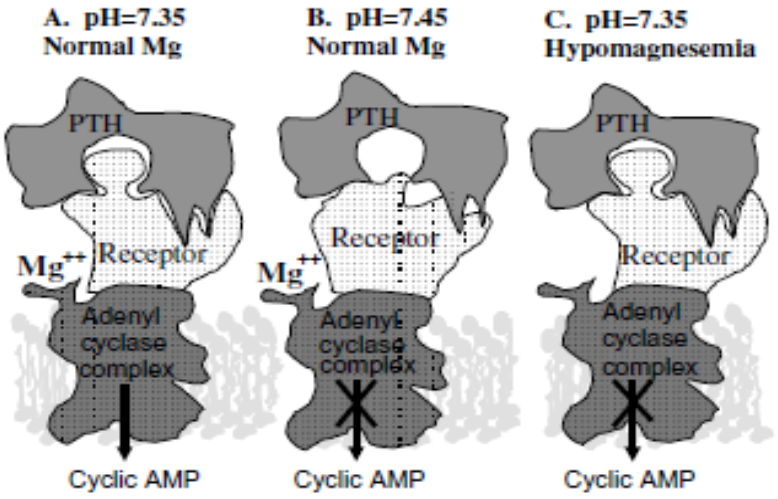

Figure 2. The effect of metabolic alkalosis (B) and hypomagnesaemia (C) on the production of the second messenger cyclic AMP. Adopted from Goff, 2007.

Hypomagnesaemia

The plasma concentration of magnesium in cows is usually maintained in the range of 0.75-1.00 mmol/l. There are no known hormonal mechanisms for control of magnesium homeostasis and the amount of magnesium provided in the diet therefore has to meet or exceed the excretion of magnesium in urine, feces and milk to prevent hypomagnesaemia from occurring (Martens & Schweigel, 2000). A serum concentration of magnesium that is <0.8 mmol/l is a sign of limited dietary absorption of magnesium. When the level of plasma magnesium falls below 0.4-0.5 mmol/l clinical signs of hypomagnesaemia such as recumbency, nystagmus and convulsions are observed (Goff, 2007). This condition is known as hypomagnesaemic tetany and is most common in grazing cattle. In Europe and North America the problem mainly occurs in the spring when cattle are let out on lush pasture (McDonald *et al.*, 2002). Cows with blood magnesium between 0.5-0.8 mmol/l experience a mild form of hypomagnesaemia. The clinical signs are more diffuse than those mentioned

above and the cows are often slow to eat and are not producing milk up to their potential (Goff, 2007).

Calcium metabolism is affected in two ways by hypomagnesaemia. Firstly, a lack of magnesium leads to reduced secretion of PTH in response to hypocalcaemia and secondly, the tissue sensitivity to PTH is reduced (Rude *et al.*, 1978; Goff *et al.*, 1991; Goff, 2007). The cause behind the defect in secretion and action of PTH is not entirely clear, but it is believed to be related to a decrease in enzyme activity. Adenylate cyclase and phospholipase C are two enzymes thought to be involved in PTH secretion and cellular responses to PTH. These enzymes act through the generation of second messengers; cyclic AMP, diacylglycerol and inositol-1,4,5-triphosphatase, that in turn initiates mechanisms such as bone resorption and renal production of $1,25(OH)_2D_3$ to restore calcium levels in blood to normal. Both enzymes have a binding site for magnesium that has to be occupied for normal function (see Fig. 2) (Goff, 2007). Hypocalcaemia is a normal expression of severe magnesium deficiency in humans as well as in most other species. In cattle it is often a result of tetany. It is only possible to restore the calcium level to normal through magnesium therapy, because vitamin D and/or calcium therapy is not effective (Rude *et al.*, 1998).

Magnesium

Function

Magnesium (Mg) is a mineral of crucial importance to the wellbeing of the animal. It is a major intracellular cation and an important cofactor for numerous enzymes such as ATPases, kinases and phosphatases. Magnesium is involved in several biochemical and physiological processes including carbohydrate and lipid metabolism, RNA, DNA and protein synthesis, cellular respiration and it is essential for the formation of cyclic AMP and other second messengers (McDonald *et al.*, 2002). Magnesium is also important in the regulation of membrane channels and it is vital for proper nerve transmission, muscle function and formation of bone mineral (NRC, 2001). Because of its involvement in these many functions, a depletion of magnesium may have serious consequences such as disturbances in voluntary feed intake, rumen fermentation, milk production, and may even result in death if hypomagnesaemia leads to tetany (Martens & Schweigel, 2000).

Normal levels

About 60-70% of the total magnesium content of the body is found in bone, 30-40% is distributed in the soft tissues and only about 1% can be found in the extracellular fluids (Martens & Schweigel, 2000). The normal level of magnesium in plasma for cows is in the range of 0.75-1.00 mmol/l or 1.8-2.4 mg/dl (NRC, 2001). In a 500 kg cow the blood contains about 0.70 g magnesium and all extracellular fluids 2.5 g. The intracellular content of magnesium is 70 g and 170 g is stored in bone mineral. The cow is almost solely dependent on a constant dietary uptake of the mineral since magnesium metabolism is not regulated by specific hormones (Martens & Schweigel, 2000). Even though bone is the major store of magnesium it does not function as a magnesium reserve since bone resorption occurs as a response to calcium deficiency and not guided by magnesium status (NRC, 2001). The

kidneys are important in adjusting the excretion of magnesium to maintain homeostasis. A possible surplus is quickly secreted into urine and in the case of hypomagnesaemia the excretion ceases completely (Martens & Schweigel, 2000).

Requirement

Dry cows weighing 500 kg have a daily dietary requirement of 1.2 g magnesium/kg DM. For cows in lactation with a milk production of ~30 kg/day the requirement increases to 2.0 g/kg DM (Spörndly, 2003). In the Nordic feeding recommendations NorFor, the requirement of magnesium is expressed as grams per day and according to them, a cow weighing 500 kg needs 9 g magnesium/d for maintenance and 33 g/day when producing 30 kg ECM/d. The fetal requirement of magnesium in dairy cattle increases from 2 g/day in the sixth month of pregnancy to 5 g/day in the last month before parturition (NorFor, 2007).

The concentration of magnesium in milk ranges from 0.12-0.15 g/kg and a high yielding cow may lose around 3-4 g through milk per day. Colostrum contains approximately 0.4 g magnesium/kg (NRC, 2001). Because the amount of magnesium in the extra cellular fluid equals the amount excreted in 17 kg of milk (0.15 g/kg 4% milk; Martens & Schweigel, 2000), milk production in high lactating animals can quickly deplete the extracellular pool of magnesium, resulting in hypomagnesaemia if not rapidly replaced (Goff, 2006).

The inflow of magnesium has to meet or exceed the outflow of magnesium through milk and endogenous secretion (2.8 g/day) to maintain magnesium homeostasis in the extra cellular fluid. A decreased absorption causes a net flow of magnesium from the extra cellular fluid and hypomagnesaemia will occur (Martens & Schweigel, 2000). In the face of high levels of potassium in the diet the amount of magnesium provided should be increased to prevent a negative magnesium balance from occurring. Under such circumstances a minimum level of approximately 3.5 g/kg for ruminants has been suggested (Ram *et al.*, 1998).

Magnesium excretion in urine

The kidneys play a major role in the regulation of magnesium homeostasis. Urine concentration of magnesium is directly affected when there is a deficiency in magnesium or when it is provided in excess and can therefore be used as a diagnostic test of magnesium balance in the animal. The concentration of magnesium in urine is often a better indicator of magnesium balance than magnesium level in serum, the reason being that a lack of magnesium immediately is reflected in urine while a severe deficiency is required before the concentration falls in serum (McDonald *et al.*, 2002). Concentration of magnesium in urine range from 0-28 mg/dl and is curvelinearly related to magnesium concentrations in plasma. Urine values decreases rapidly to zero as animals become increasingly hypomagnesaemic (Mayland, 1988).

It has been suggested that cows experiencing equilibrium in magnesium homeostasis will excrete approximately 2.5 g/day in urine and at urinary levels below 1.0 g/day cows are assumed to be at increased risk for developing hypomagnesaemia (Mayland, 1988). Since it is often difficult to use total collection of urine as a diagnostic tool in practice, the recommendations are to use the magnesium concentration of urine in a spot sample (Martens

& Schweigel, 2000). Urine levels above 10 mg/dl is satisfactory, 2-10 mg/dl is inadequate and levels less than 2 mg/dl is an indicator of severe deficiency and the animal is in danger of tetany (Mayland, 1988). It should be emphasized that magnesium concentration in urine is highly influenced by water excretion, which can be very high on diets high in potassium or in grazing animals (Martens & Schweigel, 2000). Since cattle have the ability to excrete large amounts of magnesium in their urine there is usually no risk for magnesium toxicity. A diet high in magnesium is however generally less palatable causing a reduction in feed intake and may also cause osmotic diarrhea (NRC, 2001).

Absorption of magnesium from the gastrointestinal tract

In lambs and calves the predominant site of magnesium absorption before weaning is the small and large intestine. As the fore-stomachs develop they instead become the main site of magnesium absorption (Martens & Schweigel, 2000) and in adult ruminants the reticulorumen is the principal place for magnesium uptake (Tomas & Potter, 1976). NRC (2001) calculates the amount of absorbable magnesium from different feedstuff for cows by multiplying the magnesium content with an absorption coefficient. The coefficient is set to 0.16 for all feed stuff and to 0.70 for mineral supplements.

Magnesium is transported across the rumen epithelium mainly through the transcellular pathway, which includes uptake across the luminal and extrusion across the basolateral membranes. Two models of transcellular transport of magnesium have been proposed. The first mechanism is defined as a secondary active process. Ionized magnesium is absorbed into the lumen of epithelial cells by passive transport, which is mainly driven by a large electrical potential difference (PD) across the rumen epithelium and to a lesser extent by a small chemical gradient (Schweigel *et al.,* 1999). The extrusion of magnesium through the basolateral membrane is thought to be mediated by a Na/Mg exchange system. Sodium (Na) is pumped out of the cell by a Na/K-ATPase enzyme in the basolateral membrane, creating an electrochemical gradient for sodium. The downhill influx of sodium into the cell through the Na/Mg exchange system then generates the energy powering magnesium efflux. This transcellular transport of magnesium can therefore be defined as a secondary active transport system because it is indirectly energized by the Na/K-ATPase. The second transport mechanism proposed for magnesium uptake is independent of alterations in the PD. This process is solely dependent on the chemical gradient of magnesium ([Mg] greater in rumen than in cytosol) as the major driving force for magnesium uptake. This passive transport is thought to be mediated through co-transport of magnesium with anions or through carriers exchanging one Mg^{2+} ion for two H^{+} ions in the apical membrane of the epithelial cells (Martens & Schweigel, 2000).

