Minerals to Dairy Cows with Focus on Calcium and Magnesium Balance

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Abstract

Both clinical and subclinical deficiency of calcium and magnesium may cause problems in dairy cows. Clinical hypocalcaemia most commonly occurs at calving and onset of lactation and is associated with milk fever, while clinical hypomagnesaemia occurs under certain dietary conditions. Factors affecting the calcium and magnesium status in dairy cows were examined in this thesis.

The effect of dietary magnesium (0.19 and 0.43 % of dry matter) and potassium (1.9, 2.8, and 3.7 % of dry matter) on magnesium digestibility and magnesium balance was assessed using a Latin square design with six lactating cows. The effect of supplying dietary calcium to dry cows (0.49, 0.93, and 1.36 % of dry matter) on calcium and magnesium homeostasis was investigated in 29 periparturient cows. A case-control study was used to investigate whether mineral feeding during the last part of the dry period differed between 30 herds with high incidence of milk fever, and 22 herds with no milk fever. Finally, the effect of prepartum milking for 1 to 7 days on calcium homeostasis was investigated in 15 cows around calving.

Magnesium uptake in lactating cows was found to depend on the level of dietary magnesium, but not on the potassium concentration. Dietary calcium had no effect on hypocalcaemia at calving, and was not different between herds with high milk fever incidence and herds without milk fever. However, high levels of calcium in the diet resulted in decreased magnesium absorption. High amounts of potassium in the diet were associated with increased risk of high milk fever incidence, while high amounts of dietary magnesium were associated with decreased risk of high milk fever incidence. Prepartum milking decreased plasma calcium levels and activated the calcium homeostatic mechanisms. However, there were no differences in the degree of hypocalcaemia at calving, and plasma concentration of calcium decreased within 1 h after calving, indicating that factors other than milk removal alone were responsible for the decrease in plasma calcium levels at calving. The conclusion was that cows should be fed high amounts of magnesium and low amounts of potassium during the last part of the dry period to avoid milk fever, while the potassium concentration in the diet of lactating cows is of less importance regarding magnesium uptake. Milk removal affects calcium homeostasis, but the effect on the risk of milk fever is unclear.

Keywords: Parturient paresis, CTx, parathyroid hormone, calcium, magnesium, cow

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Our knowledge is a little island in a great ocean of nonknowledge.

Isaac Bashevis Singer

Illustration: Maria Nordqvist

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Holtenius, K., Kronqvist, C., Briland, E. and Spörndly, R. (2008). Magnesium absorption by lactating dairy cows on a grass silage-based diet supplied with different potassium and magnesium levels. *Journal of Dairy Science* 91(2), 743-748.

II Kronqvist, C., Emanuelson, U., Spörndly, R. and Holtenius, K. (2011). Effects of prepartum dietary calcium level on calcium and magnesium metabolism in periparturient dairy cows. *Journal of Dairy Science* 94(3), 1365-1373.

III Kronqvist, C., Emanuelson, U., Tråvén, M., Spörndly, R. and Holtenius, K. Relationship between incidence of milk fever and feeding of minerals during the last three weeks of gestation (submitted).

IV Kronqvist, C., Ferneborg, S., Emanuelson, U. and Holtenius, K. Effects of prepartum milking of dairy cows on calcium metabolism and colostrum quality at start of milking and at calving (manuscript).

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Abbreviations

CTx	C-terminal crosslinked telopeptide of type 1 collagen
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
NRC	National Research Council

1 Background

In animals, almost no chemical elements are present in pure form. Instead, they are parts of chemical compounds or occur in ionised form. The term mineral as used in nutrition refers to the inorganic chemical elements in the diet and animal. These are the elements that, in theory, will remain after total combustion of the material, *i.e.* all elements except for carbon, hydrogen, oxygen and nitrogen. Based on the abundance in the body, the minerals are divided into macro minerals and micro minerals, the latter sometimes referred to as trace elements. The original basis for this categorisation was the analytical methods used, providing possibilities to quantify the amount of macro minerals, while the micro minerals could only be found, not quantified (Godden, 1939). The terms macro and micro minerals have been defined differently by different authors, but the term macro minerals always includes calcium, phosphorus, magnesium, sodium, potassium, chloride and sulphur.

It has been known for several hundred years that the living body contains mineral compounds, and that they are important for normal function. However, it was not until the beginning of the 20th century that the mineral nutrition of farm animals and the importance of the ash fraction of the diet were considered (Langworthy, 1910). Earlier, the feeding of ruminants with normal diets composed of grass and supplemented with grain was assumed to provide sufficient mineral compounds, as long as the animal had access to sodium chloride. However, it must be noted that the production rate of animals in those days was much lower, especially that of animals in the Western world, where production capacity has increased enormously.

Milk is the only product of animal origin that is naturally designed for consumption, to provide the newborn offspring with the nutrients needed for growth. The composition of milk from different species differs, and is adapted to the specific requirements of the young. Minerals in the milk consumed by the mammalian offspring are important for skeletal growth and development, and milk production is associated with increased mineral requirements in the mother. Changes in breeding, feeding and management of the dairy cow have resulted in a large increase in the average milk yield. This increase in yield is associated with increased demand for all nutrients, including minerals. This has made the mineral composition of the dairy ration an important issue for the farmer, regarding animal health as well as production and farm profit.

Milk from cows is not only a source of nutrients for the calf, but also a part of the diet consumed by humans, and the minerals provided in milk constitutes thus an important part of the daily intake of minerals. According to Food and Agricultural Organization statistics from 2007, the average milk consumption in the world is more than 0.2 litres per person and day, and the average consumption in the Western world is at least twice that amount (FAO, 2011). The minerals in milk, and thus the transfer of minerals from dairy cow feed, is therefore important for human health and nutrition, and in some cultures, *e.g.* in Europe, milk products may provide adults with the majority of the calcium they require (Fardellone *et al.*, 2010).

This thesis focuses on calcium and magnesium in dairy cows, as they are two of the most abundant minerals in animals and need to be provided in relatively high amounts in the diet of lactating animals. In addition, deficiency of calcium or magnesium in dairy cows is associated with specific diseases, and causes animal welfare problems and also financial losses for the farmer.

2 Introduction

Calcium is the most abundant mineral in the body, with 99 % of that present being found in the bones. There, together with phosphorus, it forms hydroxyapatite, which constitutes an important part of the skeletal structure of vertebrates. The remaining calcium is mainly extracellular, where it is important for normal muscle contractions and nerve function. The calcium in the bones is not a static mass, but is in continuous exchange with the extracellular fluids. The intracellular calcium fraction is involved in signalling within the cell. In plasma, calcium is found as protein-bound calcium, calcium bound in complexes with anions and ionised calcium. The fraction of ionised calcium must be maintained at a steady level to ensure the correct function of muscles and nerves.

