The use of Calcium Sulfate in diets with high potassium for the prevention of parturient paresis in dairy cows

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Abbreviation Key

1. 1,25-(OH)$_2$D$_3$ 1,25-dihydroxyvitamin D$_3$
2. 1α-OHD$_3$ 1α-hydroxyvitamin D$_3$
3. 25-OHD$_3$ 25-hydroxyvitamin D$_3$
4. ABB acid-base balance
5. ABS acid-base status
6. ACTH adrenocorticotropic hormone
7. AP alkaline phosphatase
8. ASAT aspartate aminotransferase
9. CAB cation anion balance
10. Creat Creatinine
11. DCAD dietary cation anion difference
12. DM dry matter
13. DPD deoxy proline
14. FE Ca Fractional excretion of calcium
15. HYP hydroxyproline
16. iCa ionized calcium
17. MF milk fever
18. NABE net acid base excretion
19. PTH parathyroid hormone
20. SBE standard base excess
21. SID strong ion difference
22. SLB Swedish Friesian breed
23. SPCA1 secretory pathway Ca-ATPase
24. SRB Swedish Red and White breed
25. TRAP plasma tartrate-resistant acid phosphatase
26. TRAP tartrate-resistant acid phosphatase
1. Introduction

One of the biggest advances in dairy health in the last 25 years has been the paradigm shift to focus on disease prevention, rather than treatment. The key contributors to progress in health management in the last generation include using epidemiology to better study the determinants of disease, integration of the disciplines of veterinary medicine and animal science, and renewed focus on using science to advance health and husbandry of dairy cattle. Major advances have been made in the last 25 years in the prevention of milk fever. During this time, average herd size and milk production per cow increased dramatically (LEBLANC et al., 2006).

Efficient milk production requires that dairy cows experience gestation and parturition each year. The transition from the pregnant, nonlactating state to the nonpregnant, lactating state is too often a disastrous experience for the cow. Most of the metabolic diseases of dairy cows such as milk fever, ketosis, retained placenta, and displacement of the abomasum occur within the first two weeks of lactation. Periparturient health disorders of dairy cows affect herd performance and may have considerable negative impact on the revenue of the producer, as well as on the welfare of the animals (GOFF, 2003).

1.1. Definition

Parturient paresis (milk fever) is a metabolic disorder of cows that is associated with parturition and the initiation of lactation. Milk fever is characterized by hypocalcaemia, general muscular weakness, circulatory collapse, and depressed consciousness (RADOSTITS et al., 1994). The inability to maintain sufficient concentrations of calcium in the blood to allow normal body functions, is the major cause of the periparturient paresis (GOFF, 2006).

1.2. Purpose of the study

The first object of this study was to monitor the influence of calcium sulfate as a new tasteless anionic salt on the acid-base-balance and calcium metabolism in dairy cows, consuming feed rations with a high concentration
of potassium. Calcium sulfate is added in different concentrations to obtain a variable DCAD.

Two hypotheses are formulated for this study:

1. Does a feed ration with a reduced, but still positive DCAD activate the calcium metabolism?

2. Is it possible to increase the amount of calcium sulfate to obtain the desired negative DCAD without negative side effects, such as a reduction in feed intake or other health problems?
2. Review of Literature

Milk fever (hypocalcaemia; parturient paresis) is still one of the diseases, of major economic importance to the dairy industry. It is caused by a temporary imbalance between calcium (Ca) supply and demand at the time of parturition. The daily body turnover of Ca changes from $< 30$ g in nonlactating cows to $> 30$ g in lactating cows. The resulting low blood Ca levels lead to classic milk fever symptoms within 72 h after calving (GOFF et al., 1991). Current evidence suggests that milk fever (MILK FEVER) may occur in cows as a result of excessive dietary cations. High cation diets may cause milk fever in dairy cows as they induce a metabolic alkalosis reducing the ability of the cow to maintain calcium homeostasis at the onset of lactation. Adding anions to the diet may offset the effect of cations forages by inducing a mild metabolic acidosis, restoring the ability to maintain calcium homeostasis. MILK FEVER (total blood Ca $< 1.4$ mmol/L) as well as sub-clinical hypocalcaemia (total blood Ca $1.4-2.0$ mmol/L) are risk factors for many other diseases connected to lactation including mastitis, ketosis, retained placenta, displaced abomasum and uterine prolapse. Hypocalcaemia is also a risk factor for reproductive disorders and is an indirect risk factor for increased culling (DEGARIS and LEAN, 2009).

2.1. Parturient paresis in cattle

MILK FEVER has been recognised in cattle for about 215 years and its clinical signs have not changed since they were described by Victorian veterinary surgeons in the mid-nineteenth century. It was only 80 years ago that abnormal parathyroid gland function was associated with the pathogenesis of hypocalcaemia characteristic for the disease and the current basis for its treatment with intravenous calcium salts was established. Although this treatment is effective, most recent research has focused on preventing the disease through an understanding of the endocrine control of extracellular calcium homeostasis. In the 1970s the synthetic vitamin D analogue 1- alpha-hydroxycholecalciferol was developed for intramuscular injection before calving, but variable results of this therapy encouraged other preventative strategies to be considered, including restricting the dietary intake of calcium, and manipulating the dietary cation-anion difference (DCAD) of cows before they calve. Currently, the role of extracellular calcium receptors in the parathyroid gland is under investigation.
as a preliminary step to devising more effective treatments and/or preventative methods for milk fever (MURRAY et al., 2008).

Throughout the 1960s and 1970s the prevailing theory was, that milk fever was caused by high calcium diets fed before calving, which was thought to cause a shutdown of parathyroid gland activity during the dry period. The inactive parathyroid gland was thought to be too sluggish to successfully stimulate the calcium release from the bones and diet to prevent milk fever. Research conducted in the 1970s and early 1980s (BEITZ et al., 1974; TAKAKIYO and MITSUAKI, 1981; BLOCK, 1984; GOFF et al., 1989) added credence to this theory by demonstrating that low calcium diets prevented milk fever by stimulating the parathyroid gland to release PTH for several weeks before calving. The induced bone calcium resorption and renal production of 1,25-dihydroxyvitamin D₃, before calving so that at the onset of lactation, those mechanisms were already primed to meet the calcium demands of lactation. Feeding a “low calcium” diet to dry cows remained the strategy for milk fever prevention in the United States and any other countries until 1984, despite the fact that most farms were incapable of getting diet calcium levels low enough to truly stimulate parathyroid gland activity (GOFF, 2006).

BLOCK (1984) evaluated the hypothesis which was put forward by ENDER and DISHINGTON (1971), the Norwegian scientists who demonstrated that milk fever could be prevented by feeding cows forages preserved with hydrochloric and sulfuric acids or by adding certain salts of these acids to the diet of the dry cow. Block (1984) fed late-gestation dairy cows a diet containing excess anions (supplied by salts such as calcium chloride, aluminium sulfate, and magnesium sulfate) or a diet that contained an excess of cations (supplied by sodium carbonate and sodium bicarbonate). Cows consuming the diet with added anions had no milk fever, but 47% of cows consuming the cationic diet developed milk fever. Furthermore, cows fed with anionic diet before calving produced around 7% more milk in the subsequent lactation than cows being fed the cationic diet before calving. These results confirmed the Norwegian hypothesis, which, unfortunately, had been largely relegated to obscurity. They also spurred on further studies, which corroborated and refined these observations (GOFF, 2006).

Additional studies identified alternative (and more palatable) anion supplements (GOFF and HORST, 1998; GOFF, et al., 2004), developed practical on-farm methods of monitoring whether diets were effective (i.e., measuring urine pH),
and provided information on the mode of action of anionic diets. Still, the 2 strategies for milk fever prevention failed to explain the root cause of milk fever. A 1997 study conducted at the USDA’s National Animal Disease Center (GOFF and HORST, 1997) suggested that metabolic alkalosis, induced by high K or Na diets reduced PTH responsiveness of bone and kidney, causing an inability of the cow to maintain adequate blood calcium concentrations. High calcium diets fed to the cows did not cause milk fever, suggesting that the older theory—dietary Ca inhibits PTH secretion and responsiveness leading to milk fever—was invalid. Thus, milk fever occurs secondary to metabolic alkalosis induced by diets, high in cations such as K or Na. (GOFF, 2006).

The strategy of feeding very low calcium diets (essentially a calcium-deficient diet supplying much less absorbable calcium than the cow requires) tricks the parathyroid gland into secreting PTH before calving. This works because prolonged exposure of tissues to elevated PTH levels can overcome tissue resistance to PTH that might be induced by a high K diet. Unfortunately, these calcium-deficient diets are often difficult to formulate. Other European researchers such as MARTENS (1995) in Germany and SCHONEWILLE et al. (1994) from the Netherlands demonstrated that hypomagnesemia will also cause a breakdown in PTH action and calcium homeostasis, which could lead to milk fever. Fortunately, these publishers have also demonstrated that high levels of magnesium supplementation can overcome factors such as high diet K, preventing efficient magnesium absorption across the rumen (GOFF, 2006).

2.2. Prevalence

Despite the significant advances in our understanding of production diseases at clinical, subclinical, biochemical and molecular levels, the incidence rates of milk fever in many well-managed herds remain similar to those published decades ago. Incidence rates remain at an unacceptably high level in many dairy herds. Some authors (DISTLA et al., 1989) concluded that this increase of the incidence rate of production diseases may be largely attributed to the increase in milk yields of dairy cattle, however, the relationship between milk yields per se and production diseases is complex (MULLIGAN and DOHERTY, 2008). INGVARTSEN et al. (2003) found that higher yielding cows do not have an increased risk of milk fever.
The incidence of milk fever differs between herds (DEGARIS and LEAN, 2009). Examination of field studies reporting incidence of milk fever from 1977 to the present day found that the incidence in 10 North American studies was 3.45 % (0-7 %), in 10 European studies it was 6.17 % (0-10 %) and in 10 Australian studies 3.5 % (0-7 %). Several epidemiological surveys of milk fever revealed an average incidence of 4 to 9 % in the United Kingdom, 2 to 5 % in Australia and 4 to 8 % of dairy cows calving in the United States (GRAY et al., 2007). Subclinical hypocalcaemia occurs in 23-39 % of cows postpartum in the United Kingdom (MCCULLOCH, 2008). The average incidence of milk fever in Finnish Ayrshire dairy cows was 5.4 % (RAJALA and GROHN, 1998). The annual incidence of milk fever was 4 to 9 % in the United Kingdom (HUSBAND, 2005), and 2 % of cows in New Zealand (MCDOUGALL, 2001). In Denmark, an overall milk fever incidence risk of approximately 5 % was found (National Cattle Office, 1998), however HANSEN et al. (2007) calculated in their study a 1.6 % milk fever incidence risk in the periparturient period.

Incidence rates of subclinical hypocalcaemia around 33 % are associated with an incidence rate of 5 % for milk fever (ROCHE, 2003). Approximately 50 % of elder cows suffer from subclinical hypocalcaemia (GOFF, 2008), which is consistent with an increased risk of milk fever of 9 % as lactation numbers increase (DEGARIS and LEAN, 2008). The incidence of milk fever was 17.92 % in 83 Jersey cows between the 1st and 6th lactation (DAS et al., 2008).

GELFERT et al. (2007) found that cows entering their first or second lactation seem to still have a very low risk of becoming recumbent around parturition, which confirms the findings of other studies (STOLLA et al., 2000; METZNER and KLEE, 2005).

2.3. Causes, pathogeneses and diagnoses

1. Hypocalcaemia is caused by metabolic alkalosis in the cow induced by high potassium diets. The higher blood pH interferes with the action of parathyroid hormone on its target tissues bone and kidney. As a result bone calcium is not resorbed, 1,25-dihydroxyvitamin D₃ is not produced and the cow cannot restore blood Ca to normal levels (GOFF, 2008). Feeding high DCAD forages to dairy cows in late gestation periods is a well-documented means of inducing hypocalcaemia and milk fever in dairy cows (GOFF and HORST, 1997). Cows fed the high-cation diet fail to
produce adequate amounts of Plasma 1,25-dihydroxy-vitamin D₃ which might be an underlying cause of milk fever (GOFF, et al., 1991).

2. A second cause of hypocalcaemia is hypomagnesaemia. Magnesium is a necessary co-factor to allow parathyroid hormone to stimulate cyclic AMP production in target tissues. In case of hypomagnesaemia, bone and kidney are not able to respond to parathyroid hormone, resulting in hypocalcaemia. By raising diet Mg to 0.4 % with a very available Mg source it is generally possible to avoid development of hypomagnesaemia at calving and thus rule out hypomagnesaemia as a cause of periparturient hypocalcaemia (GOFF, 2008; LEAN et al., 2006).

3. Increased prepartum mammary gland Ca storage may contribute to the development of milk fever. Cows that develop milk fever express significantly more SPCA1 (secretory pathway Ca-ATPase), in their mammary gland, ante partum (PRAPONG et al., 2005). Mastectomy totally eliminates blood Ca declines at parturition in dairy cows (GOFF et al., 2002). Ca may be actively transported from blood into milk via a process modulated by parathyroid hormone-related protein. Parathyroid hormone-related protein produced by the mammary gland is not involved in the pathogenesis of parturient paresis in dairy cows (KOCABAGLI et al., 1995).

4. Many authors (JONES et al., 1970; NDYANABO, 1974) suggested that the increased incidence of milk fever results from the high oxalate content, low Ca and P content of the forage. However JAMES and BUTCHER (1972) claimed that ruminants adapt successfully to increasing oxalate contents in the diet and (ALLISON et al., 1981) identified oxalobacter formigenes as the ruminal bacteria responsible for such a mechanism in sheep and cattle.

5. The risk of MILK FEVER is higher with increasing age of the cow as a result of decreased intestinal calcium absorption and responsiveness to hypocalcaemia (HORST et al., 1990), reduced bone turnover and decreased bone responsiveness to parathyroid hormone and vitamin D (GOFF et al., 1991; LEAN et al., 2006). Older cows are more likely to develop milk fever than younger ones. Tissue 1,25-dihydroxyvitamin D₃ receptor concentrations decline with age, leaving the tissues less able to respond to 1,25-dihydroxyvitamin D₃ (GOFF et al., 1991; HORST et al., 1994).

The hypothesis, that decreased concentrations of vitamin D receptors prior to calving are causing factors of milk fever was tested. No significant differences were
found in colon vitamin D receptor concentration prior to calving in cows with or without milk fever (GOFF et al., 1995).

6. The levels of both dietary calcium (Ca) and phosphorus (P) have been reported to affect milk fever incidence (BEITZ et al., 1974). The peak of milk fever risk has been associated with a dietary Ca concentration between 1.1 % and 1.3 % of dry matter (DM), and dietary P concentrations higher than required have also been reported to increase the risk of milk fever. DEGARIS and LEAN (2008) reported that the protective effect of high Ca diets on milk fever occurrence may be due to the counteracting of a hypercalcuric state after prolonged exposure to DCAD diets pre-calving. In their review DEGARIS and LEAN (2008) revealed that increasing the period of exposure to pre-calving diets increased risk of milk fever. While a protective effect of low Ca pre-calving diets is well established for milk fever prevention, it may be necessary to use Ca binders to induce a pre-calving hypocalcaemia to prevent milk fever (GOFF, 2008; MULLIGAN and DOHERTY, 2008).

7. Plasma estradiol-17-beta concentrations in heifers tended to be lower at parturition compared to other multiparous cows, suggesting that oestrogen, known as a potent inhibitor of bone resorption, may be involved in developing milk fever (KUROSAKI et al., 2007).

2.3.1. Pathogeneses

Milk fever provides a good model for the study of the effect of acute increase in the need of Ca caused by the start of colostrum production (RIOND et al., 1995). LIESEGANG et al. (1998) examined whether cows with periparturient paresis could mobilize Ca from bones as well as healthy cows. The group with periparturient paresis could mobilize Ca from the bones as well as the control group.

Cows with low plasma tartrate-resistant acid phosphatase (TRAP) activity (bone metabolic marker) are at risk of developing milk fever in comparison to cows with high tartrate-resistant acid phosphatase (TRAP) activity (SATO et al., 2002; SATO et al., 2003).

Plasma parathyroid hormone concentrations at parturition of cows with four or more lactations were the highest, but plasma hydroxyproline and alkaline phosphatase were the lowest. These results suggest that the large transfer of Ca and P to colostrum is a factor in the development of milk fever in third-and-more-lactation cows (KUME et al., 2003).
2.3.2. Diagnoses

Several indicators used in the diagnosis of milk fever and hypocalcaemia are closely associated with the calcium level in the blood, whereas other clinical observations used seem to be of inferior value as single predictors. Rectal temperature, ‘mood,’ appetite, muscle shivering, rumen motility, and paresis are - as isolated observations - fairly predictive of blood calcium status (LARSEN et al., 2001).

2.4. Economic losses

Parturient paresis is a metabolic disorder that negatively affects productivity of lactating dairy cows. This metabolic disorder potentially reduces the productive lifetime of a dairy cow by 3.4 years and increases the risk of other metabolic disorders such as mastitis or uterine prolapse after calving (HERON et al., 2009).

Economic losses due to milk fever are of major concern to the dairy industry around the world. The average total cost per cow per lactation in the UK is about 220 Pounds (KOSSAIBATI and ESSLEMONT, 1995). However, the total cost of fatal case of milk fever is very high and cumulative, reaching 2112 Pounds (KOSSAIBATI and ESSLEMONT, 1997).