The existence of two mechanisms for uptake of magnesium ensures absorption over a wide range of magnesium concentrations in the rumen. This is crucial for the homeostasis of magnesium metabolism since the concentration of magnesium in the extracellular fluid is dependent on a constant influx of magnesium from the diet. The PD-dependent transport mechanism functions primarily at very low rumen magnesium concentrations (0.024 mmol/l) ensuring that absorption of magnesium can occur even at low intakes. The PD-independent

transport mechanism functions mainly in the presence of high ruminal concentrations of magnesium, which allows magnesium to flow down its concentration gradient. This electroneutral magnesium uptake is probably more important or even predominant at high magnesium concentrations in the rumen (Martens &Schweigel, 2000). Goff (2007) recommended that the dietary concentration of magnesium should be 3.5-4.0 g/kg DM for the periparturient cow. This higher concentration makes it possible for the cow to take advantage of passive absorption across the rumen wall and ensure an adequate level of magnesium in plasma.

The presence of magnesium carriers in the basolateral membrane implies that there is a theoretical possibility for magnesium absorption to be saturated This idea has been supported by observations where magnesium absorption initially increased with increasing ruminal magnesium concentrations but became saturated at 4 mmol/l in sheep and 12.5 mmol/l in heifers (Weiss, 2004).

The effect of dietary potassium on magnesium absorption

Several studies have demonstrated that ruminants consuming diets with high concentrations of potassium experience a significant reduction in magnesium absorption from the gastrointestinal tract (Greene *et al*., 1983a; Khorasani *et al*, 1990; Dalley *et al.*, 1997; Ram *et al*., 1998; Schonewille 1999; Jittakhot *et al.* 2004a). According to Greene *et al.* (1983b) the reduced availability of magnesium is caused by a decrease in magnesium absorption from the fore-stomachs that is not compensated for by an increased absorption in the lower intestine. Studies *in vitro* have shown that an elevated level of potassium causes depolarization of the apical membranes of the rumen epithelial cells, removing the electrical driving force for magnesium absorption (Martens *et al.*, 1987). The PD-dependent magnesium absorption is therefore also called potassium-sensitive. The extent of the negative effect of potassium on magnesium absorption depends on the ruminal concentration of magnesium and it has been shown that supplemental magnesium can counteract the inhibitory effect of high levels of dietary potassium (Care *et al.*, 1984; Ram *et al.*, 1998; Jittakhot *et al.*, 2004a). In a study by Ram *et al*. (1998) the apparent absorption of magnesium in sheep was studied at three increasing concentrations of magnesium and two levels of potassium. The negative effect of a high potassium level (36 g/kg DM) was almost constant over all magnesium concentrations, decreasing magnesium absorption by 0.31 g/d, 0.36 g/d and 0.41 g/d at the low, medium and high magnesium intake respectively. However, at the low magnesium intake (1.65 g/d) potassium caused a 53% reduction in magnesium absorption compared to only a 27% reduction at the high magnesium intake (4.7 g/d). These results further indicate that magnesium absorption may be more sensitive to potassium at low ruminal magnesium concentrations and that the negative effect of potassium can be overcome by increasing magnesium intake.

In 2004 Weiss compiled magnesium digestibility data from eight different feeding experiments with lactating cows, where apparent magnesium digestibility had been measured using total collection of feces and urine. The cows were fed varying feed stuff and the mean apparent digestibility for magnesium was 0.18 ranging from -0.04 to 0.33. Weiss (2004)

found that the NRC absorption coefficient for magnesium (0.16) was similar to the one obtained from the empirical data for a variety of diets when potassium content was approximately 1 % of DM. However, magnesium digestibility decreased linearly with 0.075 units for every 1% increase in dietary potassium. An addition of 18 g magnesium per day should therefore be provided to lactating dairy for every percentage increase in dietary potassium above 1% DM to maintain the amount of digestible magnesium at the same level as with 1% potassium (Weiss, 2004). In this study the average potassium content was 16 g/kg DM, which is a relatively low value since the average content of forage usually is >30 g/kg DM (Fisher *et al.*, 1994). The NorFor recommendations are to use feedstuff during the dry period that have a lower contents of potassium than 25 g/kg DM to reduce the risk of interfering with magnesium absorption (NorFor, 2007).

Magnesium oxide

In ruminant diets, inorganic magnesium oxide is a common and widely used source of magnesium. It is the least expensive magnesium product but unfortunately also the least soluble. The absorption coefficient of magnesium oxide varies between 28 and 49 % and the availability is largely influenced by particle size (NRC, 2001). Finely grinding of magnesium oxide increases solubility and hence the absorption because of the much greater surface area (Adam *et al.*, 1996). Jesse *et al*. (1981) administered 100 g magnesium oxide of four different particle sizes to dry and lactating cows. The increase in urinary excretion of magnesium above baseline was significant in cows fed finely ground magnesium oxide (75 μm to 425 μm) compared to cows fed prilled magnesium oxide (425 to 1700 μm) where no increase was observed. That solubility and availability of magnesium oxide increase as particle size decrease has also been reported by Xin *et al.* (1989) and Adams *et al.* (1996). Availability of magnesium from magnesium oxide also depends on the heating temperature during the process of calcination when magnesium oxide is produced from magnesite rock. Adams *et al.* (1996) found that calcination at 800, 900 and 1 100 °C significantly increased apparent absorption of magnesium as well as urinary magnesium excretion. The solubility of magnesium oxide is also affected by rumen pH and decreases sharply as pH rises above 6.5 (Goff, 2007). Magnesium oxide is the most palatable source of magnesium and inclusion of 60 g in 0.5-1 kg grain is readily acceptable (NRC, 2001).

Prevention of hypocalcaemia

The dietary cation-anion difference

The dietary cation-anion difference (DCAD) concept is based on the theory of the strong ion difference (Stewart, 1983). This theory suggests that the acid-base status of an animal can be influenced by all cations and anions in a diet. To which extent an ion causes a change in acid-base balance and pH of the blood depends on the quantity of the ion entering the system. The acid-base balance is therefore determined by the difference in number of cation and anion equivalents that are absorbed into the bloodstream. If there is a higher amount of absorbable cations available the cow becomes alkalotic and if the amount of absorbable anions predominates the cow becomes acidotic. The major cations present in feed are Na^+, K^+, Mg^{2+}

and Ca^{2+} and the major anions are chloride (Cl^-), sulfate (SO_4^{2-}) and phosphate (PO_4^{3-}) (Horst *et al.*, 1997) . Other macro minerals are absorbed in such small amounts that their effect on the acid-base status is negligible. Volatile fatty acids produced in rumen fermentation are absorbed into blood in an undissociated form carrying a positive and a negative charge. They are rapidly metabolized in the liver and have thus only a small effect on general acid-base status under normal circumstances (Goff, 2006).

Manipulating the dietary cation-anion difference (DCAD) of dry cow rations has proven to be a successful strategy in the prevention of hypocalcaemia and milk fever. DCAD is usually described in terms of meq/kg DM and a number of formulae have been developed to calculate DCAD of different feeds. The most common equation used is: DCAD = ([Na+] + [K+]) – ([Cl-] + [S-]) (Horst *et al.* 1997; DeGaris & Lean, 2008). Other formulas that differ in their combinations of anions and cations, as well as in the coefficients assigned to the major cations depending on their acidifying or alkalinizing potential, have also been proposed (DeGaris & Lean, 2008).

Dry cows are often offered a forage-based diet containing high amounts of cations, especially potassium. This usually means that the DCAD value of the ration is high, which will place the cow in a state of mild metabolic alkalosis. It has been suggested that a high blood pH reduces the responsiveness of target tissues to PTH, which in turn leads to an impaired calcium metabolism (Goff *et al.,* 1991). The aim of manipulating DCAD is therefore to achieve a reduction in the alkalinity of the blood and by increasing the dietary amount of anions the cow can instead be placed in a state of mild metabolic acidosis as is evidenced by a reduced blood and urinary pH (Gaynor *et al.* 1989; Goff *et al.* 1991). It has been shown that decreasing the DCAD in rations to prepartum cows through the addition of anions reduce the severity of hypocalcaemia and the incidence of milk fever (Block, 1984; Oetzel *et al.,* 1988; Goff *et al.,*1991; Goff & Horst, 1998). In a study by Block *et al.* (1984) cows that received an anionic diet had 0% incidence of milk fever compared to an incidence of 47% for cows fed the cationic diet. According to two separate meta-analyses on milk fever by Lean *et al.* (2006) and Charbonneau *et al.* (2006) there is a linear relationship between DCAD and milk fever risk, showing that a reduction in DCAD leads to a decreased risk for milk fever.

Addition of anionic salts to reduce DCAD affects calcium homeostasis for example by increasing calcium concentration in plasma at parturition (Block, 1984; Oetzel *et al*. 1988, Goff *et al.,* 1991; Wang & Beede, 1992; Joyce *et al.,* 1997; Goff & Horst, 1998). In a study by Goff *et al.* (1991) the plasma concentration of calcium was significantly higher (1.74 mmol/l) 24 h after calving for cows fed an anionic diet compared to those fed a cationic ration (1.49 mmol/l). The absorption of calcium from the intestines has also been shown to increase as a result of lowering DCAD (Goff *et al.,* 1991; Schonewille *et al.,* 1994; Roche *et al*., 2007). Block *et al*. (1984) and Gaynor *et al.* (1989) observed elevated levels of hydroxyproline in plasma in cows fed anionic diets, which may indicate a higher mobilization of bone in these animals. The addition of anionic salts also results in increased excretion of calcium in urine, which probably reflects the excretion of excess calcium due to increased intestinal calcium absorption and release of bone calcium (Block, 1984; Gaynor *et al.,* 1989; Goff *et al.,* 1991; Wang & Beede, 1992).

There are no exact recommendations of the most appropriate DCAD level in dry cow rations but the attempt is usually to bring DCAD below 0 meq/kg DM to achieve an adequate acidification of the cow (NRC, 2001). DCAD should however not be allowed to fall below -1 000 meq/kg DM (Spörndly, 2003). Feeding a diet too low in DCAD prepartum may have a negative effect on feed intake and should therefore be avoided (Charbonneau *et al.*, 2006). A dietary DCAD of -50 to -100 meq/kg DM has been suggested as optimal for the prevention of milk fever by Horst *et al.* (1997). The NorFor recommendation is to feed rations with a DCAD of -100 to -150 meq/kg DM in the dry period at least for the last three to four weeks before parturition to prevent of milk fever (NorFor, 2007).