Magnesium is the second most abundant intracellular cation, even though, as with calcium, the majority of the magnesium in the body is bound in the bones. Intracellular magnesium is important for several enzymes that regulate the metabolism, and although the extracellular concentration is only 1 % of the total magnesium in the body, magnesium plays an important role as an extracellular ion for nerve transmission. Plasma magnesium, like calcium, is also found in ionised form, protein-bound and in complexes, with the ionised magnesium being the most active form.

2.1 Factors determining mineral requirements of the dairy cow

The mineral requirements of an animal are highly dependent on its physiological state. For most dietary minerals, the current Nordic recommendations for dairy cows are based on a factorial division of the requirements for maintenance, growth, gestation and lactation (Nielsen and Volden, 2011). The maintenance requirement is made up of the inevitable losses from the gastrointestinal tract and urine, and should also cover losses from *e.g.* sweat and hair.

The additional requirement for growth, gestation and lactation is the amount of minerals incorporated in the body, placenta, foetus and foetal membranes, and milk, and these can be quantified by determining the amount of minerals in the tissues and milk. The exact requirements for maintenance are more difficult to estimate than the requirements for production, and different techniques have been used. These include extrapolation of regression lines obtained in experiments with different mineral intakes to zero mineral intake (Field *et al.*, 1958), dilution of isotopes (House and Van Campen, 1971), and feeding diets with artificially achieved low levels of minerals (Smith, 1959).

2.2 Dietary minerals

The level of minerals in forages varies according to properties of the soil, level and type of fertiliser applied to the crop, botanical composition, and maturity of the plant (Swift *et al.*, 2007). Generally, forages contain high levels of potassium, fairly high levels of calcium and lower levels of magnesium and phosphorus. In modern Swedish dairy production, a large part of the diet consists of concentrate compounds such as cereals, oil seed residues or sugar beet products, at least for high producing cows in early lactation. The mineral composition of these varies; cereals are usually rich in phosphorus, oil seed residues are rich in phosphorus and potassium, and sugar beet products are rich in calcium and potassium. In several dairy cow diets, inorganic mineral supplements are added to fulfil the dietary demands of the cow. Limestone, calcium or magnesium phosphate, sodium chloride and magnesium oxide are common sources of minerals included in concentrate mixtures and mineral supplements and, in addition, sodium chloride is often fed at appetite.

2.3 Mineral uptake

The uptake of minerals occurs along the gastrointestinal tract and often requires the mineral to be in dissolved and ionised form (Thilsing-Hansen *et al.*, 2002a; Xin *et al.*, 1989). However, some minerals can be taken up in other forms, *e.g.* sulphur is an important part of certain amino acids and may therefore be absorbed with amino acids. The site of absorption depends on the element, and several minerals are absorbed through the epithelium in different segments of the gastrointestinal tract (Khorasani *et al.*, 1997). The net absorption is affected by the secretion of minerals into the gastro-intestinal tract, which may be considerably higher than the uptake in some parts of the intestine.

The main site for calcium absorption in ruminants is assumed to be the small intestine, at least at moderate calcium intakes (Schröder and Breves, 2006). The digestibility of calcium is dependent on the diet, mainly the level of dietary calcium, and the absorption is regulated by the hormone calcitriol (Hove, 1984). Dietary phosphorus has been shown to negatively influence the uptake of calcium, and this may result from altered calcitriol production (Tanaka *et al.*, 1973). There may also be a significant net absorption of calcium prior to the abomasum (Schröder and Breves, 2006), and this occurs mainly when the calcium concentration in the rumen is high (Khorasani *et al.*, 1997).

In adult ruminants, the main site for magnesium absorption is the rumen. Magnesium is assumed to be absorbed through two different mechanisms (Martens and Schweigel, 2000). One of these is dependent on the potential difference over the rumen epithelium, and is the main transport mechanism at low magnesium concentrations. Magnesium can also be transported over the epithelium independent of the potential difference. This transport has been suggested to occur together with anions or in exchange of hydrogen ions (Schweigel *et al.*, 2000), and is mainly driven by the concentration of magnesium in the rumen fluid (Martens and Schweigel, 2000).

There are several factors affecting the rate of magnesium transportation over the epithelium. The main factor is the concentration of ionised magnesium in the rumen fluid. This concentration is dependent on the level of magnesium in the diet, but also on the solubility of magnesium in the rumen. The ruminal pH also affects the solubility of magnesium, with better solubility at lower pH. Dietary potassium has been shown to affect the uptake of magnesium in dry cows (Jittakhot *et al.*, 2004a) and lactating cows (Weiss, 2004). This has been suggested to be due to the effect of potassium on the potential difference over the membrane in the epithelial cells, and thereby increased resistance for magnesium transport at high potassium concentrations in the rumen (Schweigel *et al.*, 1999). As magnesium is transported independent of rumen concentration of potassium when rumen concentrations of magnesium are high, this inhibition of the uptake can be fully compensated for by addition of magnesium to the diet, and potassium concentration has its main importance for the passage of magnesium over the epithelium at low magnesium intakes (Care *et al.*, 1984). The inhibition of magnesium uptake by addition of potassium is also most pronounced at low initial potassium concentrations (Jittakhot *et al.*, 2004b; Leonhard-Marek and Martens, 1996). Other dietary factors, such as the amount of ammonium in the rumen, dietary starch and dietary calcium can also influence the uptake of magnesium (Schonewille *et al.*, 1997; Care *et al.*, 1984). Magnesium can also be absorbed from the small intestine to a varying extent, although there can often be net secretion of magnesium in the small intestine, especially when the concentration of magnesium in the digesta is low (Scott, 1965). In the large intestine there may be significant absorption, occurring through passive transport of magnesium over the epithelium (Ben-Ghedalia *et al.*, 1975).

2.4 Regulation of mineral homeostasis

The levels of minerals in the body, especially in the extracellular and intracellular fluids, are often of critical importance for the normal functions of the body. Thus, it is necessary to maintain adequate levels. This can be done by regulating the inflow of minerals, *e.g.* the uptake from the intestines to the plasma, or by regulating the outflow, *e.g.* the renal filtration of minerals and excretion of excess minerals with the urine.

The level of ionised calcium in plasma is carefully regulated by hormones. The main hormones maintaining the calcium levels in plasma are parathyroid hormone and calcitriol, which is the most active metabolite of vitamin D (Horst *et al.*, 1994). Depending on the level of ionised calcium in the extracellular fluid, these hormones are produced to maintain the calcium at a balanced level. The parathyroid gland, located on the surface of the thyroid gland, has as its main purpose to maintain calcium homeostasis in the extracellular fluid by releasing the protein parathyroid hormone (Fry *et al.*, 1979). Calcium-sensing receptors in the parathyroid gland register the level of ionised Ca in the blood, and if the level decreases, parathyroid hormone is secreted within minutes. The release of parathyroid hormone has been suggested to result in a shift in the equilibrium in the bone surface, resulting in rapid release of calcium from bones (Talmage and Mobley, 2008). In addition, parathyroid hormone increases the renal threshold for calcium and stimulates the production of calcitriol in the kidneys, which in turn increases the absorption of calcium in the intestine (Yamagishi *et al.*, 2006). Braithwaite and Riazuddin (1971) demonstrated in sheep that the efficiency of calcium uptake from the diet was higher when dietary calcium was low.