Increased milk yield has been found to be a risk factor for milk fever in several studies (BIGRAS et al., 1990; BENDIXEN et al., 1987; DOHO0 et al., 1984; GROHN et al., 1989). According to the results of current literature however, milk fever is not associated with milk loss because cows with milk fever seem to be higher yielding cows. Furthermore, it is difficult to prove the milk-reducing effect of the disease, for it occurs at the onset of lactation (ROWLANDS and LUCEY, 1986). One reason for the failure of establishing a relationship between milk fever and milk production is the fact that cows which do not recover are culled before any records are taken. In cows which recover, the milk yield quickly returns to normal and losses (if any) are limited (RAJALA et al., 1999).

Only few authors found that milk fever was associated with high reduction in milk production (4.1–25.7 kg) and feed intake (6.7–14.7 kg DM). Milk production losses and feed intake decreases were estimated from 5 days before to 140 days after the diagnosis, in comparison to a cow without this disorder (BAREILLE et al., 2003). Increases in milk production of approximately 14 and 7% were reported, by preventing clinical and subclinical hypocalcaemia, respectively (BLOCK, 1984).
BEEDE et al. (1992) reduced the incidence of subclinical hypocalcaemia by adding anionic salts, hence the total lactation milk production was increased by 327 kg/cow. Similarly, to results reported by Block (1984). However, many authors also reduced the incidence of hypocalcaemia by reducing precalving DCAD and either did not report the effects on milk production (OETZEL et al., 1988; GOFF et al., 1991) or found no effects of precalving DCAD on milk production (JOYCE et al., 1997; ROCHE et al., 2003).

2.4.1. Hypocalcaemia predisposes the cow to other periparturient diseases.

1. Cows that develop milk fever have higher plasma cortisol concentrations than cows that do not develop milk fever (GOFF et al., 1989; HORST and JORGENSEN, 1982). These higher concentrations may exacerbate the immunosuppression at calving (GOFF and HORST, 1997). Nearly all dairy cows experience some degree of immune suppression during the 2 to 3 weeks before and after calving. Milk fever exacerbates this immune suppression resulting in a 60 to 80% loss of immune function (GOFF, 2008). Preventing milk fever can help to prevent major loss of immune function and therefore reduce the incidence of diseases, such as retained placenta, that occurs secondary to a poorly functioning immune system (GOFF, 2008; KIMURA et al., 2006; CORREA et al., 1993; RISCO et al., 1994; MELENDEZ et al., 2004; ROCHE, 2006, LE BLANC, 2008).

Cows with retained fetal membranes have an increased risk of metritis (VACEK et al., 2007).

2. Hypocalcaemia also results in loss of muscle tone in the teat sphincter which, combined with the immunosuppressive effects of the excess cortisol, might account for the increased incidence of mastitis that occurs for cows with milk fever. The loss of uterine muscle tone is a major cause of uterine prolapse, and this disease process is almost always due to hypocalcaemia (KIMURA et al., 2006).

3. Cows with milk fever also exhibit a greater decline in feed intake after calving, exacerbating a negative energy balance commonly occurring in early lactation. The decline in feed intake, associated with milk fever reduces rumen contents (hence the rumen sits above the floor of the abdomen), reduces the depth of the rumen mat, allowing more volatile fatty acids to enter the abomasum, and decreases abomasal contractility. All of these effects of hypocalcaemia predispose the cow to
displacement of the abomasum (GOFF and HORST, 1997; MARQUARDT et al., 1977; KIMURA et al., 2006).

4. Hypocalcaemia inhibits the secretion of insulin (LITLEDIKE et al., 1970), thus the absorption of glucose. The latter exacerbates lipid mobilization at calving thus increases the risk of ketosis (GOFF and HORST, 1997).

5. About 3.8 % to 28.2 % of all milk fever cases become alert downer cows with a case fatality of 20 % to 67.0 % (MENARD and THOMPSON, 2007).

6. Milk fever reduced fertility performance in dairy cows (BUCKLEY et al., 2003). Cows with a history of parturient paresis have a higher risk of contracting ovulatory dysfunction, retained placenta and metritis (SALONIEMI et al., 1986).

2.5. Calcium homeostasis in the dairy cow

Blood Ca in the adult cow is maintained between 2.1 and 2.5 mmol/L. Typically, the nadir in blood Ca concentration occurs between 12 and 24h after calving and blood samples obtained around this time can reveal the extent of hypocalcaemia (YAMAGISHI et al., 1996). Nearly 25 % of heifers will have blood Ca concentrations <2 mmol/L. About 50 % of older cows fall into this category (GOFF, 2008). A 500-kg cow needs up to 31 g Ca in order to meet the daily maintenance and demands of the foetus in late gestation (GOFF et al., 1991). Cows producing 10 L of colostrums loose about 23 g of Ca in a single milking. This amount is about nine times higher than the entire plasma Ca pool of the cow (HORST et al., 1997).

In order to prevent blood Ca from decreasing at the onset of lactation the cow must replace the Ca losses to the milk. She does this by withdrawing Ca from the bones and by increasing the efficiency of absorption of dietary Ca. The dairy cow is programmed to go into a state of lactational osteoporosis, mobilizing bone Ca to help her achieve normocalcaemia in early lactation. This will typically result in losses of 9–13 % of her skeletal Ca in the first month of lactation (which is reversible later in lactation). Bone Ca mobilization is regulated by parathyroid hormone (PTH) which is produced whenever there is a decline in blood Ca. Renal tubular reabsorption of Ca is also enhanced by PTH. However, the total amount of Ca that can be recovered by reducing urinary Ca excretion is relatively small as only small amounts of calcium are typically lost to urine each day. A second hormone, 1,25-dihydroxyvitamin D₃, is required to stimulate the intestine to efficiently absorb dietary Ca (YAMAGISHI et al., 1996). This hormone is made from vitamin D by the kidneys (GOFF, 2008).
2.6. Hormonal regulation

Ca regulation in mammals and birds involves coordination between parathyroid hormone (PTH), calcitonin and the hormonally-active form of vitamin-D₃, 1,25-dihydroxyvitamin-D₃ (1,25(OH)₂D₃). Failure of this system to maintain normal blood Ca concentrations at parturition is a common occurrence in ruminants leading to clinical and subclinical hypocalcaemia (HORST et al., 2003).

Both PTH and 1,25-(OH)₂D₃ are produced in response to hypocalcaemia and act to increase the entry of Ca into the extracellular Ca pool. Calcitonin is secreted in response to hypercalcaemia and acts to slow the entry of Ca into the extracellular pool. A decrease in plasma Ca causes the parathyroid glands to secrete PTH. Within minutes, PTH will rapidly increase renal reabsorption of Ca from the glomerular filtrate (GOFF et al., 1991). Two processes under the control of PTH were found, renal production of 1,25-(OH)₂D₃ and osteoclastic bone resorption. Cows fed highly cationic diets are less responsive to parathyroid hormone than those fed a highly anionic diet (GOFF et al., 1991). Active transport of Ca across the intestine is mediated by 1,25-(OH)₂D₃ (BRONNA, 1987). Inadequate production of 1,25-(OH)₂D₃ could contribute to development of milk fever (GOFF et al., 1991).

2.7. Factors impairing Ca homeostasis at the cellular level

2.7.1. Metabolic alkalosis

Metabolic alkalosis blunts the response of the cow to PTH (GOFF et al., 1991). PTH receptors are altered during metabolic alkalosis rendering the tissues less sensitive, prevents effective utilization of bone canaliculi fluid Ca and prevents activation of osteoclastic bone resorption (PHILIPPO et al., 1994). Failure of the kidneys to respond to PTH also reduces renal reabsorption of Ca from the glomerular filtrate. More importantly, the kidneys fail to convert 25-hydroxyvitamin D₃ to 1, 25-dihydroxyvitamin D₃. Therefore enhanced intestinal absorption of dietary Ca that normally would help restore blood Ca to normal, fails to be instituted (CONSTABLE, 1999; GOFF, 2000).
2.7.2. High calcium intake
The high Ca intake makes any active Ca transport unnecessary, and due to the alkaline diet, PTH metabolism is inactive due to the reduced sensitivity of the tissues to PTH (GOFF, 2004). Passive resorption in the intestine is sufficient to fulfil the requirements of a non-lactating, pregnant cow (GOFF et al., 1991).

2.7.3. Hypomagnesaemia
Hypomagnesaemia affects Ca metabolism in two ways, firstly by reducing PTH secretion in response to hypocalcaemia and secondly by reducing tissue sensitivity to PTH (GOFF, 2008; RUDE, 1998). High K concentration in the rumen fluid furthermore depolarizes the apical membrane of the rumen epithelium reducing the electromotive potential needed to drive Mg across the rumen wall (MARTENS and SCHWEIGEL, 2000).

2.7.4. Excessive blood phosphorus concentration
When blood P concentration is increased above the upper normal limit (2 mmol/L), the phosphate has a direct inhibitory effect on the renal enzyme converting 25-hydroxyvitamin D₃ to 1,25-dihydroxyvitamin D₃. Therefore, even if PTH secretion occurs and the tissues recognize the PTH, the cow will be unable to produce the hormone necessary for activation of intestinal Ca transport and the cow will suffer impaired Ca homeostasis (GOFF, 2006).

2.8. Predisposing factors
Factors known to predispose cows to milk fever are age, milk yield, breed, body condition, length of the dry period, and diet (HORST et al., 1994). Higher frequencies of milk fever cases among less technically efficient farms were reported by LAWSON et al. (2004). Milk fever was more frequent in herds housed free in barn stalls (OHGI and HATTA, 2001).

High milk yield has been found to be a risk factor for milk fever (DOHOØ et al., 1984; GROHN et al., 1995; 1989; 1986; STERGAARD and GROHN, 1999; KUSUMANTI et al., 1993; RAJALA and GROHN, 1993). Four percent of cases of parturient paresis occurred before, and 45 % within 24 hours after calving. When cases were categorized by month of calving, the risk of parturient paresis did not significantly vary by month of calving (GROHN et al., 1986).
More cases of clinical and subclinical hypocalcaemia were recorded in fat cows than in thin cows (STOCKDALE, 2007; HANSEN et al., 2002). Over-conditioned dry cows are four times more likely to experience milk fever (HOUE et al., 2001).

Climate influences the susceptibility of grazing cows to milk fever, with greater incidences when diurnal variation in temperature is high. Older, fat, or very thin cows, cows, having exhibited milk fever previously, and cows requiring assistance at calving were at increased risk of milk fever (ROCHE and BERRY, 2006).

2.8.1. Race

Finnish black and white cows have a higher risk of parturient paresis compared to Finnish Ayrshir cows. This higher risk was observed in both housing systems, it was more pronounced for cows calving during the growing period (SCHNIER et al., 2004). The Finnish black and white cows have a higher risk for paresis because of the breed's higher milk yield, since milk yield and the risk for parturient paresis are positively correlated (ERB, 1987). A comparison of the incidences of milk fever of dairy cows kept in cold and warm loose-housing systems revealed no significant differences (SCHNIER et al., 2002).

The Swedish Red and White breed (SRB) have a 1.4 times higher incidence of parturient paresis than the Swedish Friesian breed (SLB). The incidence was low in the first and second calvings and increased with parity to reach a maximum in the seventh calving (BENDIXEN et al., 1987).

The increased risk of milk fever for Jersey and Norwegian and Swedish Red and White cows compared with Holstein-Friesian cows was also detected (LEAN et al., 2006; ROCHE and BERRY, 2006). Channel Island breeds are known to be more susceptible to MILK FEVER than Holstein- Friesian cows (HORST et al., 1997), probably because of reduced concentrations of intestinal receptors for 1, 25(OH)2-vitaminD3 (GOFF et al., 1995).

Minimal variation exists among breeds such as Jersey and Danish Red & Danish White and Black for risk of milk fever. However, the difference among breeds in the fourth lactation shows some deviation. This may be due to different management procedures among breed-framers (KUSUMANTI et al., 1993).
2.8.2. Age
Progressing age increases the risk of milk fever (LEAN et al., 2006) as a result of decreased intestinal calcium absorption and responsiveness to hypocalcaemia (HORST et al., 1990), reduced bone turnover, and decreased bone responsiveness to parathyroid hormone and vitamin D (GOFF et al., 1991).

2.8.3. Feeding
Dietary Ca (0.46 vs 0.84 %) did not have any apparent effects on plasma minerals, PTH concentrations and bone turnover markers around parturition. Plasma minerals and bone turnover activity in primiparous cows were higher than those of multiparous cows in both groups fed on low and high Ca diets (KAMIYA et al., 2005).

Precalving dietary Ca may not be as important a risk factor as high intakes of K and subsequent effects on blood acidbase status. Hence the most constructive step that can be taken to prevent milk fever is to reduce the dietary K content of the prepartum diet (GOFF and HORST, 1997).

A reduction in the incidence of hypocalcaemia and an increase in milk production were recorded when Na and K concentrations in the diet were reduced (CHANDLER, 1997) and/or Cl and S concentrations were increased to produce a negative DCAD (BLOCK, 1984; JOYCE et al., 1997).

OETZEL (1991), ENEVOLDSEN (1993) and ROCHE et al. (2002) reported that S was the most important dietary constituent in determining the risk of hypocalcaemia, more important than either Cl or K. The absorption efficiency of S is known to be less than either Cl or K (UNDERWOOD and SUTTLE, 1999).

Potassium and sulfur both have a highly significant and substantial effect on milk fever incidence. Higher potassium concentrations greatly increased milk fever incidence (WALKER et al., 2006), and higher sulfur concentrations strongly and linearly reduced the risk of milk fever. These observations are consistent with the proposed effects of strong ions on milk fever incidence mediated through the DCAD (BLOCK, 1984; GOFF et al., 1989; TUCKER et al., 1991; GOFF and HORST, 1997; GOFF, 2000).

The incidence of milk fever among grazing dairy cows increased, with the consumption of the pasture higher in K and Na (SANCHEZ et al., 1998).
Clinical symptoms

Clinical symptoms of this disease include inappetence, inhibition of urination and defaecation, paresis, lateral recumbency and eventually coma and death. If left untreated, the outcome is death in approximately 60 to 70% of the cases (HORST et al., 1997). Hypocalcaemia is the major cause of recumbent paresis in dairy cows on the peripartal period (1 day a.p. – 2 days p.p.) (GELFERT et al., 2005). Concomitant diseases, such as myopathy, ketosis, or neural lesions might complicate the healing process (GELFERT et al., 2007). Plasma calcium concentrations become too low to be able to support nerve and muscle function (GOFF and HORST, 1997; GOFF, 2004). However, prior to the treatment of a recumbent cow, a detailed clinical examination must be made in order to exclude other possible causes for a recumbent cow.

Cows with milk fever may suffer lower feed intake and depressed chewing behaviour (HANSEN et al., 2003), and therefor have an increased risk of associated diseases such as dystocia, retained placenta, ketosis, metritis, displaced abomasum, and mastitis (WILSON and STEVENSON, 1998) than healthy animals (WU et al., 2008). Milk fever is characterized by progressive symptoms and starts out with the inability to remain standing, followed by nervous disorders, anorexia and digestive atonia. The stimulation of the PTH receptor in reticulo-ruminal smooth muscles reduces the motility of this tissue and may play a role in the depression of motility of the digestive tract which is characteristic of clinical milk fever in the dairy cow (CARE et al., 1999) as well as meteorism, suppressed defaecation, unconsciousness, cardiorespiratory difficulties, coma, tetany, and death (SCIORSCI et al., 2001).

Clinical signs of post-parturient hypocalcaemia of cows that died or were slaughtered with myocardial necrosis were compared to those of cows with milk fever showing recumbency and standing after Ca therapy. Cows with myocardial necrosis showed anorexia, lateral recumbency, dyspnoea characterized by oral breathing, severe tachycardia and hyperpnoea which persisted even after Ca therapy. Both groups suffered from severe hypocalcaemia, but the plasma Ca levels returned to normal after treatment with Ca (YAMAGISHI and NAITO, 1997; YAMAGISHI et al., 1999a, 1999b).

Transient clinical signs of hypercalcaemia caused by milk fever treatment with calcium borogluconate were: decreased rumination, muscle ticks, salivation and a
heart rate reduction of 20%. Rectal temperature remained unaltered. The frequency of rumen contractions was reduced up to 40% whereas the amplitude of contractions did not deviate from baseline values (JORGENSEN et al., 1998).

2.10. Treatment of parturient paresis

The diagnosis is confirmed by response to the classical therapy with 500–1000 ml (on the basis of animal's body weight) of 23% calcium-borogluconate administered intravenously and subsequent therapy with 500 ml of 23% Ca-borogluconate subcutaneously. A cow considered to have milk fever (recumbency and plasma Ca concentration < 5.5 mg/100ml) should be treated with i.v. administration of 10.5 g of Ca as Ca borogluconate (YAMAGISHI and NAITO, 1997).

Treatment with intravenous infusion of calcium salt solutions cures most clinical cases of hypocalcaemia. Comparing the effects of administration of 2 volumes of calcium solution (calcium oxide and calcium gluconate) on clinical recovery from milk fever the results did not support the need to increase the administered volume of calcium solution from 450 to 750 mL (DOZE et al., 2008).

GELFERT et al. (2007) found that the standard treatment of infusion of organic calcium solutions is still recommended. In cows suffering from hypocalcaemia only, at least 95% regained the ability to stand. In the other cows, increased activities of CK creatine phosphokinase and ASAT aspartate aminotransferase are an indication of the prevalence of muscle damage, but do not act as an indicator for prognosis of the recumbent cow. However, laboratory analyses are useful to confirm the clinical diagnosis in order to explain the failure of the first therapy and to improve the future treatment of the animal. The cure rates and success rates after the first treatment were, between 72.7–94.8%. The total rate of successful treatment was between 89.4% and 95.4%. Similar results were reported in previous studies (SHPIGEL et al., 2003; SIEGWART and NIEDERER, 2005).