Addition of anionic salts

A method commonly used to acidify rations is through the addition of ammonium-, calcium- or magnesium salts of chloride and sulfate, or through addition of acids of the strong anions. Strong cations like Ca^{2+}, Mg^{2+} and NH_4^+ are absorbed to a lesser extent from the gastrointestinal tract compared to strong anions (Cl^-, SO_4^{2-}). This leads to a relative excess of absorbed anions that reduces the strong ion difference and causes a drop in plasma pH. Feeding NaCl or KCl has no effect on the strong ion difference since sodium, potassium and chloride are absorbed with almost 100 % efficiency in the intestine (DeGaris and Lean, 2008). Goff *et al.* conducted a study in 2004 where they evaluated the relative acidifying properties of different anionic salts by feeding them to dry cows and determining their ability to reduce blood and urine pH. They found that sulfate salts had a 55-60% lower effect on blood pH compared to chloride salts. Goff *et al.* (2004) speculated that the reason for the lower acidifying effect of sulfate salts could be that sulfate does not get absorbed to the same extent as chloride, or that sulfate is cleared from the blood faster than chloride and therefore does not exert as great an effect on blood pH. Oetzel *et al.* (1991) could not find any difference in acid-base status or calcium balance when the effects of six different anionic salts, $MgCl_2$, $MgSO_4$, $CaCl_2$, $CaSO_4$, NH_4Cl and $(NH_4)_2SO_4$ were tested. This result is in agreement with the findings by Tucker *et al.* (1991) and Gelfert *et al.* (2010) where there was no indication of chloride salts in general having a stronger acidifying effect on the acid-base status than sulfate salts.

Effect of DCAD on urine pH

Urine pH is related to metabolic pH and is therefore a useful indicator of changes in the acid-base balance of the cow. Measuring urine pH is therefore regarded as a simple and efficient method in monitoring the effect of manipulating the DCAD of diets and pH can be checked 48 hours or more after a ration change (Goff, 2007). Cattle generally have a high urine pH, often above 8, and the addition of anionic salts has been shown to reduce urine pH in several studies (Schonewille, 1994; Moore *et al.*, 2000; Roche *et al.* 2007; Kurosaki *et al.* 2007; Ramos Nieves *et al.*, 2009). The relationship between DCAD and pH is curvilinear and DCAD does not exert an effect on urine pH until it reaches approximately 100-200 meq/kg DM (McNeill *et al.*, 2002). It has been suggested that pH should be brought down to between 6.2-6.8 for optimal control of milk fever in Holsteins and to 5.8-6.3 for Jersey cows (Goff, 2007). However, there are several authors that think that the target could be relaxed to a pH of 6.5-7 (McNeil *et al.*, 2000; Oetzel *et al.*, 2002). Charbonneau *et al.* (2006) concluded in a

meta-analysis of 22 published studies that a group urine pH of 7.0 would be a more appropriate goal because acidification of urine beyond this point could have a negative effect on dry matter intake without much additional benefit in the prevention of milk fever.

Feed intake

The addition of anionic salts has been associated with a decrease in feed intake. The effects on feed intake are however contradictory in the literature and some experiments have demonstrated a significant depression (Joyce *et al.* 1997; Moore *et al.*, 2000) whereas others have not observed any reduction (Block *et al.,* 1984; Oetzel *et al*., 1988; Oetzel *et al*., 1991). Goff & Horst (1998) even reported higher prepartum consumption of a ration where hydrochloric acid had been added compared to control. In a meta-analysis by Charbonneau *et al*. (2006) it was clearly demonstrated that a reduction in DCAD caused a significant decrease in dry matter intake. One theory proposed is that anionic salts reduce palatability when added to the diet. Oetzel & Barmore (1983) demonstrated in their study that if anionic salts were added to the concentrate portion of the ration it decreased the intake of concentrate. The authors then suggested that anionic salts should be mixed with large amounts of concentrate or with forages to ensure adequate feed intakes. Another explanation for the reduced feed intake could also be that the metabolic acidosis induced on high anion diets creates a discomfort that results in decreased consumption (Charbonneau *et al*., 2006). The benefits of reducing DCAD in the prevention of milk fever therefore have to be weighed against the problems that can arise from a reduced dry matter intake before calving. Moore (2000) demonstrated that addition of anionic salts to prepartum diets significantly reduced energy balance and increased the content of liver triglycerides in heifers. These effects were however not observed in cows and Moore (2000) therefore suggested that supplemental anions should be avoided in prepartum heifer rations.

MATERIAL AND METHODS

Cows and experimental design

Twenty-four dry pregnant cows (parity 1 to 3) and twelve pregnant heifers were studied during three weeks before and one week after parturition in this experiment. All animals were of the Swedish Red Breed and were housed indoors in individual tie-stalls on rubber mats with straw or sawdust as bedding. The cows were divided into four blocks based on parity number and expected date of calving and heifers were divided into two blocks based on calving date. The experimental setup was a three factor design and animals within block were randomly allocated to one of three experimental diets. The experimental design and animal procedures were approved by the Uppsala local ethics committee.

This Master thesis project is a part of a larger study and the results presented in this report will therefore not include all animals taking part in the project.

Diets

Cows and heifers were fed rations that covered their nutrient requirements according to Swedish feeding recommendations during the whole experimental period (Spörndly, 2003). During the last three weeks of gestation the animals were offered one out of three experimental diets in restricted amounts to ensure a constant intake of nutrients. The control diet (C) consisted of late harvested grass silage. The magnesium chloride diet (MCL) consisted of grass silage from the same harvest as in (C) and a silage additive, containing 8 g $MgCl_2$/kg DM in a suspension of 150 kg $MgCl_2$* 6 H_2O + 158 kg water, had been added at ensiling of the silage bales to increase the total concentration of magnesium by ~1.7 g/kg DM and to lower DCAD to ~100 meq/kg DM. The magnesium oxide diet (MO) consisted of the same grass silage as in (C) and a dose of 22.7 g magnesium oxide was manually mixed in the silage at every lunch feeding to increase the total concentration of magnesium by ~1.7 g per kg DM of silage. The magnesium oxide had a purity of 83.9% and a particle size of 0-0.2 mm (9.8%), 0.2-0.5 mm (27.4%), 0.5-0.8 mm (35.0%), 0.8-1.0 (20.2%) and >1.0 mm (7.5%).

Animals assigned to each dietary treatment started to receive their respective rations approximately 21 days before expected date of calving, starting on a Monday, and remained on the diet until parturition. All animals were fed individually with 8 kg DM per day of the experimental silages equally divided over three feeding occasions at 08.00, 12.00 and 18.00 h. Concentrate was fed separately to each animal in equal portions at 08.00, 12.00 and 16.00 by automatic feeding wagons. The concentrate portion was increased by 0.5 kg per day until the final amount of 2 kg/day was reached on Thursday.

The animals were regarded as “on treatment” after being on the ration for one week. After parturition all animals were offered silage *ad lib* suited for lactating cows and the amount of concentrate was increased by 0.5 kg per day. In addition, they were given ~100 g of mineral

supplement adapted for lactating cows. Table 1 displays the compositions of the experimental rations and total daily intake of magnesium, calcium and DM during the experimental period.

Table 1. Composition of the experimental rations control (C), $MgCl_2$-silage (MCL) and magnesium oxide (MO)

Ingredients	C	MCL	MO
Control-silage, kg of DM/d	8	-	8
$MgCl_2$-silage, kg of DM/d	-	8	-
Concentrate, kg of DM/d	1.8	1.8	1.8
MgO, g of DM/d	-	-	27.0
Mg, g/d	19.1	28.9	32.7
Ca, g/d	61.4	67.4	61.4
DM, kg/d	9.8	9.8	9.8

Collection of samples

Samples of the fresh crop were collected at ensiling from the C-silage and from the MCL-silage after the addition of $MgCl_2$. Silage samples were collected each time a new silage-bale was opened during the experimental period. The samples were dried at 60 °C for 24 h in a forced-air oven, ground in a hammer mill (1 mm screen) and stored for later analysis.

Two blood and two urine samples were taken from each cow/heifer with four days interval one week before the start of the trial to be used as reference. During the first week on the experimental diet one blood (Saturday) and six urine samples (Monday-Saturday) were collected per animal. Thereafter two blood and two urine samples were collected per week (Tuesday and Saturday) until calving. All samples were collected in the morning after the morning feeding. After calving, blood was sampled after 6, 12 and 24 hours and in the morning of day 2, 4 and 7. Urine samples were obtained at 24 h after calving and in the morning on day 2, 4 and 7 after calving.

All urine samples (~50 ml) were obtained by manual stimulation. The samples were centrifuged within one hour of collection (>1 min, 1 800 x g) and three aliquots of 1.5 ml were frozen and stored for later analysis. Blood samples were collected from the tail vein into 10 ml Vacutainer tubes containing lithium-heparin as anticoagulant. The samples were kept cool on ice and centrifuged within one hour of collection (10 min, 1800xg, 3°C). The plasma was then harvested, frozen and stored for later analysis.

Cows were milked two times a day after calving and milk production was registered during the first week of lactation.

Analysis

The ground material of the feed samples was dried at 105°C overnight for analysis of DM and ash. Crude protein (CP) was analyzed by the fully automated Kjeldahl procedure (Technicon, Solna, Sweden). The metabolizable energy (ME) in the feed was calculated using the in vitro

digestibility (Lindgren, 1979; Lindgren, 1983). The calcium, phosphorus, magnesium, potassium, sulfur and sodium concentration in feed was determined by optical spectral emission analysis with inductively coupled plasma (Spectro Analytical Instruments GmbH & Co. KG, Kleve, Germany). Analyzed composition and calculated ME values of the experimental silages, lactation silage and concentrate feed are displayed in Table 2.

Table 2. Analyzed composition and calculated ME values of the control-silage, $MgCl_2$-silage, lactation-silage and concentrate. Standard deviations are given within ( )

Dietary analysis	Control-silage	$MgCl_2$-silage	Lactation-silage	Concentrate
DM, %	59.82(±1.02)	52.75(±5.14)	30.1	88
ME, MJ/kg of DM	9.1(±0.28)	9.7(±0.35)	n.d	13.2
NDF % of DM	52.4(±1.90)	52.4(±0.07)	n.d	30.1
CP, g/kg of DM	12.5(±1.77)	12.85(±1.06)	n.d	182
Ash % of DM	7.31(±1.35)	8.76(±0.26)	n.d	6.9
Ca g/kg of DM	6.42(±0.78)	5.67(±0.50)	5.5	8
P g/kg of DM	2.02(±0.33)	2.04(±0.16)	2.7	5.1
Mg g/kg of DM	1.47(±0.15)	2.85(±0.26)	1.4	4.5
K g/kg of DM	19.36(±2.98)	22.61(±1.52)	27.6	8.5
S g/kg of DM	1.90(±0.02)	2.10(±0.10)	2.3	n.d
Cl g/kg of DM	7.99	13.27(±0.53)	n.d	n.d
Na g/kg of DM	< 0,1	< 0,1	n.d	n.d
DCAD* meq/kg DM	202.9 (±88.34)	77.6 (±47.80)	n.d	n.d

*DCAD was calculated as ([Na]+[K])-([Cl]+[S]) meq/kg DM (Spörndly, 2003).