There are no hormonal systems that directly affect the absorption of magnesium, although aldosterone, a hormone regulating sodium metabolism, exerts an indirect influence by regulating the potassium concentration in saliva and thus in the rumen (Charlton and Armstrong, 1989). Instead, magnesium is absorbed irrespective of the requirements of the cow, and it is thus only the excretion of magnesium from the body that can be regulated. The output of magnesium through urine is thus used to maintain normomagnesaemia (Ram *et al.*, 1998), as long as the absorption is sufficient.

Some minerals are stored in body tissues, and can be released when there is an increased need or decreased availability, *e.g.* from a dietary deficiency. Bone tissues contain high amounts of minerals, and most of the magnesium, calcium and phosphorus in the body forms part of the skeleton and helps maintain the resistance to compression (Ravaglioli *et al.*, 1996). The calcium in skeletal structures is of major importance for the maintenance of a stable concentration of ionised calcium in the extracellular fluids. The osteoclasts are the cells that are mainly responsible for catabolism of collagen in bone, and thus release of the minerals that are stored in bone tissue (Blair, 1998). This catabolism is increased as a result of increased levels of parathyroid hormone, but there is no known direct action of parathyroid hormone on the osteoclasts, as they seem to lack receptors for this hormone (Fuller *et al.*, 2007). Instead, the action is suggested to be mediated by cytokines released from the osteoblasts, the cells responsible for the formation of bone, as a response to parathyroid hormone (Li *et al.*, 2007a). The importance of the tissue stores of minerals was shown by Ellenberger *et al.* (1932) in an experiment where cows lost about 1 kg of calcium during the beginning of the lactation, but this amount was fully regained during the last part of the lactation and dry period. The magnesium in bones is released when the bone is resorbed and calcium is released, but deficiency in magnesium intake does not induce bone resorption, and there are studies showing that there are no possibilities to store magnesium during periods of high supply and use it when the supply is insufficient (Ritter *et al.*, 1984). Martens and Schweigel (2000) have suggested that the absorption of magnesium must therefore meet the outflow.

2.5 Mineral outputs

When the mineral metabolism of a dairy cow is in a steady state, the mineral output corresponds to the inflow of minerals through the diet. If the mineral balance is negative, the cow excretes more minerals than are contained in intake. This is commonly the situation with calcium in the beginning of lactation, and it is compensated for by a positive balance at the end of the lactation, when some of the dietary calcium is retained in the body (Taylor *et al.*, 2009; Verdaris and Evans, 1976).

2.5.1 Faeces

A fraction of the minerals excreted via faeces comes from endogenous losses, *e.g.* from sloughed intestinal cells and gastric juices. The extent of endogenous faecal mineral excretion differs for different minerals and depends on the feed intake of the cow (Martz *et al.*, 1990). The faecal mineral output that is not of endogenous origin comes from dietary minerals that are not absorbed in the gastro-intestinal tract. For magnesium, and sometimes also for calcium, the excretion of undigested minerals via faeces constitutes the main pathway of mineral losses from the body. The extent of excretion of calcium depends on the calcium content in the diet and the requirement of the animal. If dietary calcium is limited, the production of calcitriol increases (Wilkens *et al.*, 2010), and this may result in more efficient uptake of calcium in the intestine, and thus decreased faecal excretion of calcium (Hove, 1984). The digestibility of the mineral, and thus faecal excretion, differs for different minerals (Khorasani *et al.*, 1997). The digestibility also differs among mineral sources, and *e.g.* different magnesium sources used in mineral supplements have different solubility. The solubility of magnesium oxide, which is the most used inorganic source, is variable and thus the source of magnesium oxide has a high influence on the absorption and faecal losses of magnesium (Xin *et al.*, 1989).

2.5.2 Urine

The excretion of minerals in the urine is dependent on the plasma levels of minerals and on renal filtration and reabsorption. Some minerals are almost entirely absorbed and then excreted through urine, while others are only found in low concentrations in urine from healthy cows. Due to the homeostatic control of calcium absorption, the excretion of calcium in urine is normally at or below 1 g per day, so a large decrease in calcium output through urine does not reduce the total calcium output to a large extent (Ramberg *et al.*, 1976). Nevertheless, under the influence of parathyroid hormone, this excretion is decreased to almost zero. Urine output of calcium has generally been shown to be independent of calcium in the diet (Taylor *et al.*, 2009), although Ramberg *et al.* (1976) observed a decreased calcium excretion in cows fed high levels of calcium. Calcium excretion in urine can increase markedly when cows are fed acidifying rations, due to an increased supply of calcium from the bone tissues that is released when the cow tries to buffer the excess of hydrogen ions (Bushinsky, 1989). The excretion of calcium in urine can then increase several-fold and reach levels above 10 g per day (Charbonneau *et al.*, 2008).

When magnesium is absorbed in excess, the surplus is efficiently excreted in the urine, while when the diet is deficient in magnesium, the urinary excretion of magnesium decreases. Mayland (1988) suggested that magnesium excretion in urine of more than 0.1 g per litre, equivalent to about 2.5 g per day for lactating cows indicates sufficient magnesium absorption. Low rates of excretion of magnesium in urine can be associated with low levels of plasma magnesium and, thus, increased susceptibility to clinical hypomagnesaemia (O'Kelley and Fontenot, 1969).

2.5.3 Milk

The most abundant mineral in milk is potassium, with calcium in second place. The calcium and magnesium concentration of milk varies with lactation stage and is highest soon after parturition (Tsioulpas *et al.*, 2007), but the concentration is independent of mineral intake (Taylor *et al.*, 2009). The excretion of these minerals through milk is therefore highly dependent on the milk yield. A large fraction of the calcium is secreted into milk through exocytosis in association with the casein (Neville and Peaker, 1979), and the calcium concentration shows a high correlation to the casein concentration (Cerbulis and Farrell, 1976). Magnesium is to some extent also associated with the casein micelles, and it is possible to alter the concentration of calcium and magnesium in milk by genetic selection, perhaps due to the relatively high heritability of casein concentration (van Hulzen *et al.*, 2009). The calcium concentration in milk differs among cattle breeds, with higher concentrations for Jersey cows compared with *e.g*. Holsteins (Cerbulis and Farrell, 1976).