Cows with parturient paresis were treated with either a conventional single-dose intravenous infusion of a calcium + phosphorus + magnesium solution or with a slow continuous intravenous infusion using the same solution. There were no significant differences between both groups (BRAUN et al., 2004).

The effect of calcium (Ca\(^{2+}\)) combined with naloxone (Nx, an opioid antagonist; Ca\(^{2+}\)-Nx) on plasma concentrations of ACTH, \(\beta\)-endorphin (\(\beta\)E) and Ca\(^{2+}\) were evaluated. Administration of both Ca\(^{2+}\) and naloxone (Nx) resulted in a faster and
better recovery from milk fever than that achievable by administration of either Nx or Ca\(^{2+}\) alone (SCIORSCI et al., 2001; RIZZO et al., 2008).

2.10.1. Oral & rectal calcium treatment

Oral Ca treatment with 50 g of Ca could be used instead of Ca treatment administered i.v. Repeated doses may be given; each dose presumably supplies the equivalent of 4 g of Ca to the blood. The total amount of CaCl\(_2\) administered in 24 h should not exceed 288 g (120 g of Ca) to avoid severe metabolic acidosis. The oral solutions of CaCl\(_2\) may be more effective than CaCl\(_2\) gels; the risk of aspiration pneumonia precludes their use. The CaCl\(_2\) gels are a useful compromise. Development of a Ca propionate gel or mixture of Ca propionate with CaCl\(_2\) may permit more Ca to be administered without risk of metabolic acidosis (GOFF and HORST, 1993). Rectal administration of Ca salts might raise the plasma Ca concentration rapidly, but may cause serious pathological lesions, precluding their use (GOFF and HORST, 1994).

2.10.2. Udder inflation

In the past, hypocalcaemia in cows was treated by udder inflation (MAYER et al., 1967). Inflation was successful, because it reduced the demand for Ca by inhibiting milk synthesis or encouraged diffusion of Ca from mammary gland to plasma (ASLAM and TUCKER, 1998).

2.11. Downer cow syndrome

Clinical hypocalcaemia increased the risk of downer cow syndrome five fold (CORREA et al., 1993). The incidence of downer cow syndrome among milk fever cases ranged from 4.5 to 14 % (CURTIS and WILLOUGHBY, 1970), but the incidence of downer cow syndrome was 2.1 % for herds (COX and ONAPITO, 1986; CORREA et al., 1993).

A downer cow is an animal that is unable to rise to a standing position after more than 24 h of recumbency (SMITH et al., 1997). Other authors describe downer cow syndrome as a cow being recumbent, but having Ca concentrations similar to healthy cows and does not respond to Ca treatment (ALLEN and DAVIES, 1981), or as a cow that is in sternal recumbency when the reason for the recumbency is unknown (COX et al., 1968; FENWICK et al., 1969). Downer cows are common in
the periparturient period. They can be divided into 3 categories: (a) cows that are unresponsive to standard hypocalcaemic or MF therapy and do not exhibit other complications but remain alert; (b) alert recumbent animals that have traumatic musculoskeletal and nerve problems; (c) and recumbent animals that are affected with systemic diseases related to metabolic, toxic, alimentary, or neurologic conditions (SMITH et al., 1997; MENARD and THOMPSON, 2007).

Additionally, secondary metabolic disorders involving phosphorus (P), magnesium (Mg), and potassium (K) deficits have been suggested as risk factors, but without direct evidence of their involvement (CAPLE et al., 1986). To better define therapeutic strategies and measure their benefits for alert downer cows, the classification of recumbent animals into the right category and recording of biological factors are needed before relevant associations on which to base therapy can be defined (HOUE et al., 2001).

Fatty liver is associated with downer cows (ALLEN and DAVIES, 1981). In a study using slaughtered cows, nearly 70 % of downer cows were found to have fatty liver (OIKAWA and KATOH, 2002), suggesting that fatty liver is one of the underlying factors (KATOH, 2002).

MILIAN-SUAZO et al. (1988) reported that more than one half of downer cows were culled in the same lactation.

Recumbent cows around parturition are suffering mostly from hypocalcaemia and often show other concomitant diseases, such as muscle damage and ketosis. Contrary to elder cows, muscle damage is the most frequent diagnosis in heifers. Severe muscle damage is the main reason for the failure of treatment. Serum analyses at the time of the first treatment do not provide additional information to improve the prognosis at that time (GELFERT et al., 2007).

2.12. Strategy for prevention

Numerous methods for prevention of parturient paresis in dairy cattle have been used. Increased herd size, new feeding principles such as the TMR system, new housing and milking systems enhance the need for development of new strategies for controlling milk fever at herd level. Several options for controlling milk fever in the dairy herd are available (HORST et al., 1997).

The most-suitable strategy to control milk fever in a specific herd will depend on herd-specific circumstances such as the attitude and skills of the farmer, the
opportunities available in the production system and the economic consequences of a certain strategy (SRENSEN et al., 2002).

2.12.1. Oral drenching around calving with a supplement of easily absorbed calcium.

Oral administration of large amounts of Ca salts forces Ca into the blood by passive diffusion to increase the blood Ca concentration during the periparturient period (AGGER and RENNY, 2004; MCCULLOCH, 2008). CaCl₂ solutions and gels have several disadvantages. Aqueous solutions of CaCl₂ and some gel products are very caustic and cause ulceration of the mouth and digestive mucosa of some cows. Calcium propionate has the distinct disadvantage of being only 21.5 % Ca, thus requiring larger volumes of preparation to be given orally (HORST et al., 1997).

The efficacy of calcium propionate for the prevention of MILK FEVER was compared with that of calcium chloride (PEHRSON, 1998). The results of this trial confirmed preliminary results (GOFF and HORST, 1993; GOFF et al., 1996) that calcium propionate may be a satisfactory alternative to calcium chloride for the prevention of milk fever. However, if milk fever was not prevalent in the elder cows, the cost of the treatment (minimum $16 per cow) would be difficult to justify for commercial herds (GOFF et al., 1996). Milk fever was not prevented by using 50 g of CaCl₂, but it was prevented by using 75-g doses. Problems associated with this treatment included induction of metabolic acidosis and, possibly, aspiration pneumonia (GOFF and HORST, 1991).

Oral calcium drenching around calving apparently has a mean efficacy of 50-60 % in terms of milk fever prevention as well as prevention of milk fever relapse after intravenous treatment with calcium solutions (HANSEN et al., 2002).

The economic evaluation per cow-year indicated that oral calcium used for prevention of parturient paresis as well as control strategies based on combinations of two preventative actions such as oral calcium + body condition control and oral calcium + anion supplement, were too costly compared to similar but less intensive control strategies against milk fever such as body condition control, anion supplement and culling of high-risk cows (STERGAARD et al., 2004).
2.12.2. Feeding prepartum Ca deficient diet

Feeding dry cow rations low in calcium leads to an activation of the calcium homeostatic mechanisms before calving rendering the cow ready for the massive draw on blood calcium for final stages of prenatal growth and colostrum production. When dietary calcium availability is decreased below calcium requirements, the cow is brought into a state of negative calcium balance. The drop in serum calcium leads to a secretion of parathyroid hormone from the parathyroid glands, which in turn increases renal reabsorption of calcium (within minutes), stimulates calcium resorption from the bone within hours to days and renal production of 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) in the same time (GOFF et al., 1991; GOINGS et al., 1974; JORGENSEN, 1974).

Ca restriction has numerous disadvantages, particularly in that it restricts legume forage utilization in favor of utilizing corn silage, corn, or other cereal grains. This may result in excessive fattening during the prepartum period, sudden changes in forage feeding after freshening, and increased incidence of abomasal displacements (COPPOCK, 1974).

Despite the effectiveness of feeding dry cow rations low in calcium in preventing milk fever, this method of prevention has been almost abandoned because of difficulties in keeping the calcium intake sufficiently low (<20 g/d) when using commonly available feed. Studies have shown, however, that it is possible to prevent milk fever by adding a substance to the feed capable of binding dietary calcium thereby making it unavailable for absorption (HANSEN and JORGENSEN, 2001; HANSEN et al., 2002; WILSON, 2001; PALLESEN et al., 2008).

2.12.3.1. Zeolite (sodium aluminum silicate) supplementation

Zeolite binds Ca in the gastrointestinal tract and causes it to be passed out in the faeces. At present this method is unwidely used since very large amounts of Zeolite must be ingested each day (varies from 0.25 to 1 kg/day for 2 weeks before calving). Also Zeolite may have negative effects on P absorption which may not be overcome with extra P in the diet (HANSEN and JORGENSEN, 2001; HANSEN et al., 2002; KATSOULOS et al., 2005; PALLESEN et al., 2008) for Zeolite is not Ca specific. It also binds phosphorus (P) and to a lesser extent magnesium (Mg) (THILSING et al., 2006; HANSEN et al., 2002). Although hypomagnesaemia resulting from zeolite supplementation remains to be demonstrated, its occurrence cannot be
excluded in herds with a borderline Mg status and it would be unwanted even at the subclinical level since it is believed to interfere with the cow’s ability to mobilise Ca in response to hypocalcaemia (PALLESEN et al., 2008). Feed refusals recorded were 12 % or below on Zeolite supplementation studies (HANSEN et al., 2002; PALLESEN et al., 2008).

2.12.3.2. Zinc Oxide

Oral drenching with a single dose of zinc oxide of 100 mg/kg of body weight caused a decrease in total serum calcium. This decrease was not followed by overshooting, indicating that the single treatment with zinc oxide did not stimulate the calcium homeostatic mechanisms (JORGENSEN et al., 2001).

The reduction of dietary calcium availability in late pregnancy cows by zinc administration is however questionable, as the zinc dose used is around 6 times the dose recommended for facial eczema prevention and conflicts with feeding recommendations for zinc. Further more zinc toxicity has ben recorded after long term exposure of zinc in doses between 45 and 240 mg Zn/Kg body weight (HANSEN and JORGENSEN, 2001).

2.12.3.3. Vegetable oils (Soya bean oil)

The administration of vegetable oils which bind Ca to form an insoluble soap prevents the absorption of dietary Ca (WILSON, 2003). This method has been successfully used in cattle fed diets containing 30–50 g Ca/day. They irreversibly bind enough dietary Ca to cause the reaction typically seen when the diet provides <15 g absorbable Ca/day. Soya bean oil was chosen as a supplement to form poorly digestible calcium soaps in the gastrointestinal tract. Feeding Soya bean oil for 2–4 weeks prior to calving reduced dietary calcium availability as well as the incidence of clinical milk fever (WILSON, 2001; 2003).

2.12.4. Prepartum administration of vitamin D₃, vitamin D₃ metabolites and analogues.

Treatment with 1,25-dihydroxyvitamin D₃ and its analogues can be more effective and much safer than using vitamin D₃ but problems associated with timing of administration remain. The 1,25(OH)₂D₃ stimulates the active transport of calcium across the intestinal epithelial cells (HORST et al., 1994). During bone resorption,
urinary excretion of pyridinoline and deoxypyridinoline, derived from collagen breakdown, is increased (LIESEGANG et al., 1998).

The effectiveness of a combination of 1α-hydroxyvitamin D₃ and 25-hydroxyvitamin D₃ to reduce the incidence of parturient paresis was tested. The prepartum diet of alfalfa silage and hay was supplemented with a grain mixture supplying 100 g of ground limestone. Under these dietary conditions, incidence of parturient paresis was reduced from 33 to 8% (HODNETT et al., 1992).

Oral administration of 10 million I.U. of an encapsulated form of vitamin D₃ had more potent ability to prevent parturient paresis compared to the vitamin D₃ injection used widely in Japan (YAMAGISHI et al., 2000). However, the occurrence of parturient paresis in both groups suggested that the prophylaxis with Vitamin D₃ was not always complete (JULIEN et al., 1977).

1α Hydroxyvitamin D₃ (1α-OHD₃) is used to prevent bovine parturient paresis (SACHS et al., 1987). The 1α-OHD₃ is hydroxylated rapidly in the liver to form 1,25-(OH)₂D₃. In cows, plasma concentrations of 1,25-(OH)₂D₃ peaks 24 to 48 h after the intramuscular injection of 1α-OHD₃. The increase in plasma 1,25- (OH)₂D₃ is followed by a rise in plasma Ca, apparently as a result of increased intestinal Ca absorption (BAR et al., 1988). 24F-1,25-dihydroxyvitamin D₃ can also be used successfully to reduce the incidence of parturient paresis (GOFF and HORST, 1990; ZADNIK et al., 2008).

Oral and intramuscular doses of vitamin D₃ have prevented milk fever successfully. However, repeated treatments may be necessary due to the inaccurate prediction of date of parturition and may lead to toxicity problems (LITTLEDIKE and HORST, 1982; HANSEN et al., 2002).

Intramuscular injection of 1,25-dihydroxyvitamin D₃ combined with prostaglandin F₂α closely before parturition might be successful to prevent hypocalcaemia close to calving (YAMAGISHI et al., 1999).

2.12.5. Administration of parathyroid hormone

PTH administration by intramuscular injection was evaluated (GOFF et al., 1988). Intravenous infusion of synthetic bovine parathyroid hormone for 96 h before the expected date of calving increased 1,25-dihydroxyvitamin D₃, Mg, Ca, and hydroxyproline in plasma of pregnant cows. GOFF et al. (1986) concluded that
exogenous parathyroid hormone may prevent parturient paresis if administered at least 60 h prior to parturition (GOFF and HORST, 1993).

2.12.7. Reduce milking in early lactation

The milk producers focused on reduced milking during the first two days post parturition. There are few studies only (GOFF et al., 2002) investigating the effects of reduced milking on the incidence of milk fever, however, the benefit of reduced milking on milk fever incidences is therefore controversial (HANSEN et al., 2002). Moderate milking during the colostrum period may be beneficial for other reasons not related to milk fever prevention (HANSEN et al., 2007).

2.12.8. Intramammary Infusion of Calcium

Intramammary infusion of 40 ml of 50% Ca borogluconate solution containing 1.6 g of Ca three times at 12-h intervals immediately postpartum enhanced the ability of the cow to maintain a stable plasma Ca concentration and reduce the incidence of milk fever. However, side effects, such as mastitis might accompany the intramammary infusion of Ca (ASLAM and TUCKER, 1998).

2.13. Dietary Cation Anion Difference (DCAD)

2.13.1. High diet cation–anion difference (DCAD)

Reducing the number of absorbable dietary cations and/or increasing the number of absorbable dietary anions may greatly diminish the incidence of hypocalcaemia and milk fever in dairy cows (GOFF, 2008). The relative merits of the various DCAD equations proposed, are addressed (DEGARIS and LEAN, 2009).

Small changes in blood pH could have large effects on parathyroid hormone receptivity and ultimately on the risk for clinical milk fever (GOFF et al., 1991; CHARBONNEAU et al., 2006). Diets with a low but positive DCAD may improve the capability of dairy cows to maintain Ca homeostasis to some extent (KUROSAKI et al., 2007; PENNER et al., 2008; ANNICK et al., 1995), but may not completely eliminate the prevalence of hypocalcaemia. MOORE et al. (2000) reported that 50% of cows fed a diet with 0 mEq/100 g DM were diagnosed with subclinical hypocalcaemia (plasma iCa < 1.0 mM). KUROSAKI et al. (2007) reported 11% severe hypocalcaemia (total serum Ca < 1.25 mM) for cows fed 1.2 mEq/100g DM. Similarly PENNER et al. (2008) reported 35% of cows fed a diet with 1.6 mEq/100g
DM were still diagnosed with subclinical hypocalcaemia (blood iCa < 1mM). The diet (0.7 mEq/100g DM) was effective at improving Ca homeostasis (HERON et al., 2009).

Feeding diets rich in nonmetabolizable anions to dairy cows during the dry period reduces the risk of hypocalcaemic paresis puerperalis. When cows are fed a diet rich in anions instead of cations, more Ca is absorbed in the intestine and excreted in urine (SCHONEWILLE et al., 1999; FREDEEN et al., 1988; SCHONEWILLE et al., 1994; VAN MOSEL et al., 1993). Diets with a negative DCAD prevented milk fever more effectively than diets that were low in Ca (GOOF and HORST, 1997).

Anionic diets compared to cationic diets (23 vs. -8 mEq/100g DM) did not show advantages in preventing parturient hypocalcaemia when P levels were high and Ca levels were low in the ration. Addition of anionic supplements to slightly cationic (<+22 mEq) feeding regimes may not be of further help in preventing parturient paresis (ROMO et al., 1991).

2.13.1.1. Use hydrochloric or sulfuric acids

An alternative source of anions is HCl. The inclusion of HCl into the prepartum ration significantly reduced the incidence of milk fever from 63 % of control cows to 11 % of the treated cows and also reduced the degree of hypocalcaemia that was experienced by the cows during the periparturient period. The prepartum consumption of the ration with HCl was greater than the consumption of the control ration. Commercial preparations of HCl mixed into common feed ingredients as a premix could offer an inexpensive and palatable alternative to anionic salts as a mean of controlling the incidence of milk fever in dairy cows (GOFF and HORST, 1998).

Direct acidification of diets by spraying hydrochloric or sulfuric acids (FREEDEN et al., 1988) increases bone mobilization and potentially reduces the incidence of milk fever (OETZEL et al., 1988).