Urinary pH was measured within five minutes after collection with a portable pH-meter (Metler Toledo SG2) calibrated using two buffers with pH 4 and 7. The concentration of calcium and magnesium in urine and plasma was measured using quantitative colorimetric methods (The Randox method, Randox Laboratories Ltd, Crumlin, UK). Urine samples with high calcium concentration were diluted 1:4 or 1:9 and reanalyzed. Urine samples with low calcium concentration were reanalyzed using four times the initial sample volume. All urine samples for magnesium analysis were diluted 1:9 in the first analysis. Samples with low magnesium concentration were reanalyzed undiluted.

Plasma was analyzed for CTx using a commercial ELISA method. All samples were diluted 1:2 before analysis and the absorbance was read at 450 nm (Serum CrossLaps® ELISA, Immunodiagnostic Systems Nordic a/s, Herlev, Denmark). PTH in plasma was analyzed using Bovine Intact PTH ELISA Kit and the absorbance was read at 450 nm (Immutopics, Inc. San Clemente, CA). Creatinine concentration in urine was analyzed on an Autoanalyzer using Technicon 1974b, Technicon method No. SE4-0011FH4 (Technicon Instruments Corporation, Tarrytown, NY) and the total daily production of urine was calculated using creatinine excretion of 29 mg/kg body weight (Valadares *et al*., 1999).

Statistical methods

The data was analyzed using PROC MIXED (SAS institute, 2002) and correlations were analyzed using PROC CORR. The categorical variables used were cow, treatment, block, day and lactation group and cow was treated as a random effect. Time was expressed in days relative to parturition in the analysis and time variables during the prepartum period were divided into periods of three days, the first interval containing day 0, -1, -2, the second interval day -3, -4, -5 and so on. Only the samples collected after the animals had been fed the experimental diets for one week were used in the analysis. First order autoregressive covariance structure was used for cow over time.

Two statistical models were used for plasma and urine parameters. The first model included the fixed factor block. In the second model, block was replaced by the fixed factor lactation group, where heifers and cows were grouped separately:

$Y_{ijkl} = \mu + (\text{treatment})_i + (\text{block})_j + (\text{day})_k + (\text{block*day})_{jk} + (\text{treatment*day})_{ik} + (\text{cow})_{ijkl} + \varepsilon_{ijklm}$

$Y_{ijkl} = \mu + (\text{treatment})_i + (\text{lactation group})_j + (\text{day})_k + (\text{lactation group*day})_{jk} + (\text{treatment*day})_{ik} + (\text{lactation group*treatment*day})_{ijk} + (\text{cow})_{ijkl} + \varepsilon_{ijklm}$

μ = mean value of all observations
treatment = fixed effect of treatment (three levels)
block = fixed effect of block (five levels)
lactation group = fixed effect of lactation group (two levels: heifer, cow)
day = fixed effect of time relative to calving (divided into periods precalving, 12 levels for urine parameters, 15 levels for plasma parameters)
cow = random effect (25 levels for urine pH, 24 levels for urine magnesium and calcium concentration and 23 levels for plasma parameters)
ε = random error

A significance level of $p<0.05$ was used for all models and correlations and non-significant interactions were eliminated from the model. A star (*) indicates significant differences in the figures. All data is presented as LSmeans and SEM except milk production, which is presented as average daily milk yield.

RESULTS

This Master thesis project is a part of a larger study and the results presented in this report will therefore not include all animals taking part in the project. All heifers (12) are included in the urine and plasma results, 13 cows in the urine pH results (av. parity 1.46), 12 cows in the urine calcium and magnesium results (av. parity 1.33) and 11 cows are included in the plasma results (av. parity 1.36). The numbers of animals per treatment are presented in the graphs. Animals were fed the experimental diets for 24 days on average (15-33 days), the first week on the experimental diets included. Some graphs only display a part of the period before parturition because no statistical differences were observed in that time period.

Due to technical problems in the process of applying the silage additive containing $MgCl_2$ to the silage at ensiling, the analyzed magnesium content of the $MgCl_2$-silage was on average 1.38 g/kg DM higher than the contents of the control-silage instead of the expected 1.7 g/kg DM. The total magnesium intake then became 19.1, 28.9 and 32.7 g/day for the C, MCL and MO diets respectively (Table 1). The goal to reduce DCAD of the $MgCl_2$-silage to ~100 meq/kg DM was achieved (Table 2).

No differences in feed intake could be observed between the experimental rations and the daily offered feed quantity was entirely consumed by all animals.

Urine parameters

Urine pH

Urine pH was not affected by treatment (see Fig. 3), but there was a numerical difference where MCL on average had a lower pH (8.12±0.156, LSmeans ±SEM) than MO and C prepartum (8.21±0.093, 8.22±0.119), respectively. In addition, there was a significant negative correlation between urine pH and urinary calcium concentration (r = -0.165) in the period from day -21 to the day of parturition. The correlation grew stronger as the animals approached calving and was r = -0.44 in the week before parturition.

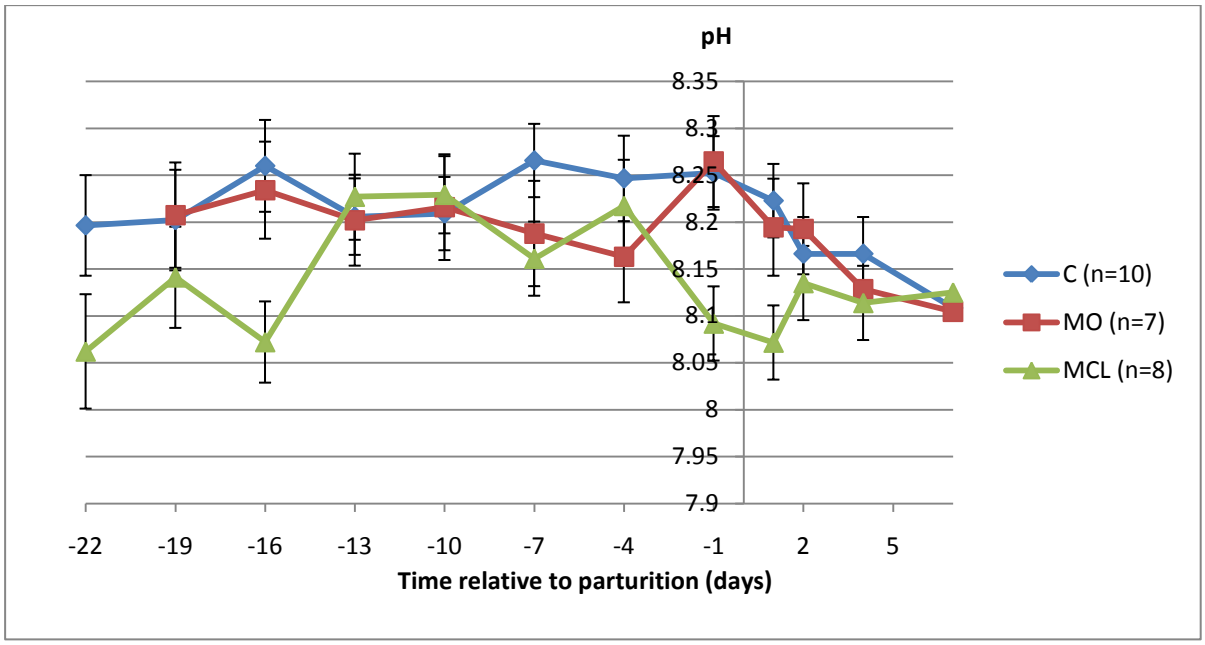

Figure 3. Urine pH on day relative to parturition for animals fed control-silage (C), magnesium oxide (MO) and $MgCl_2$-silage (MC). Error bars display the standard error of the mean.

Urine calcium

There was a significant effect of day and treatment on daily excretion of calcium in urine (g/day), where MCL-animals had a higher daily excretion compared to other treatments. Urine concentration (mg/dl) of calcium decreased abruptly for animals on all treatments on the day of calving, but slowly returned to prepartum levels on day 7 (see Fig. 4). There was a significant *day* by *treatment* interaction where the concentration of calcium in urine was higher for MCL compared to C and MO on day -8 to 0 and -5 to 0 respectively. MO had a higher urinary calcium concentration than C and MCL on day 7 after parturition.

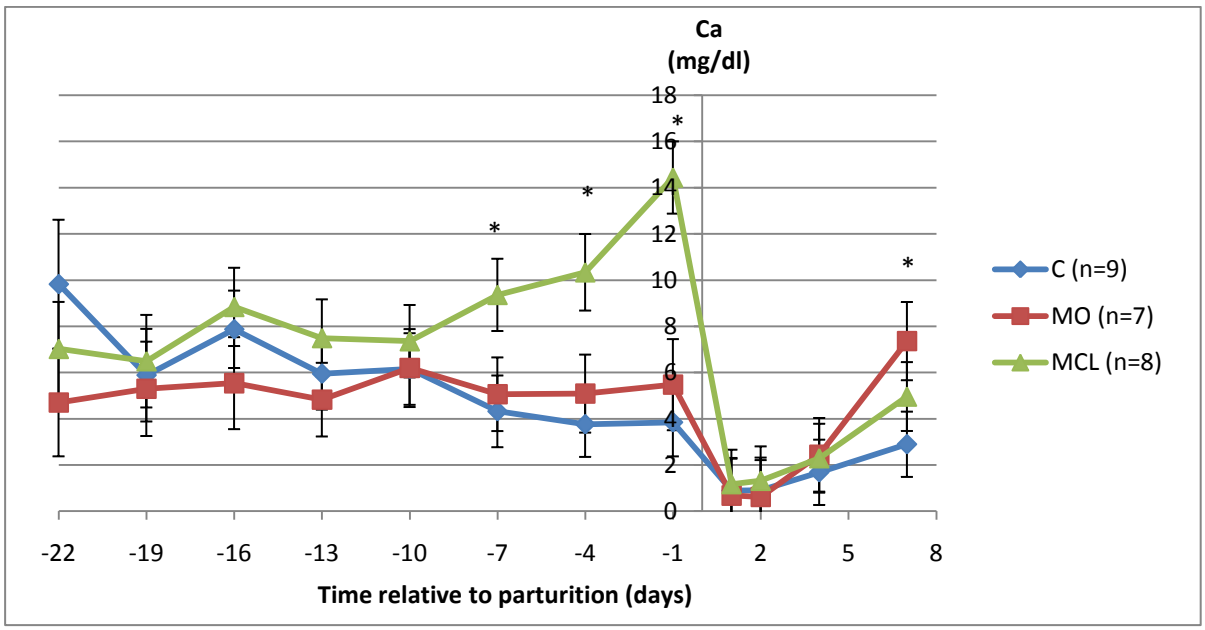

Figure 4. Concentration of calcium in urine for animals fed control-silage (C), magnesium oxide (MO) and $MgCl_2$-silage (MCL). Error bars display the standard error of the mean.