2.6 Mineral deficiencies

2.6.1 Subclinical deficiencies

Subclinical deficiencies of minerals are by definition hard to diagnose, and the definition of a subclinical deficiency is often based on normal values of minerals in *e.g.* plasma (Goff *et al.*, 1996). The diagnosis of subclinical hypocalcaemia is usually based on plasma or serum levels of calcium, and cut-off points at total calcium levels of 2.0 mM (Reinhardt *et al.*, 2011) and 1.88 mM (Goff *et al.*, 1996) have been suggested. Such levels commonly occur in dairy cows soon after calving (Reinhardt *et al.*, 2011). It has been shown that cows with subclinical hypocalcaemia have impaired rumen function and depressed feed intake (Hansen *et al.*, 2003), and it has been suggested that subclinical hypocalcaemia at calving predisposes the cow to several other diseases, due to effects of low calcium ion concentrations on the immune functions and muscle contractions (Reinhardt *et al.*, 2011; Kimura *et al.*, 2006).

The diagnosis of subclinical hypomagnesaemia can be made by measuring total concentration of plasma magnesium, where 0.74 mM has been suggested to be a cut-off point (Mayland, 1988). However, some authors suggest that total magnesium in plasma is not always decreased at magnesium deficiency, and that the ionised magnesium level in plasma provides a more accurate measure of the magnesium status (Arnaud, 2008; Speich *et al.*, 1981). Urine sampling and the determination of magnesium concentration has been suggested as a simple way of determining magnesium status in cows (Mayland, 1988), and has been shown to be linearly related to magnesium uptake (Martens and Schweigel, 2000). The cut-off point for urinary magnesium excretion indicating sufficient magnesium supply has been set at 2.5 g per day (Guéguen *et al.*, 1989; Kemp and Geurink, 1978) or 0.1 g per litre (Mayland, 1988). In human medicine, subclinical hypomagnesaemia is associated with diabetes, metabolic syndrome and cardiovascular disease (Musso, 2009; Barbagallo and Dominguez, 2006), and may also have a negative impact on the ability to cope with hypocalcaemia, since adequate magnesium level is important for both the excretion of parathyroid hormone (Suh *et al.*, 1973) and the tissue responsiveness to parathyroid hormone (Reddy *et al.*, 1973).

2.6.2 Milk fever

Milk fever, or parturient paresis, is second only to mastitis in terms of number of veterinary treatments in Sweden (Swedish Dairy Association, 2010). The disease, which has been recognised since the 18th century, is associated with severe hypocalcaemia and occurs mainly in periparturient cows. Just prior to or after calving, the cow shows symptoms including loss of appetite, reduced body temperature and anxiety (Houe *et al.*, 2001). As the disease progresses, the cow becomes unable to rise and if no treatment is given, the majority of the afflicted cows eventually die (Hibbs, 1950). Onset of lactation is related to the development of milk fever, probably because calcium is one of the most abundant minerals in cow’s milk and the concentration of calcium in colostrum is almost double that in milk later in lactation (Tsioulpas *et al.*, 2007). The daily amount of calcium required for the foetus during the last days of pregnancy corresponds to the amount of calcium required to produce less than five litres of colostrum. Modern dairy cows, unlike their ancestors, often produce much more colostrum than that, and the first milking is usually associated with a much higher calcium loss than the calcium regulatory mechanisms of the cow can replace immediately. Adaptation to the new requirements starts immediately, with increases in parathyroid hormone and calcitriol (Penner *et al.*, 2008), but despite this, plasma concentrations of ionised calcium may decrease to a level where milk fever develops. The disease is a predisposing factor for several other periparturient diseases, such as retained placenta, ketosis and mastitis (Correa *et al.*, 1990), and may have a negative influence on milk yield (Rajala-Schultz *et al.*, 1999). Milk fever is usually accompanied by increased concentrations of plasma magnesium (Klimiene *et al.*, 2005; Larsen *et al.*, 2001). Cows suffering from milk fever usually have equal or higher levels of parathyroid hormone than healthy cows

(Horst *et al.*, 1978). The reason why some cows develop such severe hypocalcaemia and are affected by milk fever is not entirely understood. Cows of certain breeds, *e.g*. Jersey cows, and cows in high parities have an increased risk of developing milk fever (Roche and Berry, 2006), while heifers rarely contract milk fever.

The treatment for milk fever today usually consists of intravenous infusion of calcium borogluconate, which provides calcium ions and keeps the plasma calcium concentration at an adequate level until the metabolism of the cow has adjusted to the new calcium requirements. Earlier, insufflation of air into the udder was successfully used to treat milk fever, see Figure 1. Although the cause of milk fever was unknown when this treatment was invented, the reason for insufflation was to increase the pressure in the udder, thus decreasing milk secretion, as explained by Mohler (1904). This was effective because the decreased milk secretion resulted in a decrease in calcium removal from plasma. Insufflation of the udder has also recently been shown to improve the recovery from milk fever in combination with intravenous infusion of calcium (Andersen, 2003).

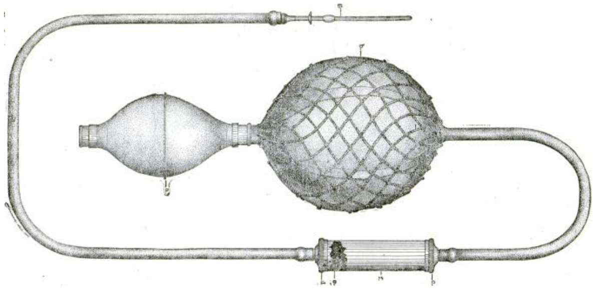

Figure 1. Equipment for insufflation of atmospheric air into the udder as a treatment for cows suffering from milk fever. From Mohler (1904).

There is high variability in milk fever incidence among herds (McLaren *et al.*, 2006), which implies that there are possibilities to affect the milk fever incidence by differences in management. Preventive measures against milk fever, such as restricted milking after calving and oral supplementation with calcium around parturition, are often applied in practice (Hansen *et al.*, 2007; Sørensen *et al.*, 2002). The levels of minerals in the diet fed to dry cows during the last part of gestation have been shown to be important for the incidence of milk fever at parturition (Lean *et al.*, 2006; Goings *et al.*, 1974). A low level of calcium during the last part of the gestation puts the cow in a calcium-saving state and facilitates the adaptation to the high calcium requirements post partum, given that the supply of calcium in the lactation ration is adequate

(Green *et al.*, 1981). The range where a decrease in calcium is efficient in preventing milk fever is not consistent among studies (Lean *et al.*, 2006; Goff and Horst, 1997; Shappell *et al.*, 1987), but Thilsing-Hansen *et al.* (2002b) reviewed a number of studies and suggested that dietary calcium had to be kept below 20 g per day to prevent milk fever. Suboptimal intake of magnesium increases the incidence of milk fever (Lean *et al.*, 2006), and this may be due to the negative effect of hypomagnesaemia on production and response to parathyroid hormone. High intakes of potassium are also associated with high incidence of milk fever (Goff and Horst, 1997), and this is explained by the impact of potassium on the acid-base balance of the cow according to the strong ion theory (Stewart, 1983). This theory suggests that when the amount of absorbed cations (*e.g.* potassium) exceeds the amount of absorbed anions (*e.g.* chloride), the cow will be shifted towards a more alkalotic state. On the other hand, when the cow absorbs more negative ions, she will be pushed towards a mild metabolic acidosis. This might have a positive effect on the tissue response to parathyroid hormone (Krapf *et al.*, 1992), and also increases the degree of calcium mobilisation from bone tissue (Bushinsky *et al.*, 2003). It has been shown that cows in a more acidotic state, which can be measured by the pH in the urine, have a decreased risk of developing milk fever (Seifi *et al.*, 2004).