2.13.1.2. Low-DCAD forages

Feeding low-DCAD forages is another management approach to decrease the severity of hypocalcaemia (CHARBONNEAU et al., 2008; PENNER et al., 2008). Timothy (Phleum pratense L.) is low in potassium concentration and DCAD value
compared to other cool-season grasses (TREMBLAY et al., 2006), and its DCAD can be further decreased with chloride fertilization (PELLETIER et al., 2007, 2008). PENNER et al. (2008) fed prepartum dry cows with low- or high-DCAD timothy hay and reported that the diet containing low-DCAD timothy hay at 63 % of dietary DM improved Ca homeostasis during the periparturient periods without decreasing DMI (HERON et al., 2009).

Fertilization with Cl increased the chloride concentration in the crop and lowered the DCAD of the hay (PEHRSON et al., 1999). Chloride application to forages may offer another mean of reducing DCAD of the prepartum dairy cow diet (GOFF et al., 2007).

DCAD, particularly milliequivalents/kg DM of (Na + K) – (Cl + S) has been examined in dry cows (BLOCK, 1984; GAYNOR et al., 1989; OETZEL et al., 1988; PEHRSON et al., 1999) for its effect on milk fever (ANNICK et al., 1995) and in growing calves for its effect on dry matter intake and growth (JACKSON and HEMKEN, 1994; JACKSON et al., 1992; TUCKER et al., 1991), and in lactating dairy cows for its effect on DMI and milk yield (TUCKER et al., 1988). Forages and by-products that may be rich in K would increase the cation relative to anion content of the diet. Conversely, the dietary inclusion of excess Cl and S will increase the anion content of the diet. The dietary inclusion of excess Cl and S has been shown to have a dramatic effect on urine pH (TUCKER et al., 1991) and Ca metabolism as it relates to milk fever. FREDEEN et al. (1988) found that postpartum incidence of milk fever in cows was reduced when dietary Cl and S anions were in excess to Na and K ions during the dry period.

The emerging cause of milk fever seems to be metabolic alkalosis caused by high dietary cation intake (GOFF and HORST, 2003). Formulation of rations to induce a compensated metabolic acidosis in the pre-partial cow has proved a useful strategy for prevention of milk fever. The concept of manipulating dietary cation–anion difference (DCAD) as a means of reducing hypocalcaemia has been widely reported (SCHONEWILLE et al., 1999; MCNEILL et al., 2002; GOFF and HORST, 2003). A reduction in the ratio of anorganic cations to anions in the diet causes a non-respiratory systemic acidosis (JOYCE et al., 1997; ESPINO et al., 2003; ROCHE et al., 2003). Such acidification improves the ability of the cow to maintain calcium homeostasis by promoting the absorption of calcium from the small intestine and mobilization of calcium from the bone (SCHONEWILLE et al., 1999; ESPINO et al.,
The bone turnover may be stimulated via the facilitation of the action of parathyroid hormone on the bone and kidney or because of the role of the bone in buffering systemic acidosis (GAYNOR et al., 1989; GOFF, 2000; ESPINO et al., 2003).

BLOCK (1984) reported a 47% incidence of milk fever in cows fed diets containing a DCAD of 449 mEq/kg of diet DM; cows, fed diets with a DCAD of -172 mEq/kg of diet DM had no incidence of milk fever. Cows, fed the -172 mEq diet maintained higher blood Ca content at and around parturition.

There was no significant effect of precalving DCAD on the production of milk or milk components. This differs from the findings of (BEEDE et al., 1992; ROCHE et al., 2003). Milk fat percentage was unaffected by DCAD, whereas, milk fat yield increased (HU and MURPHY, 2004). Low DCAD diets would allow greater capability to maintain Ca homeostasis because small changes in blood pH have large impacts on the extent of Ca resorption (ARNETT, 2007).

ROCHE et al. (2002) mentioned that much of the controlled research concerning diets containing a decreased DCAD was conducted using animals that are highly predisposed to milk fever (cows in their third or greater lactation). Therefore, the effects of a low DCAD diet in breeds that are less susceptible to milk fever and in modern dairy herds that often exceed 40% first-lactation animals need to be considered in decision-making (OVERTON and WALDRON, 2004).

**DCAD Equation**

The DCAD equation cited by ENDER et al. (1962) and used by BLOCK (1984) was \( \text{DCAD} = (\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{S}^{2-}) \). This equation 1 is the most commonly used form of the equation and most accurate to predict the risk of milk fever (LEAN et al., 2006). HORSIT et al. (1997) recommended that other anions and cations have to be included in the equation and proposed \( \text{DCAD} = (0.38 \text{Ca}^{2+} + 0.3 \text{Mg}^{2+} + \text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{S}^{2-}) \). GOFF (2000) proposed a variation of this equation based on the capacity of different salts to acidify urine and recommended \( \text{DCAD} = (0.15 \text{Ca}^{2+} + 0.15 \text{Mg}^{2+} + \text{Na}^+ + \text{K}^+) - (\text{Cl}^- + 0.25 \text{S}^{2-} + 0.5 \text{P}^{3-}) \). Following the research of SPEARS et al. (1985), who estimated that the absorption of sulfur from the gastrointestinal tract was 60% of dietary intake, TUCKER et al. (1991) suggested as formula \( \text{DCAD} = (0.38 \text{Ca}^{2+} + 0.3 \text{Mg}^{2+} + \text{Na}^+ + \text{K}^+) - (\text{Cl}^- + 0.6 \text{S}^{2-} + 0.5 \text{P}^{3-}) \).
2.13.2. DCAD value

The optimum DCAD, the amount and kinds of anionic salts to feed are not well established. However, forages high in K, would require large amounts of anionic salts to achieve a DCAD of −10 to −15 mEq/100 g DM (MOORE et al., 2000). It was suggested that the DCAD needs to be −5.0 to −10.0 mEq/100 g DM to prevent hypocalcaemia (HORST et al., 1997). Diets with low but positive DCAD can also improve Ca homeostasis around parturition (KUROSAKI et al., 2007; PENNER et al., 2008). The optimal DCAD that can account for both reducing the prevalence and severity of hypocalcaemia and minimizing depression in DMI has not yet been established (HERON et al., 2009).

Feeding of anionic salt diets of -50 and -150 DCAD reduced retained placenta when compared to the +150 DCAD diet. Reduced retained placenta was accompanied by higher plasma Ca. The target regions of DCAD should be clearly below 100 mEq/kg dry matter to ensure the desired effect on ABB and calcium metabolism. Extremely negative DCAD should be avoided to minimize the risk of clinical acidosis induced by anionic salts (GELFERT et al., 2007).

Several studies have shown that reducing DCAD prepartum has no adverse effect on hepatic (SPANGHERO, 2002; GIULIO et al., 2005) and kidney function (SPANGHERO, 2002). Overall, the reduced DCAD might improve periparturient dairy cow’s health. Further larger-scale and longer-term trials are needed to confirm the knowledge at present (WU et al., 2008).

2.14. Anionic salt

Anionic salts have been defined as salts higher in the fixed ions Cl and S (anions). They increase absorption of Ca through the gastrointestinal tract and increase bone mobilization of Ca because of their acidifying properties (GANT et al., 1998).

Since the anionic salts are relatively easy to handle, they are the components of choice for overcoming the alkalinizing potential of prepartum diet. However, anionic salts may be unpalatable and are always accompanied by a cation, which, depending on its rate of absorption, will negative some of the effects of the anions (GOFF and HORST, 1998).

Anionic salts are added to the diet of dry cows 2–3 weeks before calving to achieve a mild metabolic acidosis. The main disadvantage of anionic salts is the lack
of palatability and the resulting decrease of feed intake (GOFF and HORST, 1998). Anionic salts can be administered to prevent milk fever without danger of significantly reducing the transfer of Selenium from the dam to the calf and without compromising the Selenium status of the cow when the anionic salts are limited to administration for two to three weeks before calving (GANT et al., 1998).

Heifers do not benefit from anionic salts because their serum Ca concentration was maintained at a consistently higher level than for multiparous cows (CHAN et al., 2006).

2.15. Monitoring the use of anionic salts

The monitoring of the use of anionic salts is necessary because cows might refuse to consume the diet due to the inherent bad taste of anionic salts (OETZEL and BARMORE, 1993). As DCAD declines, blood pH decreases and calcium homeostasis improves. Monitoring changes in urine pH as an index of body acid-base status has proved a valuable and inexpensive means of monitoring the success of addition of anions to prepartal rations to prevent milk fever in the field (GAYNOR et al., 1989; DAVIDSON et al., 1995; JARDON, 1995).

The addition of anionic salts to a ration induces metabolic acidosis (GOFF and HORST, 1998), resulting in a reduction of the pH in urine (JOYCE et al., 1997; OETZEL, 1991). Monitoring urine pH has proven useful in the field as a means of monitoring the acidification of the blood caused by anion supplementation; however, it is not foolproof. Sulfate salts were able to acidify the urine to the same extent as the chloride salts but did not acidify the blood to the same extent (GOFF et al., 2004). To monitor a sufficient effect of anionic salts, urine samples have to be analysed for pH & net acid-base excretion (NABE) (GELFERT et al., 2007).

Urine pH of the cows provides a cheap and fairly accurate assessment of blood pH (HUSBAND and VECQUERAY, 2007), and can be a good judgement of the appropriate level of anion supplementation (JARDON, 1995). Urinary pH is a good indicator to monitor implementation of dietary anionic salts and blood CAD can also be a useful measure (CHAN et al., 2006).

Urinary pH was very indicative of changes in the acid-base status of dairy cows with DCAD, especially when DCAD was low or negative (VAGNONI and OETZEL, 1998; HU and MURPHY, 2004).
Monitoring urine pH is a feasible method for determining the animal's response to dietary anions. Urine pH generally reflects the acid-base status of an animal. Many investigators measure the urine pH of pre-fresh cows (cows in the final three weeks prior to their due date) to monitor the effectiveness of a ration containing anionic salts. If the appropriate amounts of anionic salts are consumed in the ration, the urine pH will be 6.5–5.5 (GAYNOR et al., 1989; GOFF and HORST, 1998; JARDON, 1995).

Urine pH on high cation diets is generally above 8.2. Limiting dietary cations will reduce urine pH only a small amount (down to 7.8). For optimal control of subclinical hypocalcaemia the average pH of the urine of Holstein cows should be between 6.2 and 6.8, which essentially requires addition of anions to the ration. In Jersey cows the average urine pH of the close-up cows has to be reduced to between 5.8 and 6.3 for effective control of hypocalcaemia. If the average urine pH is between 5.0 and 5.5, excessive anions induced an uncompensated metabolic acidosis and the cows will suffer a decline in dry matter intake (Goof, 2008).

2.15.1. Net acid-base excretion (NABE)

For determination of NABE, two variations can be used: the fractional NABE and the simple NABE (BENDER et al. 2003; LACHMANN, 1981).

The determination of NABE is based on the idea of titration. The titration determines the value or the concentration of the unknown substance, by using substances of known concentration, with a defined pH value of discoloration of the indicator.

In the fractional NABE, HCl can determine the total excreted bases, NaOH the total content of acids e.g., after fixation by formaldehyde also the quantity of titrated NH₄ (LACHMANN, 1981). Based on the received quantities, the content of excreted bases, acids, NH₄, the acid base balance and the NABE may be calculated. Using these results the acidosis or alkalosis situation of the animal could be evaluated (DAVENPORT, 1973).
NABE reflects the ratio of titratable bases and acids in the urine. In case of an acidosis, NABE decreases and the ammonium concentration increases (BENDER et al., 2003; GELFERT et al., 2006). Changes of NABE are reliable indicators to monitor the use of anionic salts (GELFERT et al., 2007).

2.15.2. Urinary calcium

Increased urinary Ca excretion in low DCAD concentration might be due to a slight metabolic acidosis. This may not only increase the intestinal Ca absorption (SCHONEWILLE et al., 1994; ROCHE et al., 2003) but also Ca resorption from bones due to more synthesis of 1,25(OH)2D3 (GOFF et al., 1991). The acidosis maintains a high Ca flux through exchangeable pools without affecting the pool size (FREDEEN et al., 1988). Reduced urinary Ca excretion with increasing values of DCAD may be due to the gradual vanishing effect of a metabolic acidosis. It is also reported that ruminant kidneys are highly sensitive to blood cation anion differences and increase the excretion of Ca during acidosis, independent of the hormonal action, usually associated with Ca metabolism. WEST et al. (1992) observed an increased urinary Ca: creatinine (0.30 versus 0.09) excretion with decreasing (120 versus 465 mEq/kg) concentration of DCAD.

2.16. Mode of action of anionic salts

The exact mechanism of how dietary anions work is still unknown. It seems that the metabolic acidosis induced by anionic salts increases tissue responsiveness to parathyroid hormone, ameliorates the absorption of calcium in the intestine and enhances calcium mobilization from bones (HORST et al., 1997). STEWART (1983) showed that the addition of anions to a solution decreased the pH. The addition of anions to body fluids via dietary supplementation should, therefore, decrease the pH of body fluids. Although blood pH is highly regulated, slight variations may affect the Ca metabolism (BUSHINSKY et al., 1993; SCHONEWILLE et al., 1994) and improve periparturient calcium homeostasis (BLOCK, 1984; GOFF et al., 1991; JOYCE et al., 1997).

The acidification of the medium surrounding the bone will cause the release of bone Ca (BUSHINSKY et al., 1993) which can be monitored by indicators of collagen
degradation (BLOCK, 1984; GOFF et al., 1991). The bone is involved as a buffering system for acid-base control of body fluids. The acidifying diets induce the release of cations (including Ca) into the blood in order to correct its pH. Metabolic acidosis first stimulates the physicochemical mineral dissolution then the cell mediated bone resorption by increasing the activity of osteoclasts and decreasing the activity of osteoblasts (RIOND, 2001).

According to SEIFI et al. (2004) the supplementation of the diet with anionic salts will increase two PTH-dependent functions: bone resorption and renal production of 1, 25-dihydroxycholecalciferol. In vitro studies demonstrate that stimulating metabolic alkalosis in tissue culture systems reduces bone Ca resorption activity in response to PTH (BUSHINSKY, 1996). There is evidence to suggest that under normal conditions, when the blood pH is around 7.35, PTH and its receptor interact in a tight "lock-and-key" fashion, allowing the PTH to stimulate the target cell adequately. In cows fed a diet high in cations, the blood pH may become more alkaline, changing the conformational structure of the PTH receptor so that PTH and its receptor do not interact efficiently.

Anionic salts prevent milk fever by acidifying the blood to restore tissue responsiveness to the parathyroid hormone (GOFF and Horst, 1998). The reduced precalving DCAD significantly increased plasma Ca concentration on the day of calving ROCHE et al. (2003). MCNEILL et al. (2002) reported reduced hypocalcaemia when anionic salts were not supplemented in sufficient quantities to change the systemic pH.

2,000 mEq/day of either CaCl₂ or CaSO₄ were administered for 9 days to nonpregnant, nonlactating dairy cows as well as a diet with a different amount of calcium (60.6 g/117.6 g). The feeding of anionic salts resulted in a decrease of the blood and urinary pH. There were no significant effects detectable due to the different anionic salts or different calcium supply on ABS (acid-base status). These results confirm the statements from all of the other studies claiming that the induction of metabolic acidosis by anionic salts is a process independent from the amount of dietary calcium (GELFERT et al., 2007).

RAMBERG et al. (1996) suggested several mechanisms by which sulfur could reduce the risk of milk fever. These include an acidogenic effect, if gastrointestinal absorption of SO₄²⁻ were preferential over Mg²⁺, and a possible laxative effect of magnesium sulphate that might increase bicarbonate losses in the feces. It will be
difficult to determine whether any of these physiological actions of sulfur, apart from a role in DCAD, influence the risk of milk fever independently (LEAN et al., 2006).

2.16.1. The effect of anionic salts on the calcium metabolism

The increased amount of [H+] displaces Ca that is bound to the proteins in blood, which results in an increased concentration of Ca^{2+}. The organism has a second possibility to get rid of the increased [H+] by generating [HCO_{3}^{-}] from the bone (LUNN and MCGUIRK, 1990). This is accompanied by an increased release of Ca (DELAQUIS and BLOCK, 1995; GOFF and HORST, 1998). In both cases, the [Ca^{2+}] increase is followed by an increased Ca-excretion via the kidneys (BLOCK, 1994; LUNN and MCGUIRK, 1990). An increased loss of Ca via urine will activate PTH, which itself will activate the Ca reabsorption in urine with a production of 1.25 dihydroxycholecalciferol in the kidneys. Both hormones activate the Ca transport mechanism in the bone and intestine (GOFF et al., 1991). This increased Ca-resorption in the intestine is described in a study (FREDEEN et al., 1988), in which an anionic (-2 mEq/kg DM) or alkaline diet (+71 mEq/kg DM) was fed to lactating goats. The anionic salt changed the flux of Ca by increasing the bone resorption, Ca absorption, and Ca clearance via the kidneys. The size of the Ca pool, however, remained unchanged. TAKAGI and BLOCK (1991) examined the effect of anionic salts in sheep and confirmed the findings of FREDEEN et al. (1988). They stated that the increased fraction of [Ca^{2+}] together with an increased excretion of Ca in urine maintain a high metabolic rate of this element.

The steady feeding of anionic salts ensures the continuity of the metabolic acidosis, which itself maintains a shift in [Ca^{2+}]. These observations were made in several studies that either dealt with the feeding of anionic salts (GELFERT et al., 2006; 2007; MOORE et al., 2000; GOFF et al., 1991) or the role of anionic salts and dietary Ca (GELFERT et al., 2007; LIESEGANG et al., 2007; CHAN et al., 2006; GOFF and Horst, 1997; SCHONEWILLE et al., 1994; ENDER et al., 1971).