Urine magnesium

Treatment had a significant effect on daily excretion (g/day) of magnesium in urine, where MCL and MO experienced a higher loss of magnesium compared to C. For urine magnesium concentration (mg/dl) the statistical analysis showed a significant *treatment* by *day* interaction. MCL and MO had a higher level of magnesium in urine than C on day -20 to -18 and MO had a higher concentration than C on day -11 to -9 (see Fig. 5.). MCL reached the highest concentration one week before calving and remained significantly higher than C and MO from day -8 to 0 and -5 to 0 respectively. This was followed by an abrupt decrease on the day of parturition that continued through day 7. C also experienced a steady decrease starting at parturition although the decrease at calving was not as pronounced as for MCL. For MO the magnesium concentration began to decrease on day 10 and kept decreasing until day 4 after calving when the curve turned upwards again. A numerical difference was observed prepartum where the average concentration for MCL, MO and C was 32.9 (±16.92), 26.2 (±12.71) and 21.4 (±11.61) mg/dl respectively.

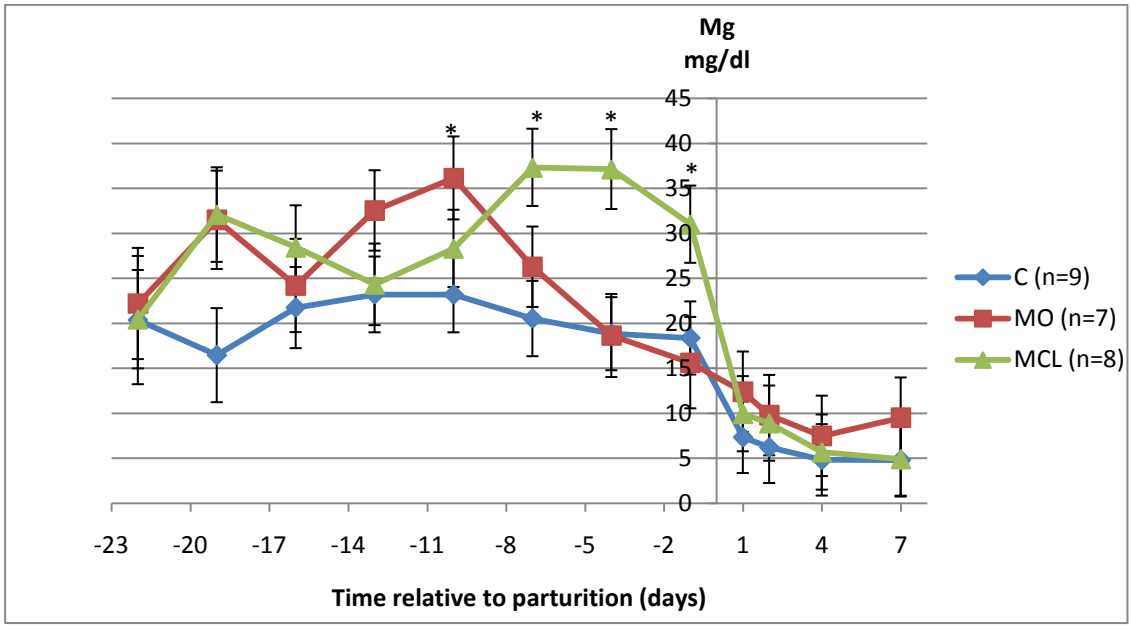

Figure 5. Concentration of magnesium in urine on day relative to parturition for animals fed control-silage (C), MgO (MO) and $MgCl_2$-silage (MC). Error bars display the standard error of the mean.

Plasma parameters

Plasma magnesium

There was a significant effect of day on magnesium concentration in plasma but no effect of treatment or block. All animals experienced a decrease in plasma magnesium concentration after parturition that reached a nadir on day 4 (see Fig. 6) and then began to increase to the level seen before calving. All animals maintained a plasma magnesium concentration within the interval considered as normal (0.75-1.0 mmol/l) for the whole trial.

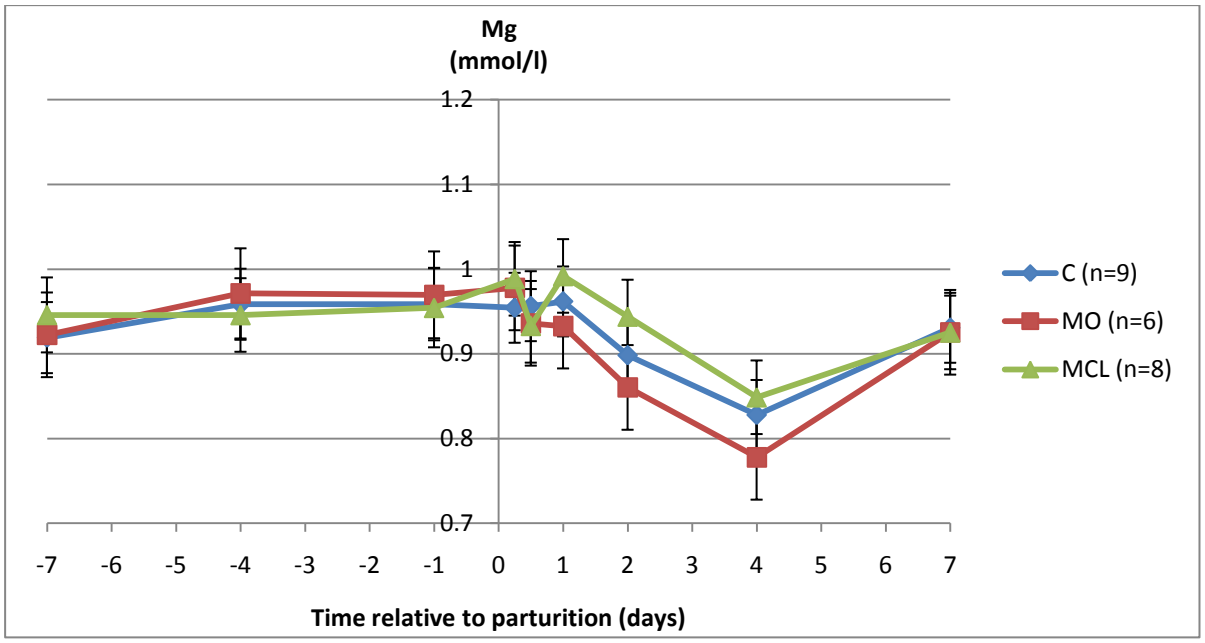

Figure 6. Concentration of magnesium in plasma on day relative to parturition for animals fed control-silage (C), magnesium oxide (MO) and $MgCl_2$-silage (MCL). Error bars display the standard error of the mean.

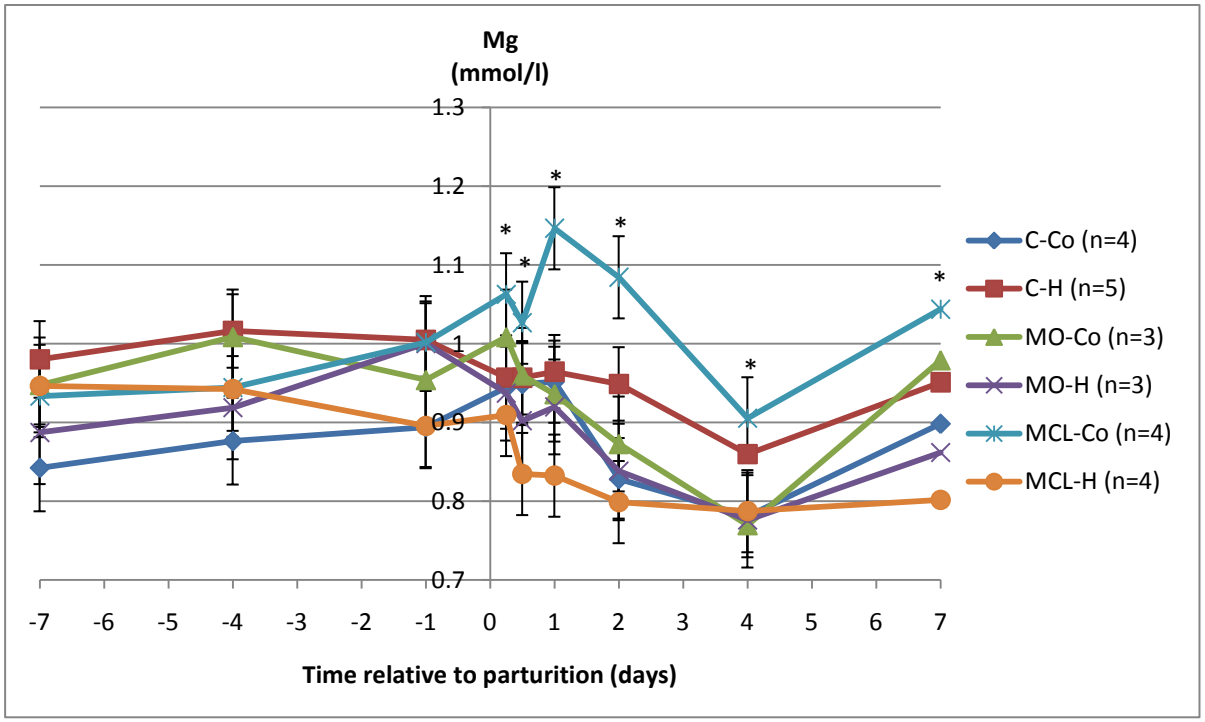

Figure 7. Concentration of magnesium in plasma for cows (Co) and heifers (H) fed control-silage (C), magnesium oxide (MO) and $MgCl_2$-silage (MC). Error bars display the standard error of the mean.

When age was included as a fixed factor in the statistical model there was a significant *treatment* by *age* by *day* interaction postpartum. MCL-cows had a higher concentration of magnesium in plasma than MCL-heifers during the first 2 days and on day 7 postpartum (see Fig. 7). In addition, MCL-cows experienced a higher plasma concentration than all other treatments/ages after 24 h (except for C-heifers) and on day 2.

Plasma calcium

Treatment did not have an effect on calcium concentration in plasma during the trial. There was a significant effect of day where the concentration of calcium decreased abruptly for all animals on the day of calving and reached a nadir 24 h (MCL) and 48 h (MO, C) after parturition (see Fig. 8). In addition there was an effect of block. No animal experienced clinical hypocalcaemia (<1.4 mmol/l) after calving, but all treatments had a mild form (1.89-1.97 mmol/l) of subclinical hypocalcaemia (1.4-2.0 mmol/l) after 24 h and on day 2 postpartum.