2.6.3 Hypomagnesaemic tetany

Clinical hypomagnesaemia may occur when cows are grazing pasture under certain conditions (Robinson *et al.*, 1989), but may also occur at other times. The disease is known by several names, such as grass tetany, grass staggers and winter tetany, depending on the circumstances in which it occurs (Fontenot *et al.*, 1989). The condition is associated with tetany and, if severe, eventually death, caused by a decrease in the magnesium levels in the cerebrospinal fluid (McCoy *et al.*, 2001), which is the result of decreased levels of magnesium in the plasma. Experiments by Meyer and Scholz (1972, cit. Martens and Schweigel, 2000) showed that plasma magnesium levels had to decrease below 0.5 mM before the magnesium concentration in the cerebrospinal fluid was decreased, while Reynolds *et al.* (1984) found that changes in plasma magnesium above that level did not affect the concentration of magnesium in the cerebrospinal fluid. Hidiroglou *et al.* (1981) found that plasma magnesium levels could decrease far below 0.5 mM without the cows showing any clinical symptoms of hypomagnesaemic tetany. Hypomagnesaemia commonly occurs as a result of impaired magnesium absorption, and may be exacerbated if the animal is stressed, *e.g.* due to weather conditions or transportation. Magnesium transport between the extracellular and intracellular compartment is affected by stress (Ali *et al.*, 2006), and it has been shown that the hormones related to stress, *e.g.* adrenaline, decrease plasma magnesium level. Increases in insulin levels in plasma are also associated with decreases in the concentration of plasma magnesium, and the rapid change in plasma magnesium level has been suggested to be caused by movements of magnesium ions from the plasma to the cells (Miller *et al.*, 1980). Clinical hypomagnesaemia, in contrast to milk fever, also occurs in beef cows and, as it is associated with dietary factors and sometimes external stressors, it may affect several cows in a herd at the same time (Odette, 2005).

3 Aims

The overall aim of this thesis work was to increase the knowledge of calcium and magnesium in the dairy cow. Specific aims were to investigate whether:

- Addition of potassium to grass silage-based diets already high in potassium results in lower uptake of magnesium in lactating cows.
- Decreasing the amount of calcium prior to calving can improve calcium status at calving, and whether it is associated with reduced incidence of milk fever.
- Differences in mineral feeding during the last part of the gestation period among herds in Sweden are related to differences in milk fever frequency.
- Milk removal prior to calving has the same effect on calcium homeostasis as the onset of lactation occurring at parturition, and whether prepartum milking can improve calcium status at calving.

4 Materials and methods

For a more detailed description of the methods used in the different studies, see Papers I-IV.

4.1 Studies conducted

The papers upon which this thesis is based describe three different experiments conducted at Kungsängen Research Centre in Uppsala, Sweden (Papers I, II and IV) and one study based on information from commercial herds in Sweden (Paper III). For the studies described in Papers I, II and IV, the animal experimental procedures were approved by the local ethics committee in Uppsala. In the following sections, the results obtained during the studies described in Papers I-IV are attributed to Studies I-IV, respectively.

Paper I describes an experiment to determine the effect of an increase in potassium concentration on the absorption and excretion of magnesium in lactating cows. The experiment was arranged as a Latin square with 6 cows and 6 treatments in a 2 x 3 factorial design, with 2 levels of magnesium (1.9 and 4.3 g per kg of dry matter) added as magnesium oxide, and 3 levels of potassium (19, 27, and 36 g per kg of dry matter) in the form of potassium bicarbonate. The adaptation period was 9 days and the sampling period was 5 days.

Paper II evaluates the effects of different calcium intakes during the dry period on calcium homeostasis at calving. The experiment contained 29 cows. The treatments consisted of 3 different levels of calcium (4.9, 9.3, and 13.6 g per kg of dry matter) obtained by addition of ground limestone manually mixed into the concentrate, and were applied during the last three weeks of gestation. The cows were followed until 7 days in milk.

Paper III sought to determine whether the feeding of dry cows, particularly in terms of the amount of minerals, was different in herds with high incidence of milk fever and herds with no milk fever. The incidence of milk fever was estimated as the reported milk fever treatments per cow per year. Paper III comprises a case-control study based on information from the Swedish official milk recording scheme and on dry cow feeding obtained through a questionnaire. All herds had more than 44 cows enrolled in the official milk recording scheme. Cases were herds that had a registered milk fever treatment incidence of more than 8.8 % (n=30), and controls were herds with no registered milk fever treatments (n=22).

Paper IV describes an experiment studying the effect of prepartal milk removal on calcium homeostasis at start of prepartum milking and at calving. The experiment contained 15 cows. Nine of the cows were subjected to twice daily prepartum milking for 1 to 7 days prior to calving, and the remaining 6 cows served as controls. The cows were followed until 7 days after calving.

4.2 Animals and management

In Papers I, II and IV, cows of the Swedish Red breed belonging to the Kungsängen research herd were used. In Paper I, the cows were in the 6th to 10th month of lactation and not pregnant. In Papers II and IV, the cows were selected based on expected calving date, and none of them had previously been treated for milk fever. They were all dry and pregnant in the 8th to 9th month on entering the experiment. All cows were kept in individual tie stalls and were fed in troughs during the experiments. The cows in Papers II and IV all calved during the experiments, and were moved to a calving pen before calving. All lactating cows were milked twice daily, and the cows were weighed once during each experiment.

4.3 Sampling and laboratory analyses

In Papers I, II and IV, plasma was sampled during the experiment. Approximately 10 ml of blood were drawn from a coccygeal vessel into a vacuum tube with heparin as anticoagulant. The blood was centrifuged within 1 h at 1800 x g, and the plasma was harvested and stored in triplicate portions at -20°C until analysis.

In Papers I, II and IV, urine samples were taken. The urine was obtained by manual stimulation or at voluntary urination. The pH was measured in the urine immediately after sampling using a pH meter that was calibrated daily using buffers with pH 7 and 4. In Paper I, daily urinary samples were pooled into one sample per experimental period. The samples were stored at -20°C until analysis. In Paper IV, the urine samples were centrifuged at 200 x g to make them cleaner by removing larger particles and transferred to new plastic tubes before freezing.