At present the following mechanisms are found to explain the effects of anionic salts (DEGARIS and LEAN, 2009).

(1) Diets high in anionic salts cause metabolic acidosis in goats (FREDEEN et al., 1988) and cattle (GAYNOR et al., 1989).

(2) Diets high in anionic salts stimulate a calciuria (GAYNOR et al., 1989; OETZEL et al., 1991; PHILLIPO et al., 1994).
Elevated hydroxyproline concentrations have been observed in cows, fed anionic salts (BLOCK, 1984; GAYNOR et al., 1989), probably indicating bone mobilisation.

Plasma ionised Ca concentrations increase with feeding of anionic salt (OETZEL et al., 1991; PHILLIPO et al., 1994).

Diets high in anionic salts stimulate higher plasma levels of 1,25(OH)₂D₃ before calving (GAYNOR et al., 1989; PHILLIPO et al., 1994). Calciuria may be induced by acute acidosis in a number of species. Metabolic acidosis increases mobilisation of Ca from rat liver mitochondria (AKERMAN, 1978) and mobilisation of Ca from the bone, independent of, and in conjunction with, PTH (BECK and WEBSTER, 1976).

2.17. Differences between anionic salts

GELFERT et al. (2008) did not find differences between the effect of CaCl₂ and CaSO₄. VAGNONI and OETZEL (1998) found that both NH₄Cl and (NH₄)₂SO₄, are effective in preventing milk fever and that their prophylactic effect can be produced in a 21-d ante partum period instead of the 45-d period (OETZEL et al., 1988). Different anionic salts did not reveal any significant differences in the relative changes of ABB (acid-base balance) parameters in blood and urine (GELFERT et al., 2006; 2007).

When there are no differences in palatability or potential efficacy, selection of anionic salts to feed during the prepartum period for prevention of parturient paresis may be based on commercial availability of the salts, price, and avoidance of toxicity. Combinations of salts are recommended because they decrease the potential for toxicity due to excessive amounts of NPN, S, or Mg. An exact dose of anionic salts necessary to prevent parturient paresis has not been established; however, doses between approximately 1.9 and 3.4 eq/cow/1 d have been used effectively to reduce the incidence of parturient paresis (OETZEL et al., 1991).

GOFF et al. (2004) found a stronger influence of chloride salts on the blood parameters and on urine pH. However, feeding calcium sulphate in a daily dose of 1500 mEq resulted in a stronger decrease of urinary pH (P<0.05) than the same dose of calcium chloride. TUCKER et al. (1991) found a significantly different impact between the 2 anions only in urine. Chloride salts induce a higher decrease of urine pH. WANG and BEEDE (1992) compared the impact of NH₄Cl and MgSO₄ on ABB
(acid base balance) and state, that MgSO\textsubscript{4} is less acidogenic because of the weaker reactions of ABB monitored. Dietary sulfate anions are less potent acidifiers of the blood, than dietary chloride anions, as there is some blockage of sulfate absorption at higher doses, while chloride absorption continues unabated. Another possibility is that sulfate is cleared from the blood faster than chloride, especially at higher blood levels. The addition of chloride to prepartal diets would prove more effective than sulphate because sulfate has about 60 % of the blood’s acidifying activity of chloride (GOFF et al., 2004). Supplemental sulfates are less efficient than Cl in their urine acidifying effect (WANG and BEEDE, 1992; GOFF et al., 1997; GOFF and HORST, 1997; 2000). Moreover, the total dietary S also include organic components (e.g. cystine, cysteine and methionine) which produce sulfates only when catabolyzed. As sulfur is difficult to determine precisely in dietary samples, its exclusion from the DCAD calculation simplifies the analytical procedure required and reduces costs (SPANGHERO, 2004).

**Sulfate anions potent and toxicity**

Some authors (OETZEL et al., 1988) have used salts containing Cl and S but not Mg (ammonium-based salts) to reduce DCAD to -70 mEq/kg, resulting in 13 % less hypocalcaemia than measured in salts based on Mg, such as MgCl\textsubscript{2} and MgSO\textsubscript{4}, (assuming a 100 % absorption and a common acidity for both Cl and S). This would result in a final DCAD of approximately +140 to +200 mEq/kg DM (ROCHE et al., 2002).

Other authors reported that Sulfur and sulfate are potentially toxic because they can be reduced to hydrogen sulfide, a potent neurotoxin, in the rumen (GOULD et al., 1991). Therefore the amount of sulfate added to the diet must be limited. The current maximum tolerable amount of dietary sulphur in cattle is thought to be 0.4 % of the diet DM (National Research Council, 2001). Since low doses of sulphate, coming from CaSO\textsubscript{4} and H\textsubscript{2}SO\textsubscript{4}, appear to be equipotent to low doses of chloride sources, adding small amounts of these salts would be useful as long as the inclusion does not bring the total sulfur content above 0.4 % (GOFF et al., 2004).

**2.18. Duration of feeding anionic salts**

Anionic salts supplied once daily confined the risk of an interrupted effect of the anionic salts on the acid–base status, as well as the calcium metabolism after 12
h (GELFERT et al., 2008). Anionic salts induce a 24 h lasting effect on the acid–base status if the salts are given twice daily (ROCHE et al., 2007).

It is possible to feed anions for only 1 or 2 d before calving to prevent milk fever. This procedure is currently not practical because of problems predicting the date of calving and the introduction of new feed immediately before calving (GOFF and HORST, 1998). It is suggested that a feeding period of at least 10 days perpartum is required (HANSEN et al., 2004). Long periods of anionic salt supplementation have not been demonstrated to be necessary. The acidogenic effect was apparent after 14 d, indicating that the period of supplementation could be restricted to the last 2 to 3 weeks before calving (PEHRSON et al., 1999).

Feeding anionic diets for 21 d (TUCKER et al., 1991; GOFF et al., 2004; OETZEL, 2002; CHAN et al., 2006), 28 days (WANG and BEEDE, 1992) and 45 days (BLOCK, 1984; HUSBAND et al., 2002; GOFF and HORST, 1998) perpartum was effective in preventing hypocalcaemia by inducing a mild metabolic acidosis; reduced urinary pH reflected the influence of the dietary anion content fed. Exposure of the dry cows to acidifying anionic salts for 10 days to 2 weeks before calving, might be enough to stimulate calcium homeostatic effects and these mechanisms may persist even when the exact dates of calving are not known. Two-day anion supplementation improved calcium homeostatic mechanisms but it was not clear experimentally whether the cows might be able to maintain their improved calcium homeostatic efficacy when anion supplementation was stopped after 2 days of supplementation (MELLAU et al., 2002).

The feeding of anionic salts to non-pregnant, non-lactating dairy cattle over a period of 5 weeks caused significant alterations in ABB and calcium homeostasis. In the middle of the treatment period, blood and urinary ABB returned to initial levels, but decreased more extensively in the fifth week of the experiment, indicating a non-compensated metabolic acidosis. Anionic salts should be fed for a minimal period of 7 to 14 days in order to guarantee the desired effect on the calcium metabolism. Feeding anionic salts for more than 4 weeks caused hypocalcaemia, indicating that after the third week the damage could be greater than the advantages (GELFERT et al., 2006).
2.19. Doses

The amount of anionic salts needed depends on the concentration of potassium in the diet, which is the major component in the formula of the dietary cation anion difference DCAD. This is especially true in Middle-European regions, where grass silages are a major component of dairy cow diets. Other cations like sodium, calcium and magnesium play only a minor role due to their lower concentrations. In case of high concentrations of potassium in the diet, the amount of anionic salt has to be much higher than the recommended dose of 2000-3000 mEq/day (GELFERT, 2006).

The influence of a daily dose of anionic salts, above the valid upper limit, on the metabolism of dairy cows was investigated. Acid-base balance was strongly influenced by anionic salts. Blood pH dropped steadily and reached values around 7.23. Urine pH dropped quickly below 6 and remained at that level regardless of the increased dosage of the anionic salt. Net acid base excretion (NABE) fell continuously, with increasing dosage of the anionic salt and reached values below -200 mmol/l. The results showed that with an increasing amount of anionic salt fed, the risk of clinical acidosis increased (GELFERT et al., 2006).

2.20. Palatability and dry matter intake

Six anionic salts were evaluated for their effects on dietary DM (dry matter) intake, systemic acid-base balance, and urinary excretion of Ca. Each of the six salts was fed for a one weak period. Anionic salt treatments did not decrease DM intake compared to the control diet fed without salts (OETZEL et al., 1991). The dry matter intake was not lower in the cows fed NH₄Cl and (NH₄)₂SO₄ in the trial (OETZEL et al., 1988).

Anionic salts resulted in a depression of DMI (dry matter intake) of a concentrate mixture to which they were added (VAGNONI and OETZEL, 1998). The delivery of anionic salts in 2.27 kg of a concentrate mixture did not result in acceptable intakes. Anionic salts may need to be mixed with larger amounts of concentrates or with forages to ensure adequate intakes (OETZEL and BARMORE, 1993).
3. Materials and Methods

3.1. Animals

40 late pregnant cows (more than 240d), having completed three or more lactations, with an expected calving date within the next three weeks were selected from the herd. The animals were randomly allocated to 4 groups which were offered the same diet and no anionic salts (control group) or defined amounts of anionic salts in the treatment groups (TG).

Mean age and body weight did not differ between the groups. Group one consisting of 10 cows used as control, the second group consisting of 10 cows and treated with 800 g/day of the salt, the third group consisting of 10 cows and treated with 1000 g/day of the salt and the forth group also consisting of 10 cows and treated with 1200 g/day of the salt.

Table A: Mean value of lactation numbers, age of cows at beginning of the experiment, daily milk production, and races.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Lactation number</th>
<th>Age of cows at beginning of the experiment</th>
<th>Race</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Days  Months Years</td>
<td>Daily milk production</td>
</tr>
<tr>
<td>CG</td>
<td>2,9</td>
<td>17  2  5,4  5,6</td>
<td>31,4</td>
</tr>
<tr>
<td>TG1</td>
<td>3,6</td>
<td>17  4,7  5,8</td>
<td>24,13</td>
</tr>
<tr>
<td>TG2</td>
<td>4,4</td>
<td>16,6  5,2  6,5</td>
<td>24,72</td>
</tr>
<tr>
<td>TG3</td>
<td>2,9</td>
<td>14,4  5,7  5,3</td>
<td>24,4</td>
</tr>
</tbody>
</table>

3.2. Housing and feeding management

3.2.1. Housing

The experiment was conducted at the school farm in Kremesberg of the University for Veterinary Medicine of Vienna at 2006/07. This farm was chosen for the ability of a computerized single place feeding. The cows were housed in a stable during the dry period until 7 days before the expected day of parturition. Then, the cows were housed in an individual pen until 3 days after calving. The cows were individually fed for the duration of the experimental period. Feed was offered twice
daily (at 0500 h and 1600 h). The cows were held in single groups on a bare paddock.

The cows calved indoors and were monitored to determine the duration of calving. The calving time was determined, as the time from the first signs of the calf’s hooves until the calf was born.

3.2.2. Feeding

All cows were offered a daily diet of alfalfa silage 8 kg, hay 1st cut 5 kg, 2 kg hay and concentrates. The concentrates feeding for lactation start with 0.5 kg/cow/d and increased to 1.5 kg/cow/d three weeks before the expected date of parturition. The diet was divided into two parts morning and in the afternoon before calving. After calving, the cows were switched to the other ration. The nutritive characteristics and mineral concentration of the feed offered are shown in table 1. Water was available ad libitum.

Table 1: Composition of the dry ration in Kg, for pregnant dairy cows and the dietary cation-anion difference (DCAD) in the control group (CG) and treatment groups (TG) after adding different amounts of calcium sulfate.

<table>
<thead>
<tr>
<th></th>
<th>CG</th>
<th>TG1</th>
<th>TG 2</th>
<th>TG 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium sulfate</td>
<td>0 g</td>
<td>800 g</td>
<td>1000 g</td>
<td>1200 g</td>
</tr>
<tr>
<td>Alfalfa Silage</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Hay 1st Cut</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Hay</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Concentrates</td>
<td>1,5</td>
<td>1,5</td>
<td>1,5</td>
<td>1,5</td>
</tr>
<tr>
<td>Dry matter intake</td>
<td>10,92 kg/d</td>
<td>11,66</td>
<td>11,89</td>
<td>12,05</td>
</tr>
<tr>
<td>Quantities of Elements in %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td>3,51</td>
<td>3,51</td>
<td>3,51</td>
<td>3,51</td>
</tr>
<tr>
<td>Sodium</td>
<td>0,19</td>
<td>0,19</td>
<td>0,19</td>
<td>0,19</td>
</tr>
<tr>
<td>Chloride</td>
<td>0,63</td>
<td>0,63</td>
<td>0,63</td>
<td>0,63</td>
</tr>
<tr>
<td>Sulfur</td>
<td>0,22</td>
<td>0,52</td>
<td>0,67</td>
<td>0,82</td>
</tr>
<tr>
<td>DCAD (mEq)</td>
<td>+610</td>
<td>+163</td>
<td>+51</td>
<td>-60</td>
</tr>
</tbody>
</table>
The cows received their morning and afternoon salt mixed with concentrates.

All cows received 100 g of calcium carbonate daily to the basic ration according to the recommendations of WILDE (2006). The total dry matter intake was 10.92 kg/d of CG and DCAD +610 mEq.

The diet of TG1 was 800 g of anionic salt daily together with the basic ration. The total dry matter intake was 11.66 kg/d and DCAD +163 mEq.

The TG2 diet consisted of 1000 g of anionic salt daily to the basic ration. The total dry matter intake was 11.89 kg/d and DCAD +51 mEq.

The TG3 diet consisted of 1200 g of anionic salt daily to the basic ration. The total dry matter intake was 12.05 kg/d and DCAD -60 mEq.

### 3.2.2.1. Chemical Analyses of Feed

Samples of feed stuff were ashed (500 °C, for 6 h) and dissolved in 4M HCl before determination of total K and Na contents. K was estimated by atomic absorption spectroscopy, and Na was estimated by atomic emission spectroscopy using the modified methods of Trudeau. The chloride content of the feed was determined according to the method of Schales and Schales. The amount of S in the feed and ingredients was measured (VDLUFA, 1997).

The DCAD was calculated according to the following formula (Na + K) - (Cl + S). Monthly samples of feed components were taken for analysis and recalculation of DCAD to give the possibility to correct the amount of anionic salt added.

### 3.2.3. Anionic salt

Natural calcium sulfate (CaSO₄) with a grain size of 10 μm was used in the present study. The salt is available in Germany by the name, Transifit* (Dr.Pieper GMBH, Wuthenow Germany).

**DCAD Calculation**

In the present study the following equation (Na + K) - (Cl + S) was used for calculation of DCAD. This equation may not be the proper equation for the calculation of the DCAD especially when using calcium sulfate in very high concentrations, to lower the value of DCAD of high potassium fed.

DCAD has been defined as (Na + K) - (Cl + S), an equation originally proposed by ENDER et al. (1962). This equation has been evaluated as the most
relevant by LECLERC and BLOCK (1989) and TUCKER et al. (1992). Since then numerous equations have been published for the calculation of DCAD in dairy cattle diets. These longer equations were

\[(\text{Na} + \text{K} + 0.38 \text{ Ca} + 0.30 \text{ Mg}) - (\text{Cl} + 0.6 \text{ S} + 0.5 \text{ P})\] (HORST and GOFF, 1997).

\[(\text{Na} + \text{K} + 0.15 \text{ Ca} + 0.15 \text{ Mg}) - (\text{Cl} + 0.2 \text{ S} + 0.3 \text{ P})\] (HORST and GOFF, 1997).

\[(\text{Na} + \text{K} + 0.15 \text{ Ca} + 0.15 \text{ Mg}) - (\text{Cl} + 0.6 \text{ S} + 0.5 \text{ P})\] (National Research Council, 2001).

\[(\text{Na} + \text{K}) - (\text{Cl} + 0.6 \text{ S})\] (GOFF et al., 2004).

Other researchers reported that the most appropriate form of the DCAD equation was \((\text{Na} + \text{K}) + (\text{Cl} + 0.6 \text{ S})\). On the basis that this equation a prediction of both milk fever risk and urine pH could be made (PEHRSON et al., 1999). According to the results of GOFF et al. (2004), sulfate is between 55 and 60% as effective as chloride at changing blood pH and SBE (standard base excess), confirming the study of TUCKER et al. (1991).

LEAN et al. (2006) said that \((\text{Na} + \text{K}) + (\text{Cl} + \text{S})\) was the best formula to predict the milk fever risk, based on the simplified strong ion model and the metaanalyses performed by the authors. Goff (2008) stated in his review that DCAD equations provide a theoretical basis for dietary manipulation of the acid-base status. They are not necessary for the formulation of mineral content of prepartum dairy cow rations, because, with the exception of K and Cl, the rate of inclusion of the other macrominerals may be set at fixed rates. In dry dairy cows fed diets, in which S was always supplemented, the DCAD equation containing Na, K and Cl was more predictive of urinary pH, than the DCAD equation including S (SPANGHERO, 2004).

The use of the DCAD5 equation \([(\text{Na} + \text{K}) - (\text{Cl} + 0.6 \text{ S})]\) has proven to be most efficient in decreasing the urinary pH and preventing clinical milk fever, since it was most highly correlated to clinical milk fever incidence and urinary pH (CHARBONNEAU et al., 2006).