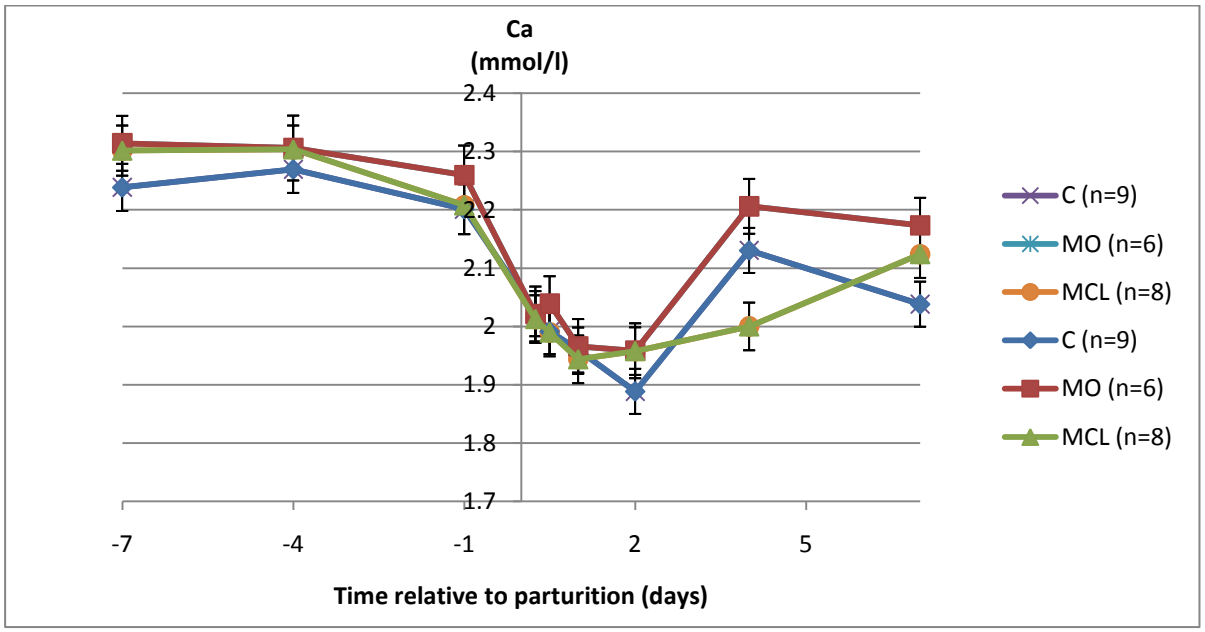

Figure 8. Concentration of calcium in plasma on day relative to parturition for animals fed control-silage (C), magnesium oxide (MO) and $MgCl_2$-silage (MCL). Error bars display the standard error of the mean.

Plasma CTx

There was a significant effect of day on plasma concentration of CTx, which increased rapidly for all animals on the day of calving and reached the highest level on day 1 after parturition (see Fig. 9). The CTx concentration then remained on a higher level than seen before calving for the rest of the trial. There was no effect of treatment but a significant effect of block was noticed, where block 2 (heifers) had a higher concentration of CTx than the other blocks. There was a significant *day* by *age* interaction where heifers had a higher level of CTx than cows from day -11 to parturition and on day 4 and 7 postpartum (see Fig. 10).

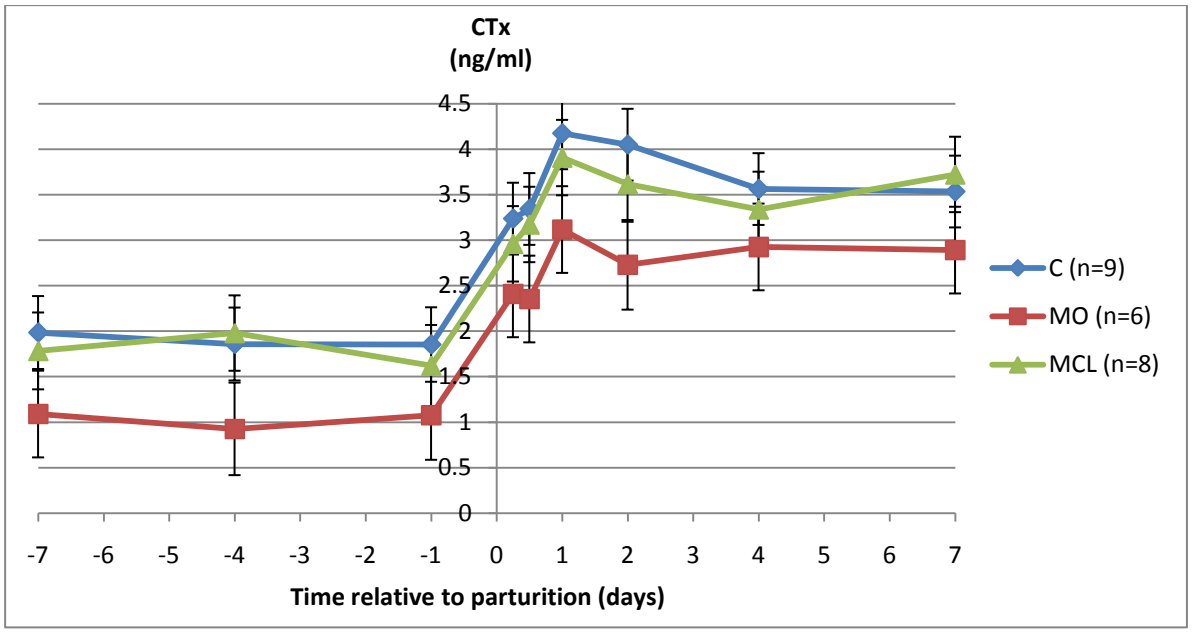

Figure 9. Concentration of CTx in plasma for animals fed control-silage (C), magnesium oxide (MO) and $MgCl_2$-silage (MCL). Error bars display the standard error of the mean.

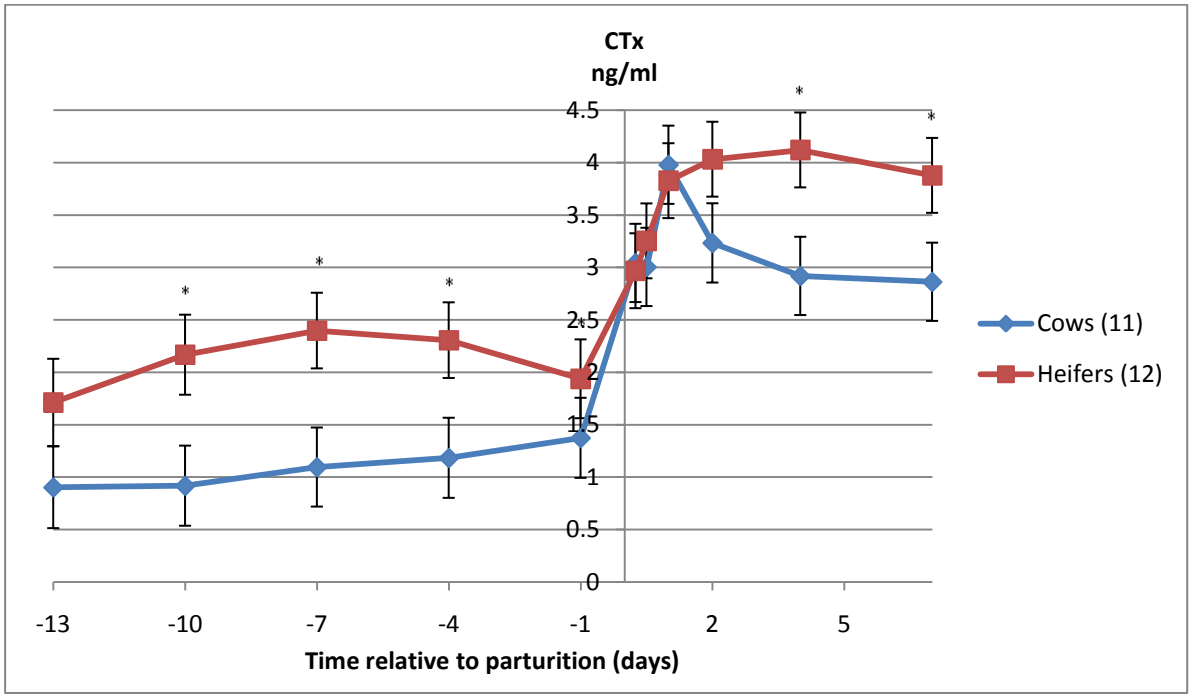

Figure 10. Concentration of CTx in plasma for cows and heifers on day relative to parturition. Error bars display the standard error of the mean.

Plasma PTH

Neither treatment nor day had an effect on plasma concentration of PTH (see Fig. 11).

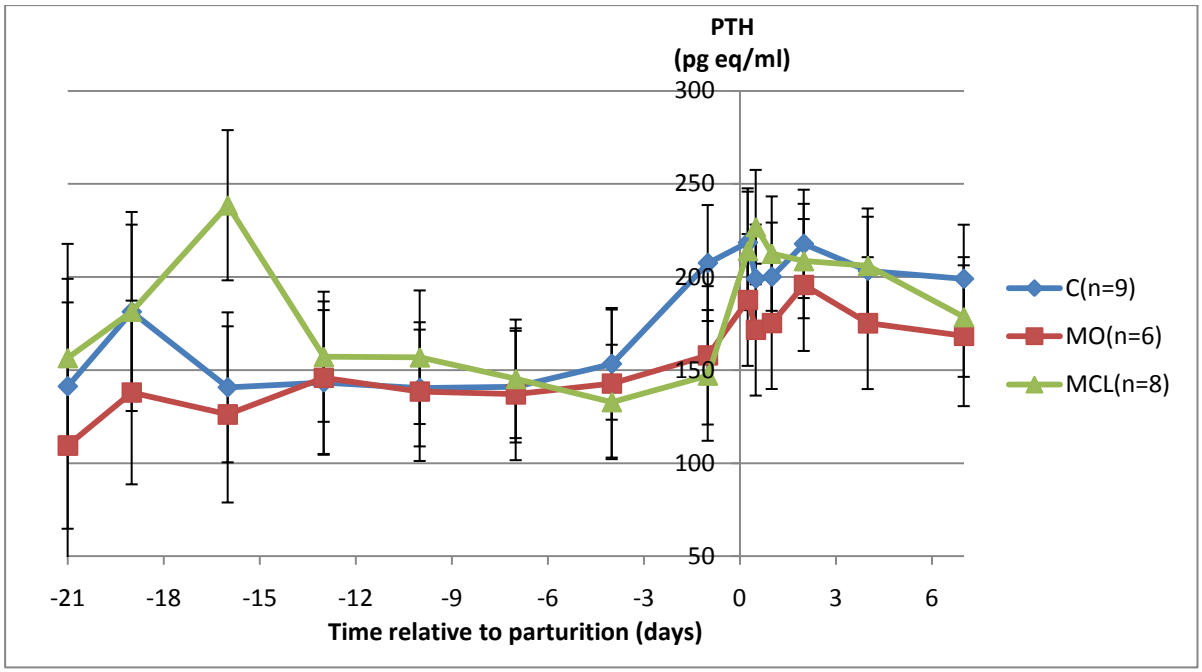

Figure 11. Concentration of PTH in plasma for animals fed control-silage (C), magnesium oxide (MO) and $MgCl_2$-silage (MCL). Error bars display the standard errors of the mean.