In Papers I and II, faecal samples were taken to determine the concentration and uptake of the minerals. Equally sized grab samples of faeces were taken at defecation or by rectal stimulation twice daily during five subsequent days and stored in plastic bags at -20°C. Samples from each cow and experimental period were thawed and mixed, and a pooled sample was freeze-dried and ground using a 1 mm sieve. In Paper I, faecal texture was determined using a 10-point scale on the faecal samples obtained in the morning of the last day of each experimental period.

In Papers I and IV, milk yield was recorded and samples were taken. Milk yield was measured for each milking during the last five days of the experimental period (Paper I) or during the entire experiment (Paper IV). After calving, when the cow had left the calving pen and later in lactation, yield measurements and milk sampling were carried out using equipment from de Laval (Tumba, Sweden), while prior to calving until the first milking after calving, yield measurements and sampling were performed manually. A well mixed sample of the milk was taken, preserved with bronopol (2-bromo-2-nitro-1,3-propanediol) and stored at 4°C until analysis of fat, protein, lactose, and density with mid-infra-red spectroscopy. In Paper I, equal amounts of the milk samples preserved with bronopol were pooled into one sample per cow and period, and stored at -20°C until analysis of magnesium. In Paper IV, an additional sample from each milking was stored without additives at -20°C until analysis of calcium and magnesium.

Samples of rumen fluid were collected once per cow and period in Paper I using an oesophageal tube connected to a water jet pump, as described by Odensten (2006). The rumen fluid was collected in a thermos and pH was measured to ensure that there was no extensive contamination of saliva before the sample was transferred to a plastic container and stored at -20°C until analysis.

Analysis of calcium and magnesium in plasma, urine and rumen fluid was performed using colorimetric methods with O-cresophthalein and Xylidyle Blue (Randox, Antrim, UK; Stanbio, Boerne, USA). The analysis of magnesium in urine was performed on acidified urine in Paper II, to dissolve possible insoluble compounds binding to magnesium, according to the instructions from the kit manufacturer. However, a comparison between results obtained in untreated urine and acidified urine showed a high correlation and equally high magnesium concentration in untreated urine and in acidified, and thus analysis of untreated urine was considered to be equally accurate. Rumen fluid was centrifuged at 13200 x g after thawing and the supernatant was analysed.

Parathyroid hormone and C-terminal crosslinked telopeptide of type 1 collagen (CTx, a marker of collagen catabolism) in plasma were analysed using commercial enzyme-linked immunosorbent assay (ELISA) kits (Immutopics, San Clemente, USA; Nordic Biosciences, Herlev, Denmark, respectively). For analysis of CTx, samples were diluted in the buffer provided in the kit, according to the concentration of the samples and the analytical range of the kit.

In Paper III, the experimental units were commercial herds. Data on herd size, average parity, reported incidence of total treatments and milk fever treatments and breed composition of the herds were obtained from the Swedish Dairy Association, and data on feeding and management of the dry cows were obtained from the farmer using a written questionnaire. Mineral analysis of the silage fed to the dry cows during the last 3 weeks of gestation was required, and data on mineral values of the other components of the diet were obtained from the feed manufacturers or from Swedish feed tables (Spörndly, 2003). Herds where the amount of forage fed was unknown were assigned the average value of the other herds. Herds where the amount of mineral supplements fed was unknown were not included. Maximum dry matter intake was assumed to be 15 kg per cow and day, and herds where a higher intake was stated were not included unless they fed a total mixed ration or high amounts of silage. The intake was then recalculated to 15 kg. The farmers were asked about the use of prophylactic treatment with oral calcium-containing preparations to prevent milk fever and the categories of cows to which such treatment was applied.

4.4 Estimations of daily mineral losses

To estimate the daily excretion of minerals through faeces and urine in Papers I, II and IV, intrinsic markers were used. For estimation of the digestibility of minerals in Papers I and II, acid-insoluble ash was used as an indigestible marker in the feed. The analysis was performed on dried feed components and freeze-dried faeces according to Van Keulen and Young (1977). Estimations of the daily urinary excretion to make it possible to calculate the loss of minerals in the urine for Papers I, II and IV were made using the creatinine concentration of the urine, analysed using a colorimetric method with picric acid in an auto-analyser.The output of creatinine was assumed to be constant and proportional to the body weight, with 29 mg creatinine per kg excreted daily (Valadares *et al.*, 1999). For the analysis of minerals in milk, the same kits as for plasma, urine and rumen fluid were used. In the analysis of magnesium in milk in Papers I and IV, the protein was precipitated with trichloroacetic acid and the supernatant was analysed. In the analysis of calcium in milk in Paper IV, the calcium in the sample was bound to ethylenediaminetetraacetic acid (EDTA) to create a sample blank.

4.5 Statistical analyses

In all papers, the statistical software SAS (Version 9.1, SAS Institute, Cary, USA) was used. In Paper I, the effects of the levels of magnesium and potassium on the different variables regarding magnesium metabolism were analysed using the MIXED procedure. The model contained treatment and period as fixed factors and cow as a random effect. For Papers II and IV, the MIXED procedure was used to model the effect of calcium level or prepartum milking over time, with cow as a random effect and the covariation among samples within cow over time modelled so that samples taken closer in time were assumed to be more correlated. For Paper III, the risk of being a herd with high milk fever incidence was modelled using a logistic regression in the GENMOD procedure. More detailed descriptions of the statistical analyses can be found in Papers I-IV.

5 Results

Detailed descriptions of the main results from the different studies can be found in Papers I – IV.

5.1 Uptake and excretion of minerals

The apparent absorption of calcium was not significantly affected by different calcium levels when fed to dry cows (Paper II), but cows fed the lowest level of calcium excreted more calcium in the urine in the dry period. The potassium intake did not affect the apparent absorption of magnesium in lactating cows (Paper I). High magnesium intakes decreased the apparent absorption of magnesium, independent of the level of potassium. Daily magnesium output in urine was higher when the cows were fed high amounts of magnesium. High intakes of calcium during the dry period decreased the apparent absorption of magnesium (Paper II), and a low daily urinary output of magnesium further indicated decreased absorption. After calving, urinary excretion of calcium decreased rapidly, but returned to prepartum levels by one week postpartum (unpublished data from Study II; Paper IV; Figure 2). Urine magnesium excretion was increased compared with prepartum levels at the sampling 2 days after calving, and then decreased to levels below prepartum levels. Prepartum milking did not affect daily urinary excretion of calcium or magnesium (Paper IV).

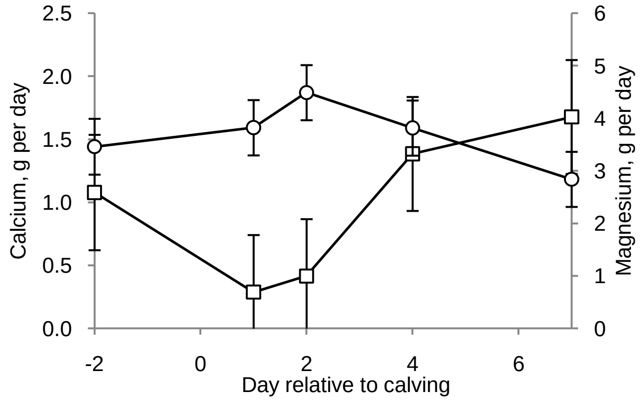

Figure 2. Urine excretion around calving of calcium (squares) and magnesium (circles) in g per day in 14 cows from Study IV. Data are shown as least squares means with standard errors of the means.