The debate of the right formula of DCAD is still going on and more research is necessary.
3.3. Sampling

3.3.1. Blood Samples
Blood samples were drawn from the vena jugularis into Vacutainer tubes (Vacutainer, silicone coated). A sample of blood was collected from each cow weekly, on the day of calving and the next two days.

All blood samples were taken at 0700 about one hour after the cows finished the morning feed.

Samples were stored on ice during transport and centrifuged immediately after arrival at the laboratory. All blood samples were centrifuged for 10 min at 4000 g. The serum was separated into polyethylene tubes serum and stored at -20 °C immediately until the analyses were performed.

3.3.2 Urine Samples
The cows were manually stimulated to urinate by gentle massaging of the perineum, one day each week before parturition, at the day of parturition and daily after parturition for 2 consecutive days, at approximately the same time of blood sampling for the duration of the experiment. When stimulation to urinate failed, urine samples were collected using a sterile Rüsch® catheter after washing the vulva with a septical soap (Betadine, Provet AG, Lyssach, Switzerland).

A sample of midstream urine was collected in a 30 ml container. 2 ml of the sample were separated in Eppendorf tubes and frozen for subsequent Ca and Creatinine analysis. The rest was frozen in the container for further analysis of pH and net acid base excretion (NABE).

3.4. Analysis of calcium and creatinine in blood and urine

3.4.1. Calcium
Chemical urine analyses were performed on centrifuged urine samples by a fully selected chemistry Autoanalyzer Hitachi 911® (ROCHE Diagnostics, Vienna, Austria). The methods were applied according to the manufacturers'
recommendations. Quality control material was analyzed prior to each run to check adequate function of the assays.

3.4.2. Creatinine

Urine creatinine was determined by an enzymatic assay with automated predilution and Urine-Calcium by a chromogenic test with o-Kresolphthalein.

3.4.3. Fractional excretion

Fractional excretion of Ca was calculated using the following formula:

\[ \text{FE}_{\text{Ca}, \%} = \frac{\text{urinary Ca}}{\text{serum } \text{Ca}^{2+}} \times \frac{\text{urinary creatinine}}{\text{serum creatinine}} \times 100 \]

3.4.4. Urine pH

Urinary pH was determined immediately, using a pH meter calibrated, with pH 7.0 and 10.0 buffers (WTW, Weilheim, Germany).

3.4.5. Net acid-base excretion (NABE)

Urinary bases, urine acids and ammonium were measured by titration (table 1) of urine samples according to KUTAS (1965). After titration NABE was calculated by using the concentrations of bases, acids and ammonium as follows:

\[ \text{NABE (mmol/l)} = 10 \times (10 \times \text{bases (mmol/l)} - \text{acids (mmol/l)} + \text{ammonium (mmol/l)}) \]
Table 2: Determination of NABE by KUTAS (1965)

<table>
<thead>
<tr>
<th>Fractional NABE</th>
</tr>
</thead>
<tbody>
<tr>
<td>- 10 ml urine is titrated with 1n HCl to a pH of 3.5</td>
</tr>
<tr>
<td>- 30 seconds heating</td>
</tr>
<tr>
<td>- cooling</td>
</tr>
<tr>
<td>- titration with 0.1 n NaOH to a pH of 7.4</td>
</tr>
<tr>
<td>- 10 ml 20% Formaldehyde solution added</td>
</tr>
<tr>
<td>- titration with 0.1 n NaOH to a pH of 7.4</td>
</tr>
</tbody>
</table>

Bases (mmol/l) = Volum of HCl × 100

Acids (mmol/l) = Volum of NaOH1 × 10

NH₄ (mmol/l) = Volum of NaOH2 × 10

NABE (mmol/l) = (Volum of HCl × 10 – (Volum of NaOH1 + Volum of NaOH2)) × 10

3.5. Statistical analysis

Studies with rumen fistulated cows have shown that 10 cows per group are sufficient for statistical analysis (FRÖMER, 2004; LÖPTIEN, 2004). The changes in the parameter of acid-base-balance and major element metabolism, induced by the anionic salts, are sufficient to minimize the risk of an error of type 2. To compare the reaction of acid-base-balance and calcium metabolism a repeated ANOVA was used. In this analysis, the amounts of the anionic salt added to the feed ration were considered the main effects and the cow was a random factor. Dunnett-t-test was carried out as a post-hoc-test to compare the changes of each test day, to day zero. The level of significance was fixed at p = 0.05 for each single parameter.
3. RESULTS

4.1. Feeding

All cows received 100 g of calcium carbonate daily to the basic ration according to the recommendations of WILDE (2006). The total dry matter intake was 10.92 kg/d of CG and DCAD +610 mEq.

The diet of TG1 was 800 g of anionic salt daily together with the basic ration. The total dry matter intake was 11,66 kg/d and DCAD +163mEq.

The TG2 diet consisted of 1000 g of anionic salt daily to the basic ration. The total dry matter intake was 11,89 kg/d and DCAD +51mEq.

The TG3 diet consisted of 1200 g of anionic salt daily to the basic ration. The total dry matter intake was 12,05 kg/d and DCAD -60 mEq.

4.2. Incidence of parturient paresis

Of the 40 sampled cows, 6 developed recumbency after calving and were considered to be affected by milk fever. The diagnosis was based on clinical diagnosis and confirmed by the serum Ca levels prior to treatment. All parturient cows responded to intravenous treatment using Ca-borogluconate.

The results are summarized in table 3.

Table 3: Total numbers of cows, incidence of milk fever and intake of calcium sulfate.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total numbers</th>
<th>Recumbancy</th>
<th>Refusal</th>
<th>Recumbancy + Refusal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (No salt)</td>
<td>10</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TG1 (800g)</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>TG2 (1000g)</td>
<td>10</td>
<td>2</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>TG3 (1200g)</td>
<td>10</td>
<td>1</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>6</td>
<td>6</td>
<td>1</td>
</tr>
</tbody>
</table>

4.3. Intake of calcium sulfate

Cows were fed the calcium sulfate not in combination with the total mix rations, all feeding components were fed separately for the last 3 weeks before the expected date of parturition. There was a special diet that exists for transition cows in the last 3 weeks before parturition and the anionic salts were additionally administered and mixed in by hand among the other feed, the salt was given twice daily. A reduction of feed intake or only selection of the concentrates resulted in a
decrease of the amount of the anionic salt consumed by the animals. The number of cows, who refused the anionic salt, was 2 from TG1. One of them developed milk fever and recumbancy just after parturition. 3 cows from TG2 and 2 cows from TG3 were recorded (Table 3).

4.4. Urine Analysis

4.4.1. pH

Before feeding the anionic salt the urine pH was within the normal range in all groups. No marked changes in urine pH occurred in the control group. The mean value of urine pH did not change before calving, at parturition or the next two days after parturition.

After feeding the anionic salt a marked lowering in urine pH in the other three groups in the late gestation period, was observable. At the day of parturition the mean urine pH values reached the lowest value. In the next two days after parturition the urine pH increased in all groups.

The urine pH of the 4 groups (table 5) was significantly different ($p < 0.05$). The difference was only prominent between the control group and TG1 ($p = 0.031$). Figure 1 shows the changes in urine pH in the three treatment groups and the control group during the time of the experiment.

Figure 1: changes in urinary pH in the 4 groups during feeding anionic salts to pregnant dairy cows (n=40)

4.4.2. Base concentration
Before feeding the anionic salt, the urine base concentration was within normal range. No marked changes were observed in urine base in the control group. The mean value of the urine base concentration mildly decreased at the day of parturition, but in next two days the urine base concentration increased to the same range as before calving.

After feeding the anionic salt, urinary base concentration in the other three groups dropped during the late gestation period. At the day of parturition the mean urine base concentration values reached the lowest value. In the next two days after parturition the urine base concentration was increased in all groups (table 4, page 57).

Urine base concentrations of the 4 groups were significantly different (p < 0.05). The difference was only marked between the control group and TG2 (p =0.011). Figure 2 shows the changes in urinary base concentration in the three treatment groups and the control group during the period of the experiment.

**Figure 2:** changes in urinary base concentration in the 4 groups during feeding anionic salts to pregnant dairy cows (n=40)

4.4.3. Acid concentration

Before feeding the anionic salt the urine acid concentration was within the normal range. No marked changes were seen in urine acid in the control group. The
mean value of the urine acid concentration decreased mildly at the day of parturition, however it returned to the normal value prior to calving within the next two days.

After feeding the anionic salt a marked increase in urinary acid concentration in the other three groups was visible during the late gestation period. At the day of parturition the mean urine acid concentration value reached the lowest value in TG2 and was also low to some extent in TG1 but remained high in TG3. In the next two days after parturition the urine acid concentration returned to normal ranges in all groups.

The urine acid concentrations of the 4 groups were significantly different (p<0.05). The difference was only prominent between TG2 and TG3. (p=0,019). Figure 3 shows the changes of urine acid concentration in the three treatment groups and the control group during the period of the experiment.

**Figure 3:** changes in urinary acid concentration in the 4 groups during feeding anionic salts to pregnant dairy cows (n=40)

![Graph of urine acid concentration changes](image)

4.4.4. **Ammonium NH₄**

Before feeding the anionic salt the urine ammonium concentration was within the normal range in the four groups. No marked changes of urine ammonium in the control group were detectable. The mean value of urine ammonium concentration during the late gestation period and the day of parturition was not markedly changed. A severe increase of the urine ammonium concentration was found but in the next
two days the urine ammonium concentration decreased to the same range as before calving.

The urinary ammonium concentrations of the 4 groups (table 5, page 57) were not significantly different (p > 0.05).

4.4.5. Net Acid Base Excretion (NABE)

Before feeding the anionic salt the net acid base excretion was within the normal range. In the CG, the mean value of NABE period did not change during the late gestation. There was a small decrease visible at the day of parturition, however, in the next two days NABE increased to the same range as before calving.

Feeding the anionic salt induced a marked lowering of NABE in the other three groups during the late gestation period. At the day of parturition, the mean net acid base excretion values reached the lowest value (table 4, page 57). After parturition, NABE increased in all groups, but without reaching the value of the control group.

The net acid base excretion of the 4 groups differed significantly (p<0.05). The difference was only prominent between the CG and the TG 2 (p =0.007). Figure 4 shows the changes in NABE concentration in the three treatment groups and the control group during the period of the experiment.

**Figure 4:** changes in urinary net acid-base excretion (NABE) in the 4 groups during feeding anionic salts to pregnant dairy cows (n=40)
4.5. Calcium

4.5.1. Calcium concentration in serum

Before feeding the anionic salt, the serum calcium concentration was within the normal range. During the late gestation period, no changes in serum calcium concentrations were visible in CG. At the day of parturition there was a moderate decrease in calcium concentration which continued to the first day after calving. The second day after calving, serum calcium concentrations increased again however without returning to the normal range.

No marked changes of the mean serum calcium values, between the 3 treatment groups, were found in the late gestation period after substituting the anionic salt (table 4, page 57). At the day of parturition the calcium concentration in the serum dropped remarkably in the three TG and remained low for the first two days after calving. The serum calcium concentration of the 4 groups was not significantly different (p > 0.05). Figure 5 shows the changes in the serum calcium concentration in the three treatment groups and the control group, during the period of the experiment.

Figure 5: changes in serum calcium concentration in the 4 groups during feeding anionic salts to pregnant dairy cows (n=40)

4.5.2. Urinary calcium concentration
Before feeding the anionic salt the urinary calcium concentration was within the normal range. The urinary calcium concentration remained nearly the same in the CG during the late gestation period. It decreased at the day of parturition and increased anew in the first two days after calving.

After feeding the anionic salt a marked increase of urinary calcium concentrations in the three TG was visible, during the late gestation period (table 5). At the day of parturition urinary calcium concentrations dropped. No significant differences were detectable (p>0.05). Figure 6 shows the changes of the urinary calcium concentration in the three treatment groups and the control group during the period of the experiment.

**Figure 6**: changes in urinary calcium concentration in the 4 groups during feeding anionic salts to pregnant dairy cows (n=40)

4.5.3. **Fractional excretion of calcium (FE\textsubscript{Ca})**

Before feeding the anionic salt FE\textsubscript{Ca} was within the normal ranges. The changes of FE\textsubscript{Ca} were the same as urinary calcium concentrations (table 4, page 57). The fractional excretion of calcium of the 4 groups was not significantly different (p>0.05).
Table 4: Mean concentration of different parameters in serum and urine of dairy cows fed with different amounts of calcium sulfate as anionic salt in the last three weeks before parturition

<table>
<thead>
<tr>
<th>Group</th>
<th>Day</th>
<th>Urinary pH</th>
<th>Base mmol/l</th>
<th>Acid mmol/l</th>
<th>NH₄⁺ mmol/l</th>
<th>NABE mmol/l</th>
<th>Serum Ca mmol/l</th>
<th>Urinary Ca mmol/l</th>
<th>FE_Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>21 d</td>
<td>8,52</td>
<td>328</td>
<td>77</td>
<td>3,8</td>
<td>274</td>
<td>2,37</td>
<td>0,44</td>
<td>0,002</td>
</tr>
<tr>
<td>(CG)</td>
<td>14 d</td>
<td>8,41</td>
<td>361</td>
<td>96</td>
<td>4,4</td>
<td>261</td>
<td>2,36</td>
<td>0,61</td>
<td>0,003</td>
</tr>
<tr>
<td> </td>
<td>7 d a.p.</td>
<td>8,48</td>
<td>341</td>
<td>91</td>
<td>3,1</td>
<td>247</td>
<td>2,34</td>
<td>0,85</td>
<td>0,005</td>
</tr>
<tr>
<td> </td>
<td>1 d p.p.</td>
<td>8,47</td>
<td>339</td>
<td>85</td>
<td>6,4</td>
<td>247</td>
<td>1,87</td>
<td>0,55</td>
<td>0,002</td>
</tr>
<tr>
<td> </td>
<td>2 d p.p.</td>
<td>8,32</td>
<td>325</td>
<td>75</td>
<td>13,3</td>
<td>237</td>
<td>2,16</td>
<td>1,59</td>
<td>0,007</td>
</tr>
<tr>
<td>TG 1  </td>
<td>21 d</td>
<td>8,37</td>
<td>314</td>
<td>95</td>
<td>4,2</td>
<td>215</td>
<td>2,31</td>
<td>1,18</td>
<td>0,006</td>
</tr>
<tr>
<td>+163 mEq/kg TS</td>
<td>14 d</td>
<td>8,34</td>
<td>276</td>
<td>102</td>
<td>4,3</td>
<td>169</td>
<td>2,37</td>
<td>2,40</td>
<td>0,010</td>
</tr>
<tr>
<td> </td>
<td>7 d a.p.</td>
<td>8,28</td>
<td>262</td>
<td>97</td>
<td>11,7</td>
<td>153</td>
<td>2,27</td>
<td>1,22</td>
<td>0,005</td>
</tr>
<tr>
<td> </td>
<td>1 d p.p.</td>
<td>8,25</td>
<td>271</td>
<td>82</td>
<td>4,9</td>
<td>184</td>
<td>1,55</td>
<td>0,44</td>
<td>0,005</td>
</tr>
<tr>
<td> </td>
<td>2 d p.p.</td>
<td>8,29</td>
<td>338</td>
<td>102</td>
<td>4,6</td>
<td>231</td>
<td>1,92</td>
<td>0,46</td>
<td>0,004</td>
</tr>
<tr>
<td>TG 2  </td>
<td>21 d</td>
<td>8,32</td>
<td>239</td>
<td>75</td>
<td>3,9</td>
<td>160</td>
<td>2,36</td>
<td>0,90</td>
<td>0,004</td>
</tr>
<tr>
<td>+51 mEq/kg</td>
<td>14 d</td>
<td>8,26</td>
<td>230</td>
<td>90</td>
<td>4,8</td>
<td>135</td>
<td>2,34</td>
<td>1,39</td>
<td>0,010</td>
</tr>
<tr>
<td> </td>
<td>7 d a.p.</td>
<td>8,25</td>
<td>217</td>
<td>85</td>
<td>4,1</td>
<td>129</td>
<td>2,30</td>
<td>1,11</td>
<td>0,006</td>
</tr>
<tr>
<td> </td>
<td>1 d p.p.</td>
<td>8,41</td>
<td>252</td>
<td>64</td>
<td>37,6</td>
<td>151</td>
<td>1,71</td>
<td>1,06</td>
<td>0,008</td>
</tr>
<tr>
<td> </td>
<td>2 d p.p.</td>
<td>8,35</td>
<td>261</td>
<td>78</td>
<td>7,6</td>
<td>176</td>
<td>1,94</td>
<td>0,34</td>
<td>0,003</td>
</tr>
<tr>
<td>TG 3  </td>
<td>21 d</td>
<td>8,40</td>
<td>335</td>
<td>86</td>
<td>3,7</td>
<td>245</td>
<td>2,35</td>
<td>1,08</td>
<td>0,006</td>
</tr>
<tr>
<td>-60 mEq/kg</td>
<td>14 d</td>
<td>8,46</td>
<td>304</td>
<td>89</td>
<td>3,5</td>
<td>211</td>
<td>2,34</td>
<td>2,21</td>
<td>0,010</td>
</tr>
<tr>
<td> </td>
<td>7 d a.p.</td>
<td>8,30</td>
<td>249</td>
<td>89</td>
<td>8,8</td>
<td>151</td>
<td>2,43</td>
<td>2,86</td>
<td>0,020</td>
</tr>
<tr>
<td> </td>
<td>1 d p.p.</td>
<td>8,47</td>
<td>279</td>
<td>81</td>
<td>29,1</td>
<td>169</td>
<td>1,64</td>
<td>0,45</td>
<td>0,003</td>
</tr>
<tr>
<td> </td>
<td>2 d p.p.</td>
<td>8,40</td>
<td>283</td>
<td>87</td>
<td>18,0</td>
<td>178</td>
<td>1,70</td>
<td>0,43</td>
<td>0,003</td>
</tr>
</tbody>
</table>

Table 5: Results of the Post-hoc-Test showing the statistical significance between the four treatment groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Urinary pH</th>
<th>NABE</th>
<th>Base</th>
<th>Acids</th>
<th>Ca serum</th>
<th>Urinary Ca</th>
<th>FE_Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG1</td>
<td>0,031</td>
<td>0,079</td>
<td>0,323</td>
<td>1,000</td>
<td>1,000</td>
<td>0,770</td>
<td>0,856</td>
</tr>
<tr>
<td>TG2</td>
<td>1,000</td>
<td>0,007</td>
<td>0,011</td>
<td>0,790</td>
<td>1,000</td>
<td>1,000</td>
<td>0,255</td>
</tr>
<tr>
<td>TG3</td>
<td>1,000</td>
<td>0,056</td>
<td>0,132</td>
<td>0,677</td>
<td>1,000</td>
<td>0,167</td>
<td>0,045</td>
</tr>
<tr>
<td>TG1</td>
<td>0,401</td>
<td>1,000</td>
<td>1,000</td>
<td>0,285</td>
<td>1,000</td>
<td>1,000</td>
<td>1,000</td>
</tr>
<tr>
<td>TG3</td>
<td>0,226</td>
<td>1,000</td>
<td>1,000</td>
<td>1,000</td>
<td>1,000</td>
<td>1,000</td>
<td>1,000</td>
</tr>
<tr>
<td>TG2</td>
<td>1,000</td>
<td>1,000</td>
<td>1,000</td>
<td>0,019</td>
<td>1,000</td>
<td>1,000</td>
<td>1,000</td>
</tr>
</tbody>
</table>
5. Discussion

The prevention of hypocalcaemia should be a major goal for dairy farming. Hypocalcaemia is essentially caused by metabolic alkalosis in the cow induced by high potassium diets. The higher blood pH interferes with the action of parathyroid hormone on its target tissues in the bone and kidney. As a result bone calcium is not resorbed and 1, 25-dihydroxyvitamin D\textsubscript{3} is not produced. Therefore the cow cannot restore blood Ca to normal levels. To prevent hypocalcaemia, it is necessary to reduce diet cations - in particular potassium and to increase diet anions - particularly chloride and to a lesser extent sulfate. This will induce a compensated metabolic acidosis in the cow restoring the ability of parathyroid hormone to regulate blood calcium levels (GOFF, 2008).