Milk production

Milk production rapidly increased for the first three days after parturition and then remained on approximately the same level until the end of the trial (see Fig. 12).

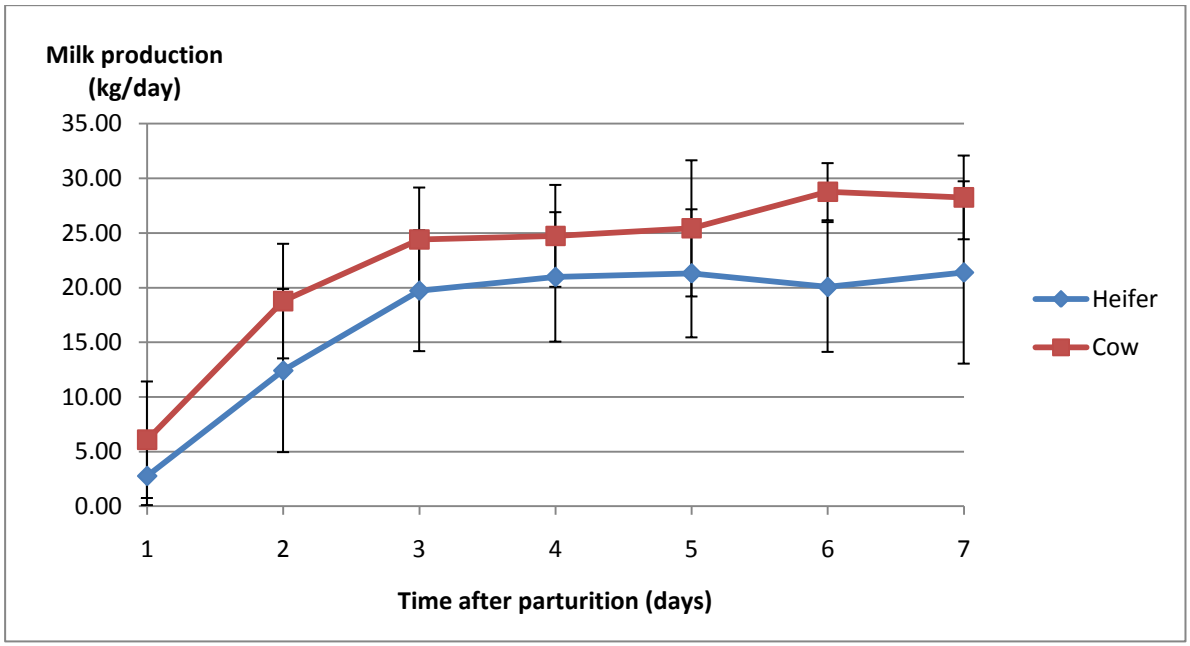

Figure 12. Average daily milk production ± standard deviations for cows and heifers during the first week postpartum.

DISCUSSION

Reducing DCAD of dairy cow rations has been shown to decrease urine pH in several studies (Schonewille, 1994; Goff & Horst; 1998: Moore *et al.* 2000; Kurosaki *et* al., 2007; Roche *et al.* 2007; Penner *et al.*, 2008). In our experiment there was no significant effect of treatment on urine pH although there was a numerical difference prepartum among treatment groups where animals fed the MCL ration had a lower average pH than animals fed MO and C rations. The numerical difference may imply that the low DCAD ration had an acidifying effect in the MCL-animals even though a significant effect could not be observed.

The lack of treatment effect on urine pH may be explained by the relatively small difference in DCAD (125 meq/kg DM) between the $MgCl_2$- and control-silage, as well as by the high variation in DCAD within each silage ($MgCl_2$ ±47.8, control ±88.3). The high variation was due to differences in potassium contents between the silage bales, which made the DCAD difference between treatments very variable during the experimental period. The number of animals (25) included in the urine pH results may therefore have been too small to be able to get a statistical significance between treatments. In studies where a decrease in urine pH has been achieved on low DCAD rations the difference between experimental diets has been greater than in our study (130-446 meq/kg DM) and DCAD has usually been negative or very close to zero in the anion diet. The response of urine pH to declines in dietary DCAD is curvelinear rather than linear (Oetzel, 2000). If a cow has a very alkaline urine (>8) it is unlikely that it will fall to any greater degree until DCAD reaches a level of approximately 100-200 meq/kg DM (McNeil, 2002). Roche (2000) reported urine pH in three cow herds in Australia during one year to be consistently above 8 for most of the year. In two herds DCAD successively decreased during summer and autumn months from 400-600 meq/kg DM to close to zero. The urinary pH remained above 8 until a 2-3 week period in May when urine pH declined to 6-7. This was associated with a reduction in DCAD to 0-150 meq/kg DM. The experimental diets in our study had a DCAD of +78 and +203 meq/kg DM for the MCL and MO/C diets respectively. Considering the curvelinear relationship between DCAD and urine pH and the results found by Roche *et al.* (2000), our DCAD levels might have been too close to each other and on a too high level for the treatments to be able to have different effects on urine pH.

The plasma concentration of CTx increased rapidly on the day of parturition for all animals, with cows experiencing a peak in plasma CTx 24 h after parturition. Postpartum CTx remained on a higher level than seen before calving for the rest of the trial. An increase in plasma CTx during the first week after parturition was also experienced by Holtenius & Ekelund (2005) and is consistent with the general view that parturition induces bone resorption in dairy cows (Goff, 2000; Liesegang *et al.*, 2000). First calf heifers had a higher CTx concentration both before and after parturition compared to the cows, which probably can be explained by that first calf heifers have a considerable faster rate of bone turnover than older cows (Sjaastad *et al*. 2003) that results in a greater release of CTx into circulation.

PTH plasma concentrations were unaffected by treatment, a result corresponding to findings in other studies (Joyce *et al.* 1997; Kurosaki *et al*. 2008). PTH commonly increases at

parturition (Horst *et al.*, 1994), but an effect of day could not be observed in the statistical analysis. A reason for this may be that the analyzed PTH values were low in relation to the standard curve and that there was a high insecurity in the data which made it difficult to obtain a statistical significance.

Hypercalciuria is typical in ruminants fed a low-DCAD ration (Fredeen, 1988; Gaynor *et al.* 1989; Goff & Horst, 1998; Vagnoni *et al.*, 1998; Charbonneau, 2008) and was also experienced by MCL-animals in our study. The source of the additional calcium in urine when low-DCAD diets are fed is however still not entirely clear.

It has been suggested that hypercalciuria in animals fed an acidifying diet reflects excretion of excess calcium as a result of increased bone mobilization and/or increased intestinal absorption of calcium. Bone is one of the major buffers in the body required to resist systemic changes in pH and there is one theory suggesting that by feeding a low DCAD diet to induce metabolic acidosis, the pH will decrease in the medium surrounding the bone and cause release of bone calcium in the buffering process (Bushinsky *et al.*, 1993). It therefore seems possible that a part of the increased calcium excretion may be a result of increased bone mobilization when low DCAD diets are fed. In the literature there are not many studies on DCAD where measurements of specific bone parameters have been performed. The bone mobilization theory is supported in some manner through research indicating increased bone resorption through an elevated level of hydroxyproline, an indicator of bone collagen degradation (Block, 1984; Gaynor *et al.*, 1989; Goff *et al*. 1991). However, in several studies there has only been a tendency for or no increase in hydroxyproline in cows fed anionic diets (Schonewille, 1994; Joyce *et al.*, 1997; Roche, 2003a,b; Roche, 2007). The reason for the inconsistency is unclear, but hydroxyproline concentration seems to be affected by pregnancy and stage of gestation because changes have been most apparent in cows nearing parturition (Block, 1984; Goff *et al*. 1991). An explanation in particular may be that hydroxyproline can be derived from other tissues than bone (Liesegang *et al.*, 2003). Results from studies were hydroxyproline is used as bone resorption marker should therefore be considered with caution, especially when research has been performed on periparturient cows where the rate of tissue catabolism may be high (Roche *et al.*, 2003b).

Liesegang (2008) conducted a study where the influence of anionic salts on bone metabolism was studied in periparturient dairy goats and dairy sheep. Goats receiving additional anions had a greater concentration of plasma CTx from day seven prepartum to parturition compared to sheep receiving anions and compared to control goats and control sheep. This indicated a higher rate of bone resorption in goats while on this feeding regimen. The goats also had an increased calcium concentration in urine prepartum. According to Liesegang (2000) and Liesegang & Risteli (2005) dairy goats seem to react in a similar manner as dairy cows according to bone markers around parturition and could therefore be an appropriate animal to model calcium metabolism in the dairy cow. This hypothesis was however not supported by the results in our study where the low DCAD diet did not have an effect on CTx. Holtenius & Ekelund (2005) found that the concentration of CTx at parturition in dairy cows was not directly related to the lactation curve. This implies that the demand of calcium for lactation is not the sole factor governing bone turnover and other factors, such as the level of estrogens,

has also been demonstrated to influence bone resorption. There are no studies to be found in the literature where the effect of anions on CTx concentration in cows around calving have been monitored and further investigations in this area are required before a conclusion about the suitability of CTx as a bone marker in DCAD-experiments on cows can be stated. It can therefore not be ruled out that the hypercalciuria in the MCL-animals was a result of increased bone resorption induced by the low DCAD diet and that there may be other bone markers more appropriate for monitoring the effect of low DCAD diets on bone turnover in dairy cows.

It has also been suggested that hypercalciuria in part may be explained by increased calcium absorption from the gastrointestinal tract in cows fed a low-DCAD diet (Fredeen, 1988b; Gaynor *et al*. 1989; Schonewille, 1994; Roche, 2007; Charbonneau *et al.*, 2008), but the literature is contradictory also on this point.

The urine concentration of calcium increased greatly for MCL-animals in the last week before parturition. This is similar to what has been observed in other studies where the highest value of urine calcium concentration have been noticed during the last days before calving in cows fed low DCAD diets prepartum (Joyce *et al.*, 1997; Kurosaki, 2007). The hypercalciuria may have been the result of enhanced calcium absorption and bone resorption as discussed above, but since the MCL-effect on urine calcium did not become significant until the week before calving it is also likely that physiological changes associated with the onset of parturition, in addition to the low DCAD diet, were the cause of hypercalciuria. Feeding rations high in anions precalving to induce a mild metabolic acidosis has been demonstrated to increase the responsiveness of the kidney and bone tissue to PTH (Goff *et al.*, 1991; Horst, 1997). Because the MCL-diet had a lower DCAD compared to MO and C, the absorption and resorption mechanisms may already have been activated in the MCL-animals prepartum and may therefore have been more responsive to PTH secretion before calving. This resulted in a higher contribution of calcium to the calcium pool in the MCL-animals with the excess excreted in urine.