There was a clear relationship between the magnesium excretion in urine (g per day), estimated by the creatinine concentration and the body weight of the cow, and the concentration of magnesium (g per litre) in urine samples from lactating cows in Study I and dry cows in Studies II and IV (unpublished results). These results are shown in Figure 3.

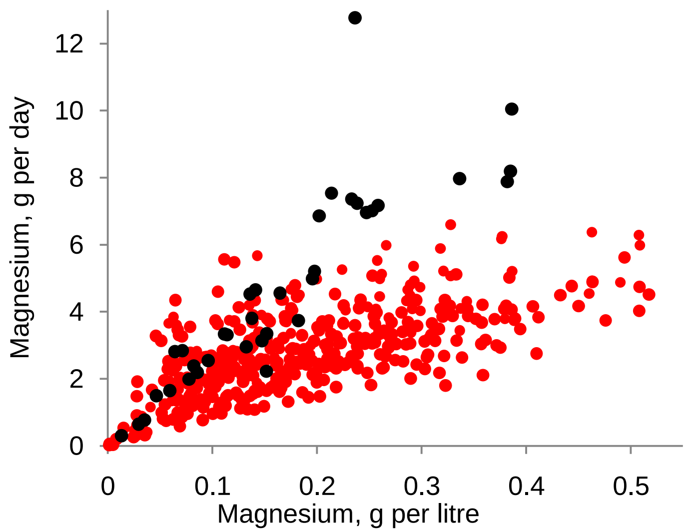

Figure 3. Relationship between total daily excretion of magnesium estimated by creatinine excretion and concentration of magnesium in the urine of lactating cows in Study I (black) and dry cows in Studies II and IV (red).

The concentration of magnesium in milk from cows in mid to late lactation was affected by dietary magnesium intake and the effect depended on the potassium level. The concentration was higher at a low potassium level when the magnesium intake was high, but higher at a high potassium level when the magnesium intake was low. However, these differences were small and the concentration of magnesium in milk was on a level of 0.09 g per litre (Paper I). In periparturient cows, the initial magnesium concentration in milk was high both when the cow was milked prepartum and postpartum for the first time, 0.34 and 0.24 g per litre in the first two milkings for prepartum and postpartum milked cows, respectively. However, 1 week after calving the concentration had decreased to 0.14 g per litre (Paper IV). The calcium concentration in colostrum and milk was higher prepartum compared with postpartum, and was higher on the day after calving compared with the rest of the first week postpartum. Cows milked only after calving excreted between 8 and 35 g calcium in the first milking which occurred 1 to 13 h after calving whereas cows milked prepartum excreted between 0.3 and 18 g calcium in their first milking, which occurred 1 to 7 days prior to calving (Figure 4, unpublished data from Study IV). The corresponding amounts of magnesium were 1 - 7 g for control cows, and 0.2 - 4 g for prepartum milked cows. The difference was due to a much lower yield prepartum compared with the postpartum milked cows. Milk yield was not affected by differences in magnesium or potassium intake in mid to late lactation (Paper I), and prepartum milking did not affect the milk yield during the first week postpartum (Paper IV).

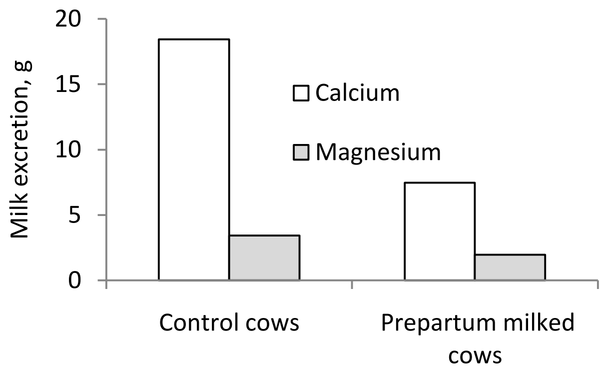

Figure 4. Excretion of calcium and magnesium in the first milking from cows milked prepartum and control cows milked after calving. Data are shown as mean values.

5.2 Plasma minerals, parathyroid hormone and CTx

Magnesium intake had an effect on the concentration of magnesium in plasma in lactating cows, with a higher intake resulting in higher magnesium levels (Paper I). The different calcium intakes prior to calving had no effect on the concentration of plasma calcium prior to, at, or after calving; the calcium homeostatic mechanisms measured by plasma parathyroid hormone level; or bone mobilisation measured by the level of the collagen residual plasma CTx, assumed to be a marker of calcium release from bones (Paper II). Cows fed high concentrations of calcium prior to calving experienced hypomagnesaemia after calving, as indicated by a plasma magnesium level below 0.74 mM, to a greater extent than cows fed lower concentrations of calcium. Milk removal prior to calving resulted in decreased plasma calcium concentration and increased level of plasma parathyroid hormone at the sampling two days after the start of prepartum milking and until calving (Paper IV). Plasma CTx level increased from the sampling 24 h after milking until calving. After calving, there was a further decrease in the level of plasma calcium and an increase in plasma CTx concentration, while plasma parathyroid hormone level did not increase further. However, there were no differences in the levels of calcium, parathyroid hormone or CTx between prepartum milked cows and control cows after calving. In Paper IV, there was no effect of prepartum milking on the concentration of plasma magnesium, but in both Papers II and IV plasma magnesium levels increased at calving, followed by a decrease to a minimum level at the sampling 4 days after calving (results from analysis of data from Studies II and IV, shown in Figure 5). At 7 days after calving, plasma magnesium had returned to prepartum levels in Study II, while the level in Study IV was still depressed compared with prepartum levels.

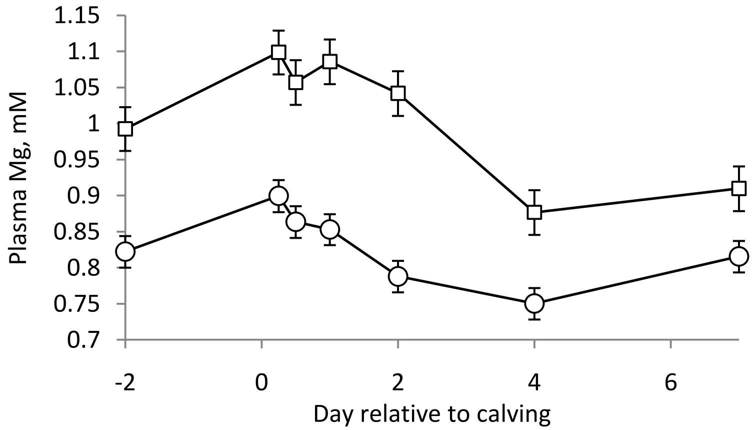

Figure 5. Plasma magnesium (mM) around calving in cows from Study II (circles) and Study IV (squares). The treatments in each study are combined. Results are presented as least squares means and standard error of the means.