5.1. Palatability

According to the results of this experiment a reduction of feed intake, or selection of the concentrates was found in 2 cows from group 2. One of them developed milk fever just after parturition. 3 cows from group 3 and 2 cows from group 4 were also recorded. The result was a decrease of the amount of anionic salt consumed. These results disagree with the work of OETZEL et al. (1991). They screened calcium sulphate and 5 other anionic salts for their potential use in preventing parturient paresis. All six salts were palatable, and no significant depression of DM intake was detectable. On the other hand, the results of the present work agree with the findings of other studies by OETZEL and BARMORE (1993). Anionic salts are added to a TMR are incorporated into a grain pellet. OETZEL and BARMORE (1993) found that some anionic salts reduced the concentrate intakes of cows by 50%. HORST et al. (1997) stated that the palatability becomes a problem when high amounts of anionic salts are added to diets with a DCAD >25 mEq/100g. GANT et al. (1998) found potential health problems, associated with long-term administration of anionic salts. (GANT et al., 1998). The addition of traditional anionic salts to prepartum diets may reduce palatability, which sometimes results in reduced feed intake prior to calving (GOFF and HORST, 1998).
Depressed feed intake in low, or negative DCAD diet, may be due to unpalatability of the anionic salt supplemented diets or metabolic acidosis resulting from an increase of dietary anions (HU and MURPHY, 2004).

Due to the high content of K in most roughage, very large quantities of supplemental anions will be needed to reduce the DCAD significantly, which may reduce the palatability of the concentrate (PEHRSON et al., 1999). The DMI prepartum has been reported to be reduced by inclusion of anionic salts to the diet (OETZEL and BARMORE, 1993; JOYCE et al., 1997; VAGNONI and OETZEL, 1998). The anionic salts are acidogenic and their addition to the diet of prepartum dairy cows may depress voluntary feed intake. It was evident that the most acidogenic mixtures caused the highest depression of voluntary intake in a comparison among several anion sources (VAGNONI and OETZEL, 1998; HU et al., 2007). Salts reduce palatability. Cows preferred the diets treated with acid versus the diet containing salt (GOFF, 1996). A different explanation for the reduction of DMI, may be the metabolic acidosis, induced by anionic salts supplemented feed (VAGNONI and OETZEL, 1998). TUCKER et al. (1988) and WEST et al. (1991), (1992) indicated that acidosis reduced the feed intake of steers.

Decrease in DMI occurs in most pregnant cows during the final week of gestation (~ 30% or greater) (BERTICS et al., 1992; DRACKLEY, 1999). This decrease in nutrient intake is associated with changes of the metabolic and hormonal status (DRACKLEY, 1999; DRACKLEY and DANN, 2005). The changes triggered by reduced DMI result in increased mobilization of adipose tissue lipids and decreased availabilities of volatile fatty acids, glucose and acetic acid (GULAY et al., 2007).

All three groups, receiving calcium sulphate supplement, revealed feed refusal values well below 25%. In the present trial several factors deviated from current literature, such as a higher level of anionic salt supplementation per cow and the type of calcium sulphate formulation used. The anionic salt was administered in powder form mixed by hand with concentrate. This gave the cows the chance to select the concentrates and leave the salt. In the present study the metabolic acidosis may be not the cause of reduction of the DMI. The lower basic ration palatability may have been the case in the previous experiment.

The problem of formulation and palatability was solved by piling or mixing the anionic salt with palatable solution (WU et al., 2008). DMI prepartum was not significantly affected by the DCAD treatments in the present study. This is possible, because the
offered anionic salts were pelletized and mixed with molasses, distillers and dried grains, masking the unpalatable taste of anionic salts. Pilling the Cl (and SO₄) salts reduces the unpleasant taste of the salts. In these experiences hydrochloric acid has been proved to be the most palatable source of anions. Hydrochloric acid can be extremely dangerous to handle when it is procured as a liquid concentrate. Several North American companies now manufacture hydrochloric acid based anion supplements, which are safe to handle (GOFF, 2008).

5.2. Urine pH

In the present study the mean value of urine pH did not change during the late gestation period, at parturition or the next two days after parturition in the control group. After feeding the anionic salt a marked decrease of urine pH occurred in the other three groups in the late gestation period. At the day of parturition the mean urine pH values reached the lowest value. In the next two days after parturition the urine pH was increased in all groups, the urine pH of the 4 groups was significantly different (p < 0.05). The difference was only prominent between TG 1 and TG 2 (p = 0.03).

These results agree with the study of GOFF et al. (2004). Urine pH is easily measured and has proven useful in the field to adjust dietary cation-anion difference (DCAD). However, it does not always accurately assess the degree of acidosis induced by chloride or sulfate addition to the diet. For routine monitoring of the dry cow, the ease of measuring urine pH, more than makes up for its inaccuracy.

Also in agreement with the results of this study, SEIFI et al. (2004) reported high urine pH in normal cows (>8.0). A decrease from the normal pH values from 8.0 to 7.4 indicates an increase in dietary acidity. The optimal pH in the urine for the prevention of milk fever has not been clearly defined. JARDON (1995) considered that a pH of 6 to 7 was optimal, whereas HORST et al. (1997) proposed 5.5 to 6.2. HORST et al. (1997) also considered that a pH <5.5 should be avoided because it might indicate that the metabolic acidosis is close to being uncompensated. Urinary pH of 6–7 was optimal for Holstein cattle and a pH of 5.5–6.5 was optimal for Jersey cattle to indicate metabolic acidosis. CHARBONNEAU et al. (2006) concluded that a urinary pH of 7.0, regardless of breed, may be more appropriate (DEGARIS and LEAN, 2009).
The kidney can efficiently eliminate excess anions from the blood, thus addition of anionic salts induces a sharp reduction in urinary pH. This was associated with a mild metabolic acidosis (JOYCE et al., 1997; PEHRSON et al., 1999). Similar results have been observed in previous studies for dairy cows (VAGNONI and OETZEL, 1998; MOORE et al., 2000; LIESEGANG et al., 2007) and buffalos (SHAHZAD et al., 2008). On the other hand, DCAD has been proven to be associated with fluid acid–base balance. SPANGHERO (2004) found a strong relationship between DCAD and urinary pH in support of CHARBONNEAU et al. (2006). The established weak association between DCAD and blood pH may be due to the difference in buffering capability between the blood and urine. Urinary pH, due to its high sensitivity and ease of assessment on the farm, may be a simple and efficient monitor of the acid–base balance in extracellular fluids (WU et al., 2008).

In conclusion, the use of anionic salts results in a decrease of urine pH, the unexpected value of urine pH in this study may be due to the use of high amounts of anionic salt to lower the DCAD of the ration, while the normal hay used contain very high amounts of K. The high amount of anionic salts decrease food intake, hence the cows received less amount of anionic salt.

A possible explanation was an incorrect mixing of the anionic salt containing premix, an inconsistent intake of feed, because of the cows being able to separate the premix from the other feed components.

Urinary pH was very indicative of changes in the acid–base status of dairy cows with DCAD, especially when DCAD was low or negative (VAGNONI and OETZEL, 1998; HU and MURPHY, 2004).

GOFF and HORST (1997) proved urinary pH to be an easy and sensitive mean of monitoring the acid–base status of cows, shortly before calving. Urine pH has the advantage of being more stable and less expensive than blood gas and pH analysis. Urine pH may also prove more sensitive than blood pH, because blood pH was unable to distinguish between cows, fed the 2.1 and 3.1% K diets.

The pH of urine generally reflects the acid-base state of an animal, monitoring the pH of urine is an inexpensive and sensitive method to monitor the effect of the diet on the pH of blood and assess the risk of milk fever (GOFF and HORST., 1998).

Blood pH, theoretically less able to discern the effects of diet on metabolic alkalosis and acidosis, is more commonly measured. Urine pH is easily measured and has proven useful in the field to adjust the dietary cation-anion difference
However, it does not always accurately assess the degree of acidosis induced by chloride or sulfate addition to the diet. For routine monitoring of the dry cow, the ease with which urine pH can be measured more than makes up for its inaccuracy (GOFF et al., 2004).

Commonly, urine pH is considered an adequate parameter for monitoring the anionic salt intake of cows (OETZEL, 2002).

The urinary pH is an effective indicator of the extracellular fluid acid–base balance, and multiparous Holstein cows in late gestation may benefit from consuming negative DCAD diet, for blood calcium homeostasis and improvement of the health status (WU et al., 2008).

5.3 Base concentration

Before feeding the anionic salt the urine base concentration was within the normal range. The mean value of urine base concentration during the late gestation period and at the day of parturition was mildly decreased but in next two days the urine base concentration increased to reach the same range as before calving.

After feeding the anionic salt a marked decrease in urine base concentration in the other three groups occurred during the late gestation period. At the day of parturition the mean urine base concentration values reached the lowest value. In the next two days after parturition the urine base concentration increased in all groups.

Urine base concentrations of the 4 groups were significantly different ($p < 0.05$). The difference was only prominent between the control group and TG 2.

The results of the present experiments agree with the research of GELFERT et al. (2006). The consequence of adding anionic salt was a decrease of base concentrations, but the reduction was significant from day 2 only.

Lowered DCAD has been associated with a compensated metabolic acidosis, evidenced by reduced plasma bicarbonate (GOFF and HORST, 1997; JOYCE et al., 1997; PEHRSON et al., 1999), lower urinary pH (MOORE et al., 2000), and higher urinary net acid excretion (WANG and BEEDE, 1992; VAGNONI and OETZEL, 1998).

5.4. Acid concentration

Before feeding the anionic salt the urine acid concentration was within the normal range. The mean value of the urine acid concentration during the late
gestation period and at the day of parturition was mildly decreased in the control group but in the next two days the urine acid concentration increased to the same range as before calving.

After feeding the anionic salt a marked increase in urine acid concentration in the other three groups during the late gestation period was found. At the day of parturition the mean urine acid concentration values reached the lowest value in TG 2 and were also low to some extent in TG 1 but remained high in TG 3. In the next two days after parturition the urine acid concentration increased to normal ranges in all groups.

The urine acid concentrations of the 4 groups were significantly different (p < 0.05). The difference was only prominent between TG 2 and TG 3.

5.5. Ammonium NH₄

Before feeding the anionic salt the urine ammonium concentration was within the normal range in the four groups. The mean value of the urine ammonium concentration severely increased at the day of parturition in the CG, but returned to the range prior to calving within the next two days.

After feeding the anionic salt a mild increase of the urine ammonium concentration was present in TG 1 during the late gestation period. At the day and in the next two days of parturition the mean urine ammonium concentration value did not change in TG 1. In TG 2 the mean urine ammonium value did not change during the gestation period however the urine ammonium value increased at the day of parturition and the next day, then at the second day of parturition it reached the normal value. In TG 3 the mean urine ammonium value did not change during the late gestation period, but a high increase at the day of parturition and the next two days was present.

Current literature on urine ammonium concentration did not reveal enough basis for discussion.

5.6. Net Acid Base Excretion (NABE)

Before feeding the anionic salt the net acid base excretion was within the normal range. The mean value of urine net acid base excretion in CG during the late gestation period did not change. At the day of parturition a decrease in net acid base
excretion was found but in the next two days the urine base concentration increased to reach the same range as before calving.

After supplying the three testgroups with the anionic salt, a marked decrease of the urine net acid base excretion was observed during the late gestation period. At the day of parturition the mean net acid base excretion values reached the lowest value. In the next two days after parturition the urine base concentration was increased in all groups but without reaching the value of the control group. The net acid base excretion of the 4 groups was significantly different \((p < 0.05)\). The difference however was only marked between CG and TG 2 \((p = 0.007)\).

The results of this study are in accordance with the results of current literature. NABE reflects the ratio of titratable bases and acids in the urine. In the case of an acidosis, NABE decreases and the ammonium concentration increases (BENDER et al., 2003; GELFERT et al., 2006). Urinary pH decreases when the buffer capacity of the kidneys is depleted, which may serve as a field test to monitor the use of anionic salt in transition cows’ feeding (GOFF and HORST, 1997; GOFF et al., 2004).

A negative NABE is an indicator for a non-compensated acidosis (BENDER et al., 2003). In that case, the risk of a breakdown of the compensating capacity which may result in clinical acidosis, is increased. The cows stop eating (GELFERT et al., 2006), which leads to a severe negative energy balance, followed by an increased risk of ketosis and fatty liver.

GELFERT et al. (2007) examined the impact of anionic salts on ABS (acid-base status) depending on the varying DCAD. They found that metabolic acidosis was detectable when DCAD was still positive. Anionic salts induce metabolic acidosis regardless the reached DCAD.

5.7. Calcium

Feed, containing anionic salts and still revealing a positive DCAD, induces a metabolic acidosis and activates calcium mobilization, as an effect of a compensation mechanism (BENDER et al., 2003). The results of such field studies have to deal with fluctuations in the composition of feed and feed intake of cows (GOFF et al., 2004) and reliable results are dependent of optimal feeding management (HUSBAND et al., 2002). Unnoticeable acidogenic effects would result in similar effects regarding urine composition (OWENS et al., 1998). Clinical acidosis occurs
when the dosage of anionic salts is too high, or the resulting DCAD is too low (GELFERT et al., 2006). A certain level of acidosis is necessary to activate the calcium metabolism.

5.7.1. Calcium concentration in serum

After feeding the anionic salt, no changes in mean values of serum calcium concentration during the gestation period were noticed between the different TG. At the day of parturition calcium levels decreased remarkably in all groups.

The results disagree with the research of BLOCK (1994) and TUCKER et al. (1991). In response to the metabolic acidosis, the authors found slight and significant increases of serum concentrations. Significant changes in total calcium concentrations were only observed in studies on pregnant cows (BLOCK, 1984; OETZEL et al., 1988; MOORE et al., 2000). TAKAGI and BLOCK (1991) obtained similar results of calcium concentrations by simulating a greater calcium loss by an EDTA infusion.

The nadir of plasma Ca observed on the day of calving is due to the highly increased demand of blood Ca for colostrum production (KUME et al., 2003). The declined degree of plasma Ca was lower in TG 2, when the cows consumed 1000 g of calcium sulfate. Similar findings were reported by CHARBONNEAU et al. (2006) and LEAN et al. (2006).

The findings of the present study agree with studies by other authors. They did not find changes in serum calcium concentrations (TAKAGI and BLOCK, 1991; TUCKER et al., 1992; LEITE et al., 2003; VAGNONI and OETZEL, 1998). As the calcium metabolism is strictly controlled by hormones (HARTMANN and BANDT, 2000; MARTENS, 1995), it might depend on the time the check for alterations of calcium concentrations is carried out, whether the findings are statistically provable. GELFERT et al. (2006) monitored the impact of anionic salts in a similar study. According to their results, DCAD must be lower than 160 mEq/kg DM to affect ABB (acid-base balance) and calcium mobilization sufficiently (GELFERT et al., 2007).

5.7.2. Urinary calcium concentration

After feeding the anionic salt a marked increase in mean values of urinary calcium concentration in the three TG during the late gestation period was visible.
However, there were no significant differences between the treatment groups and the CG.