The negative correlation, r=-0.165, between urine pH and urine calcium concentration that was observed in the period from day -21 to calving grew stronger closer to parturition where it became r=-0.44 in the last week. The same correlation has also been demonstrated in pregnant cows not near calving in a study by Roche *et al.* (2007). They reported a strong negative relationship (r=-0.81) between urine pH and urine calcium nine days after the cows were started on a low DCAD ration. This suggests that there might be a minimum period required for a low-DCAD ration to have an effect on calcium absorption and bone resorption that can be noticed in urine.

As has been observed in a number of studies calcium concentration in plasma decreased at calving probably due to the increased demand of calcium for lactation (Block, 1984; Goff *et al*., 1991; Joyce *et al*., 1997; Goff & Horst; 1998). Plasma concentrations of calcium were not affected by treatment or age in our study, but there was a significant effect of block. It would have been expected to see a higher level of calcium in plasma in the heifers and in the young cows compared to the old cows. However, the old and young cows had higher concentrations

than heifers in block 2, and heifers in block 1 had a higher concentration than heifers in block 2 and one of the "young cow" blocks. The heifers within block 1 then acted as predicted, but that the heifers in block 2 experienced a lower plasma level compared to them and the other cows was somewhat unexpected and we do not have a reasonable explanation for this.

Feeding low-DCAD rations prepartum has been shown to reduce the extent to which calcium concentration in plasma is lowered after parturition (Block, 1984; Oetzel *et al*., 1988; Goff *et al.* 1991; Joyce *et al.*, 1997; Goff & Horst; 1998; Moore *et al.*, 2000). The lack of response on plasma calcium level of the treatments in our study may again be explained by the relatively small difference in dietary DCAD (~125 meq/kg DM) between the diets. In studies where the decline in plasma calcium has been prevented the DCAD differences have been in the range of +264 to +1 206 meq/kg DM. In addition, the DCAD of the anion diet have been less or very close to zero, which is the recommended level to prevent hypocalcaemia (NRC, 2001; Spörndly, 2003). No animal developed clinical hypocalcaemia and all treatments experienced a relatively mild subclinical hypocalcaemia (1.89-1.96 mmol/l) after 24 hours and on day 2 postpartum. This may imply that the animals were able to efficiently activate the calcium regulating mechanisms at parturition to handle the increased demand of calcium for lactation. The relatively low age for all animals taking part in the experiment may be the explanation for their quick adaption to milk production. Another reason for the lack of treatment effect could then be that it is not possible to improve calcium metabolism even further in cows that are already able to adapt well to lactation, by feeding a low DCAD diet prepartum.

It is worth noting that the studies mentioned above where DCAD reduced the degree of hypocalcaemia postpartum, were conducted on cows of higher risk for hypocalcaemia, such as multiparous cows and cows of the Jersey breed. Horst *et al.* (2003) showed that the incidence of subclinical hypocalcaemia increased with advancing age and was >50% for cows in or beyond their third lactation. Half of the animals in our study were heifers, who rarely develop hypocalcaemia postcalving, and the cows were relatively young, most of them entering their second lactation and with only one cow entering her fourth. In addition, none of them had a previous record of milk fever. The animals in our study were therefore probably less susceptible for developing hypocalcaemia at calving and this may again explain why there was no effect of treatment on plasma calcium concentration.

There is a close relationship between magnesium absorption and renal excretion of magnesium and it is generally accepted that a surplus is rapidly excreted in urine (Martens & Schweigel, 2000). The higher amount of magnesium provided in the MCL and MO rations was probably the cause of the higher urinary excretion of magnesium in these animals compared to the C-animals. It has been proposed that a urine magnesium concentration of 10 mg/dl indicates a positive magnesium status (Mayland, 1988). It can then be suggested that the level of 19 g magnesium provided in the diet to the C-animals was adequate since their urine concentration (21.4±11.61 mg/dl) was higher than 10 mg/dl in the prepartum period and since it seems as if the extra load of dietary magnesium given to the MCL and MO-animals was excreted. Magnesium has been demonstrated to be a mineral important in calcium metabolism and a deficiency could be a contributing factor of hypocalcaemia postcalving (Goff *et al.*, 1991). The apparently adequate supply of magnesium through the diets may then

also be a part of the explanation to why no treatment effect on calcium plasma concentration could be observed after calving.

Even though MO-animals were provided approximately 3.8 g and 13.6 g more dietary magnesium per day than MCL and C-animals respectivley, their average daily excretion of magnesium, expressed as percent of intake, was lower than for MCL and C. An explanation for this is probably the lower availability of magnesium from magnesium oxide due to its greater particle size and lower solubility compared to magnesium chloride. According to NRC (2001) the absorption coefficient for magnesium oxide varies between 28-49% and is influenced by particle size (NRC, 2001). However, Jesse *et al.* (1981) reported that magnesium oxide is almost totally unavailable for absorption in the range of 425-1700 μm and in the magnesium oxide used in this study only about 30 % had a particle size less than 500 μm with the remaining part in the range of 500 to >1000 μm. The solubility of magnesium is also affected by rumen pH and decreases sharply as pH rises above 6.5 (Goff, 2007). Rumen pH is usually high in ruminants fed a high forage diet and this may be another reason for the low availability of the magnesium oxide in our study.

The urine magnesium concentration decreased for all treatments at calving. This can be explained by the increased secretion of PTH that commonly occurs around parturition to control hypocalcaemia (Goff, 2007). There is no known hormonal control over magnesium status (Martens & Schweigel, 2000), but since PTH typically raises the threshold for renal excretion of calcium the threshold for magnesium excreted in urine is also increased raising the level of magnesium in plasma if there is magnesium to spare from the diet (Goff, 2007). For the animals on the MO diet the magnesium concentration started to decrease earlier and slower than for C and MCL animals that on the contrary experienced an abrupt decrease on the day of calving. The reason for the slow decrease in MO-animals could not be explained.

Following parturition all animals experienced a decrease in plasma magnesium concentration with the lowest level measured on day four. The decrease in magnesium concentration appeared to lag the decrease in calcium, where the lowest values were obtained 12 and 48 hours after calving. Similar results have been demonstrated by others (Goff & Horst, 1998; Ramos-Nieves *et al*., 2009) and in the study by Ramos-Nieves *et al*. (2009) 20% of the cows were classified as having clinical (<0.45 mmol/l) and 80% as having subclinical (0.45-0.75 mmol/l) hypomagnesaemia two to five days postpartum. These classifications were made without cows showing any clinical signs that warranted treatment and magnesium was fed at a much higher level than recommended both before (4.7-5.1 g/kg DM) and after (3.0 g/kg DM) parturition. The concentrations of potassium in the diets were also relatively low (12.9 g/kg DM). Since there were no clinical outcomes, the low plasma magnesium level was apparently without consequence and the authors speculated that the low magnesium status in the face of the apparently adequate dietary supply was either due to poor bioavailability or that the feeding recommendations needed reevaluation.

The experimental diets in our study also provided magnesium at a level above recommendations for all treatments, as was confirmed by the increased magnesium excretion in urine for animals fed MCL and MO rations. The decrease in plasma magnesium level

postpartum was therefore probably not a result of a poor magnesium status precalving. The reduction in urine excretion postpartum corresponded to the nadir in plasma magnesium on day four after calving, suggesting that even though less magnesium were being lost in urine it could not counteract a decrease in plasma magnesium concentration. Magnesium is excreted in colostrum at a level of approximately 0.4 g/kg (NRC; 2001) and the onset of lactation may therefore be one explanation to why plasma magnesium concentration was reduced. However, the dip in plasma magnesium lagged behind the dip in plasma calcium and it should have been reasonable to believe that both calcium and magnesium would decrease at the same time if onset of lactation was the reason for the reduction of magnesium in plasma also.

The balance between the amount of intra- and extracellular magnesium has been shown to be under the influence of several hormones. Generally any type of stress leads to activation of the sympathetic nerve system and enhanced release of epinephrine and norepinephrine. Both cathecolamines can cause a decrease in plasma magnesium, suggesting a shift of magnesium from the extracellular to the intracellular space (Martens & Schweigel, 2000). An explanation for the reduction in plasma magnesium postpartum may therefore be the stress caused by parturition, altering the relationship between intra and extracellular magnesium.

Interestingly MCL-cows had a higher plasma concentration of magnesium than MCL-heifers 6-48 h after parturition and higher than all other ages/treatments after 24 h and on day 2. MCL-cows were provided extra magnesium in the diet and since this seemed to cause a higher excretion of magnesium in urine these cows may have been experiencing a more positive magnesium status compared to MO and C which could explain their higher plasma concentration at parturition. However, MCL-heifers received the same diet as MCL-cows but had on the contrary the lowest magnesium level in plasma. It could be that these heifers were more stressed at parturition, which lead to an increased flow of magnesium from the extracellular to the intracellular space and thereby reduced magnesium level in plasma. No exact explanation for this result has been found though.

CONCLUSIONS

Reducing DCAD and increasing the amount of magnesium above feeding recommendations in the prepartum diets did not result in a lower degree of hypocalcaemia at calving compared to control. Feed intake was not affected by the addition magnesium chloride.

A significant increase in urinary excretion of calcium in the week before calving was observed for animals fed the magnesium chloride silage. This could indicate that feeding magnesium chloride prepartum resulted in the mechanisms regulating calcium homeostasis becoming more active and/or more responsive to the secretion of PTH close to parturition.

The magnesium oxide and magnesium chloride diets supplied more magnesium per day compared to control and caused a higher urinary excretion of magnesium prepartum. This implies that the extra load of magnesium was excreted and that the provision of 19 g magnesium per day in the control diet was sufficient to meet magnesium requirements in prepartum dairy cows.

Plasma level of CTx was affected by lactation group (heifer or cow) but not by treatment. Further investigations are needed to evaluate its suitability as an indicator of the effect of low DCAD diets on bone resorption in dairy cows.

Future research

The hypothesis that dry cows consuming silage preserved with an additive containing magnesium chloride will have a lower degree of hypocalcaemia at calving was not supported by the results. Magnesium chloride silage did however have an effect on calcium metabolism prepartum. Further research could focus on investigating the effect of magnesium chloride silage in animals considered as being at higher risk of developing hypocalcaemia than those in this study, for example cows in or beyond third lactation. In addition it would be interesting to study if a higher amount of magnesium chloride added at ensiling, to achieve a DCAD close to zero, would have a more pronounced effect on hypocalcaemia at calving.

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ACKNOWLEDGEMENTS

I would like to send a great thank you to my supervisor Cecilia Kronqvist for all her support during the project and for all her helpful criticism and advices during the writing of this thesis. I would also like to thank my assistant supervisor Kjell Holtenius for his help on the project. In addition I want to send a thank you to the laboratory personal and the personal in the stable for helping me with sample collection and analysis.

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