The results of the present study disagree with the findings of ROCHE et al. (2002), who reduced DCAD from +400 to +350 mEq/kg DM in linearly increased (P < 0.05) Ca/Creat. This may be an indicator for increased intestinal absorption, bone resorption or a reduced renal reabsorption, despite unaffected plasma Ca concentration. Such an effect was unexpected because such a small decrease in DCAD did not affect the systemic pH (as measured by urine pH) and would not be expected to influence Ca homeostasis (ROCHE, 1999; UNDERWOOD and SUTTLE, 1999; GOFF, 2000). In the same experiment ROCHE et al. (2002) found larger changes in DCAD without significant changes of Ca/Creat, even though there was a linear decline of urine pH.

In other studies calcium concentrations in urine increased and differed significantly, beyond the feeding of anionic salts. The increase of calcium concentrations in urine is described by FREEDEN et al. (1998), VAGNONI and OETZEL (1998), BENDER et al. (2003) and ROCHE et al. (2003). In some studies the increase of calcium excretion is found to be a result of the oversupply of feeding lime, to fulfill the need of calcium when anionic salts are fed (BYERS, 1994; BEENING, 1998). The oversupply of calcium increases the rate of passive absorption in the intestine, resulting in an oversupply of calcium in the blood, which is regulated by the kidney (WANG and BEEDE, 1992; HARTMANN and BANDT, 2000).

SHAHZAD et al. (2008) increased urinary Ca excretion in buffaloes by feeding a diet with -11 DCAD concentration. This may be due to the slight metabolic acidosis that not only increased intestinal Ca absorption (SCHONEWILLE et al., 1994; ROCHE et al., 2003) but also Ca resorption from the bones due to an increased synthesis of 1,25(OH)2D3 (GOFF et al., 1991). The acidosis maintains a high Ca flux through the exchangeable pool without affecting the pool size (FREDEEN et al., 1988). Reduced urinary Ca excretion with increased concentrations of DCAD may be due to the gradual vanishing effect of metabolic acidosis.

5.7.3. Fractional excretion of calcium

After feeding the anionic salt, no changes in mean values of fractional excretion of calcium occurred in TG 2 and TG3 during the late gestation period. At the day of parturition the mean fractional excretion of calcium values were low in TG2
and TG3. The drop of the fractional excretion of calcium continues to be found in the next two days after calving in TG2 and TG3. The mean value of fractional calcium excretion in TG3 markedly increased during the late gestation period, however started to drop at the day of parturition and remained low for the following 2 days.

The results of this study agree with studies by WANG and BEEDE (1992), who researched the effects of supplementation of ammonium chloride and sulfate on the fractional excretion of Ca. GELFERT et al. (2008) found that fractional excretion increased by adding anionic salts (CaCl₂, CaSO₄). However the increase of Ca excretion was greater in cows supplied with CaCl₂ in regard to CaSO₄ (BLOCK, 1994; WON et al., 1996; KUROSAKI et al., 2007)

OETZEL et al. (1991) found a high fractional excretion of Ca by adding six different anionic salts to the diet.

5.8. DCAD

5.8.1. DCAD Calculation

In the present study the following equation (Na + K) – (Cl + S) was used for calculation of DCAD. This equation may not be the proper equation for the calculation of the DCAD especially when using calcium sulfate in very high concentrations, to lower the value of DCAD of high potassium fed.

DCAD has been defined as (Na + K) – (Cl + S), an equation originally proposed by ENDER et al. (1962). This equation has been evaluated as the most relevant by LECLERC and BLOCK (1989) and TUCKER et al. (1992). Since then numerous equations have been published for the calculation of DCAD in dairy cattle diets. These longer equations were

(Na + K + 0.38 Ca + 0.30 Mg) – (Cl + 0.6 S + 0.5 P) (HORST and GOFF, 1997).\n
(Na + K + 0.15 Ca + 0.15 Mg) – (Cl + 0.2 S + 0.3 P) (HORST and GOFF, 1997).

(Na + K + 0.15 Ca + 0.15 Mg) – (Cl + 0.6 S + 0.5 P) (National Research Council, 2001).

(Na + K) – (Cl + 0.6 S) (GOFF et al., 2004).

Other researchers reported that the most appropriate form of the DCAD equation was (Na + K) + (Cl + 0.6 S). On the basis that this equation a prediction of
both milk fever risk and urine pH could be made (PEHRSON et al., 1999). According to the results of GOFF et al. (2004), sulfate is between 55 and 60% as effective as chloride at changing blood pH and SBE (standard base excess), confirming the study of TUCKER et al. (1991).

LEAN et al. (2006) said that \((\text{Na} + \text{K}) + (\text{Cl} + \text{S})\) was the best formula to predict the milk fever risk, based on the simplified strong ion model and the metaanalyses performed by the authors. Goff (2008) stated in his review that DCAD equations provide a theoretical basis for dietary manipulation of the acid–base status. They are not necessary for the formulation of mineral content of prepartum dairy cow rations, because, with the exception of K and Cl, the rate of inclusion of the other macrominerals may be set at fixed rates. In dry dairy cows fed diets, in which S was always supplemented, the DCAD equation containing Na, K and Cl was more predictive of urinary pH, than the DCAD equation including S (SPANGHERO, 2004).

The use of the DCAD5 equation \([(\text{Na} + \text{K}) - (\text{Cl} + 0.6 \text{ S})]\) has proven to be most efficient in decreasing the urinary pH and preventing clinical milk fever, since it was most highly correlated to clinical milk fever incidence and urinary pH (CHARBONNEAU et al., 2006).

The debate of the right formula of DCAD is still going on and more research is necessary.

5.8.2 Ranges of DCAD

According to the results of recent studies, a sufficient activation of the calcium metabolism occurs when DCAD is still positive but below 160 mEq/kg DM (ROCHE et al., 2003; GELFERT et al., 2007; KUROSAMI et al., 2007; ANICK et al., 1995).

HERON et al. (2009) suggested that the DCAD needs to be \(-5.0\) to \(-10.0\) mEq per 100 g of DM to prevent hypocalcaemia (HORST et al., 1997), but diets with low but positive DCAD may also improve Ca homeostasis around parturition (KUROSAMI et al., 2007; PENNER et al., 2008). The optimal DCAD, that accounts for both reducing the prevalence and severity of hypocalcaemia and minimizing the depression of DMI, has not yet been established. The use of low-DCAD forages instead of anionic salts does not drastically decrease the DCAD in prepartum diets. Thus, it is of interest to determine the extent to which DCAD needs to be reduced to exert physiological responses to prevent hypocalcaemia.
Other authors found that DCADs close to or below 0 mEq/kg DM are necessary for an optimal prophylactic effect against milk fever (PEHRSON et al., 1999; GANT et al., 1998). Roche et al. (2003) identified a threshold DCAD of +15 mEq/100 g, below which the blood pH would be reduced and the Ca absorption would increase.

MOORE et al. (2000) stated that adding anionic salts to the feed must result in a DCAD below zero, to lower the incidence of parturient paresis. The reduction of the urine pH to 7.3 was not sufficient according to their results. TAKAGI and BLOCK (1991) found that an active reabsorption of calcium in the intestine did not occur until DCAD was below zero.

Feeding a diet with DCAD of -209 mEq/kg DM did not result in severe clinical acidosis. If DCAD decreases below this value, animals individually react with signs of clinical acidosis, refusal of feed and bradycardia. Changes in urinary pH and NABE clearly reflect the acid load of the organism and the compensating work of the kidney (GELFERT et al., 2006).

5.9. Anionic salt

The anionic salt used in these experiments was calcium sulfate in powder form.

5.9.1. Sulfate anions potent and toxicity

There were no cases of clinical health problems, due to using calcium sulfate at very high doses. The results of present experiments agree with the findings of SPANGHERO (2004).

Dietary sulfate anions are less potent acidifiers of the blood, than dietary chloride anions, as there is some blockade of sulfate absorption at higher doses, while the chloride absorption continues unabated. Another possibility is that sulfate is cleared from the blood faster than chloride, especially at higher blood levels (GOFF et al., 2004).

Some authors (OETZEL et al., 1988) used salts containing Cl and S but not Mg (ammonium-based salts) to reduce DCAD to -70 mEq/kg, resulting in 13% less hypocalcaemia than measured in salts based on Mg, such as MgCl₂ and MgSO₄ (assuming a 100% absorption and a common acidity for both Cl and S). This would result in a final DCAD of approximately +140 to +200 mEq/kg DM.
Other authors reported that sulfur and sulfate are potentially toxic because they can be reduced to hydrogen sulfide in the rumen, a potent neurotoxin (GOULD et al., 1991). Therefore the amount of sulfate added to the diet must be limited. The current maximum tolerable limit for dietary sulfur in cattle is thought to be 0.4% of the diet DM (National Research Council, 2001). Since low doses of sulfate, coming from CaSO4 and H2SO4 appear to be equipotent to low doses of chloride sources, adding small amounts of these salts would be useful - as long as the inclusion does not result in a total sulfur content above 0.4% (GOFF et al., 2004).

ROCHE (1999) reported no decrease in either blood or urine pH and no increase in urine Ca concentrations (an indicator of increased Ca absorption) unless DCAD was -150 mEq/ kg DM. GOFF (2000) recommended a similar value (-50 mEq/kg DM). Although the DCAD was reduced in these experiments by supplementing cows with MgCl2 and MgSO4, levels of +140 to +200 mEq/kg DM are not sufficient to reduce the systemic pH and increase Ca absorption or resorption. The lack of a difference in Ca/ Creat before and after calving supports this theory as well as the lack of a difference of the urine pH in cows supplemented with either MgSO4 or MgO. The difference of the urine pH between cows supplemented with MgCl2 and MgSO4, indicating less an effect of MgSO4 on acid base balance, also suggests an effect of treatment unrelated to DCAD. The extent of the decline of urine pH (7.82 to 7.41) in cows receiving MgCl2 prior to calving is too small to suggest a change in blood pH (ROCHE, 1999; GOFF, 2000), further supporting the idea that a reduction in blood pH, by reducing DCAD was not the reason for the improved periparturient Ca homeostasis observed in this experiment.

WU et al. (2008) found, feeding anionic salt diets of -50 and -150 DCAD reduced the risk of retained placenta when compared to the +150 DCAD diet. Reduced retained placenta was accompanied by higher plasma Ca. Hypocalcaemia has been shown to blunt theca signals within the immune cells (KIMURA et al., 2006). Maintaining the blood Ca homeostasis may improve the strong tone of the uterus and enhance the muscle motility, or improve the cows' immune response (KIMURA et al., 2002) to expel the retained placenta. Several studies show, that reducing DCAD preparrum has no adverse effect on hepatic (SPANGHERO, 2002; GIULIO et al., 2005) and kidney function (SPANGHERO, 2002). Overall, the reduced DCAD diet may improve periparturient dairy cow's health. Further larger-scale and longer-term trials are needed to confirm the data presented here.
RAMBERG et al. (1996) suggested several mechanisms by which sulfur could reduce the risk of milk fever, including an acidogenic effect, if gastrointestinal absorption of \( \text{SO}_4^{2-} \) was preferential over \( \text{Mg}^{2+} \) and a possible laxative effect of magnesium sulfate that might increase bicarbonate losses in the faeces. It will be difficult to determine whether any such physiological actions of sulfur, apart from a role in DCAD, influence the risk of milk fever independently of the role in DCAD (LEAN et al., 2006).

MCNEILL et al. (2002) reported reduced hypocalcaemia, when anionic salts have not been supplemented in sufficient quantities to change systemic pH.

5.9.2. Amount of anionic salt

In the present study 3 different doses of calcium sulfate were used: TG 1 received 800 g of the anionic salt daily, TG 2 received 1000 g daily and TG 3 received 1200 g daily.

The amount of the anionic salts needed depends on the concentration of potassium in the diet, which is the major component in the formula of the dietary cation anion difference DCAD. This is especially true in Middle-European regions, where grass silages are a major component of dairy cow diets. Other cations like sodium, calcium and magnesium have only a minor role due to their lower concentrations. In case of a high concentration of potassium in the diet the amount of anionic salt has to be much higher than the recommended dose of 2000-3000 mEq/day (GELFERT et al., 2006).

5.10. Conclusions

The failures in the present experiment may be due to mistakes in feeding management. Feeding anionic salts (in powder form) is difficult, as the cows are administered the diet and the anionic salts must be administered additionally and mixed by hand. This gives the cows the chance to select the concentrates and leave the anionic salt.

1. The use of large amounts of calcium sulfate to lower the value of DCAD proved to be harmless to the health state of the animals. However, the high amount of anionic salt lead to increases of the total DM given to the animal
to more than 10 Kg DM/day/cow, in addition to the normal physiological
decrease of feed intake at the last week before parturition.

2. According to results of the present study, no improvement of the acid-base-
balance and calcium metabolism could be demonstrated by the influence of
calcium sulfate, as a new tasteless anionic salt, on the acid-base-balance
and the calcium metabolism in dairy cows, consuming a feed ration with
high concentration of potassium.

Feed rations with a reduced but still positive DCAD did not activate the
calcium metabolism.

An increase of the amount of calcium sulfate, to obtain the desired negative
DCAD, leads to a marked reduction in feed intake.
6. Summary

Hypocalcaemia in dairy cows is still one of the most frequent metabolic diseases in the peripartum period. In the present study we tried to prevent parturient paresis by using calcium sulfate. The natural calcium sulfate was used in dairy cow's diets with a high potassium content of 3.51 K/ DM. 40 dairy cows with at least three lactations were randomly distributed to four study groups: TG1: control (610 mEq/kg DM) +163 mEq/kg DM, 800 g calcium sulfate; TG2: +51 mEq/kg DM, 1000 g calcium sulfate and TG3: -60 mEq/kg DM, 1200 g calcium sulfate.

The daily feed of the treatment groups and control group consisted of alfalfa silage 8 kg, grass silage 7 kg, hay 4 kg and concentrate 1.5 kg. Twice a day calcium sulfate was offered. Blood samples (serum) and urine samples were taken at days: -21, -14, -7, of calving, +1, +2. The serum calcium, creatinine and urine calcium, creatinine, pH, net acid-base excretion (NABE) and renal fractional excretion of calcium were measured or calculated. All parameters were recorded within the physiological ranges.

The results of the present study showed that the use of large amounts of calcium sulfate to lower the value of DCAD proved to be harmless to the health status of the animals.

The results of the present study showed that the use of high amount of calcium sulfate supplementation ration was a direct reason to a clear decrease in ration dry matter intake.

According to the study condition, there was no sufficient effect of calcium sulfate to prevent milk fever and recumbancy in dairy cows, consuming a feed ration with a high concentration of potassium. It is not possible to increase the amount of calcium sulfate to obtain the desired negative DCAD without reducing the feed intake of cows. Further studies are needed to investigate the antihypocalcaemic effects of acidic salts in high potassium dairy rations.
7. Zusammenfassung

Die Hypokalzämie ist bei Milchkühen eine der häufigsten und wirtschaftlich schwer wiegendsten Stoffwechselrkrankungen im peripartalen Zeitraum. In der vorliegenden Studie wurde untersucht, ob Kalziumsulfat in Rationen mit hohen Kaliumgehalten von 3.51 g/dry TM eingesetzt werden kann, um die Gebärdparese zu verhindern. 40 hochtragende und nicht laktierende Milchkühe mit mindestens drei Laktationen wurden auf vier Gruppen nach dem Zufallsprinzip verteilt. Die Kühe der Kontrollgruppe (CG) erhielten die Grundration bestehend aus 8 kg Luzerne silage, 7 kg Grassilage, 4 kg Heu und 1,5 kg eines kommerziellen Kraftfutters. Die Kühe der drei Versuchsgruppen erhielten unterschiedliche Mengen in Form von Kalziumsulfat. Dabei ergaben sich folgende Werte für die Dietary Cation-Anion Difference (DCAD): TG1: +163 mEq/kg Trockensubstanz (TS), TG2: +51 mEq/kg TS und TG3: -60 mEq/kg TS. Die zugeführte Menge Kalziumsulfat waren 800 g in TG1, 1000 g in TG2 und 1200 g in TG3.


Weitere Untersuchungen bezüglich des möglichen Einsatzes saurer Salze in Milchviehrationen mit hohen Kaliumkonzentrationen sind angezeigt.
8. References


CHANDLER, P. (1997): Milk fever may be caused by potassium and sodium, not
calcium. Feedstuffs 69, 10–23.


GAYNOR, P.J., MUeller, F.J., MILLER, J.K., RAMSEY, N., GOFF, J.P., HORST,


HARTMANN, H., BANDT, C. (2000): Pathophysiological mechanisms of metabolism of calcium (Ca) and magnesium (Mg), and the importance of renal fractional excretion (FE) for the diagnosis of electrolyte disturbances in cattle. Tierärztliche Praxis 28, 190–198.


ROCHE, J.R., DALLEY, D., MOATE, P., GRAINGER, C., RATH, M., O'MARA, F.


SATO, J., OKADA, K., SATO, R., YASUDA, J., NAITO, Y. (2002): Serum activity of tartrate-resistant acid phosphatase in cows from farms where milk fever was prevalent and in farms where it was rare. Journal of the Japan Veterinary Medical Association 55, 580-583.


SCHONEWILLE, J.T., VAN A. T., KLOOSTER T., DIRKZWAGER A., BEYENEN. A.C.


of Physiology and Pharmacology 61, 1444–1461.


YAMAGISHI, N., NAITO, Y. (1997): Calcium metabolism in hypocalcaemic cows with