Plasma calcium levels decreased within 1 h after calving compared with prepartum levels (unpublished results from 11 cows from Studies II and IV that were not milked prior to sampling), and the level of CTx increased compared with prepartum levels at that time (Figure 6). There were no tendencies for a decrease in plasma calcium concentration or an increase in the concentration of CTx in the cows that were sampled just before calving (data not shown).

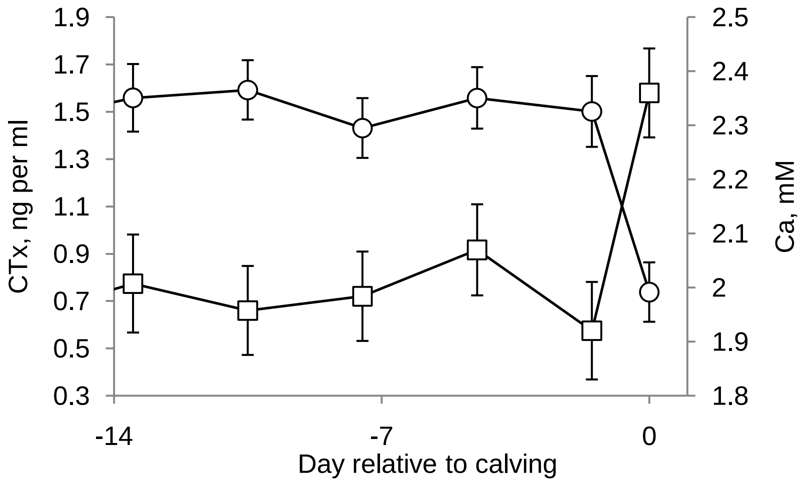

Figure 6. Plasma calcium (circles) and C-terminal telopeptide of type 1 collagen (CTx, squares) around calving in 6 cows from Study II and 5 control cows from Study IV. The sample taken at day 0 was taken within 1 h of calving, and none of the cows were milked or suckled at that time. Data are shown as least squares means and standard error of the means.

5.3 Milk fever

Paper III indicated that dietary calcium was not of major importance for the incidence of milk fever when feeding diets composed of commonly used feedstuffs. However, an increase in potassium feeding during the last three weeks of gestation was associated with a linear increase in the risk of being a case herd, with potassium in the range 118 - 304 g potassium per day. Herds feeding more than 26 g magnesium per day, corresponding to almost twice the recommended amount (Nielsen and Volden, 2011), during the last three weeks prior to calving had a decreased risk. Age, milk yield and breed composition did not differ significantly between herds with high incidence of milk fever and herds with no milk fever. There were no differences between case herds and control herds in Paper III regarding the prophylactic use of oral calcium to prevent milk fever. In most herds (87 %), the farmer stated that oral calcium was used. In a few herds it was used to all cows but most often to a subset of the cows, where the selection of cows given prophylaxis was based on *e.g.* age, body condition and previous milk fever history. Only one cow in the animal experiments in Papers II and IV was treated for milk fever with calcium gluconate and magnesium chloride, and this was one of the control cows in Paper IV, that had plasma Ca and Mg levels at calving of 1.5 and 1.2 mM, respectively. Samples from that cow that were taken after treatment were not included in the analysis of the results.

6 General discussion

6.1 Calcium

The high degree of regulation of calcium homeostasis in the cow maintains plasma calcium at an adequate level in most situations, and as long as the regulatory systems are functioning, plasma calcium level is maintained independent of dietary calcium level. This was shown in Papers II and IV, where plasma calcium was kept at a stable level until parturition or onset of lactation, even when the dietary concentration of calcium was more than doubled. This is further supported by results from studies in lactating cows reported by Belyea *et al.* (1976) and Taylor *et al.* (2009). However, in Paper II, increased intake of calcium resulted in decreased urine output of calcium. This is in agreement with results for cows reported by Ramberg *et al.* (1976), but the reason for the change in calcium excretion is not known. Theoretically, increased intake of calcium ions would shift the cow towards a more alkalotic state and thus decrease the calcium excretion through urine, but there were no differences in urine pH among treatments in Paper II, indicating that the acid-base status was similar in all treatments. The differences in calcium excretion were small and did not have any important effect on total calcium balance. The main period of interest regarding calcium supply in dairy cows is the periparturient period, when a large proportion of cows develop transient hypocalcaemia, as found in Papers II and IV and also by Reinhardt *et al.* (2011). More than 3 % of dairy cows contract milk fever each year in Sweden (Swedish Dairy Association, 2010).

6.1.1 Calcium regulation when homeostasis is challenged

In Papers II and IV, the level of CTx was increased already at the sampling 6 h after calving, and remained on the same level for the rest of the first week of lactation, indicating activated mobilisation of calcium from bones. The results from cows that were sampled within 1 h of calving also showed increased levels of CTx. Resorption of bone at calving has been shown to be of great importance for maintaining plasma calcium above the level where clinical symptoms of milk fever appear (Yarrington *et al.*, 1976). However, the rapid increase in CTx in the first 6 h after calving up to a level that seemed to be stable during the rest of the first week after calving (Papers II and IV) is to some extent different from measurements of the pattern of other bone resorption markers in dairy cows (Liesegang *et al.*, 1998), where the markers for bone resorption showed a more gradual increase in concentration after calving. Goff *et al.* (1986) suggested that 48 h of parathyroid stimuli were needed to increase bone mobilisation, although they measured a urinary marker of bone resorption. However, results from studies in humans reported by Guillemant *et al.* (2003) and Prestwood *et al.* (2000) indicate that CTx is a marker that is affected by both calcium status and alterations in *e.g.* steroid hormones, and that the response is more evident and rapid compared with other markers of bone catabolism. There are different mechanisms underlying the metabolism of collagen (Everts *et al.*, 1992), and the catabolism can be mediated by matrix metalloproteinases or by cystein proteinases, one of these being cathepsin K. In cell cultures, when cathepsin K is active, there is a release of CTx from the collagen (Garnero *et al.*, 2003), while the matrix metalloproteinase activity releases other markers. The choice of marker can thus be used to evaluate which pathway of collagen catabolism is active. The rapid increase in the concentration of CTx that was found at calving in Papers II and IV could be due to the decrease in plasma calcium level and/or differences in the hormones associated with pregnancy. However, in Paper II, no significant increase in parathyroid hormone level could be seen, while when prepartum milking was started in Paper IV, there was a response in CTx already at 24 h after the first milking, but the responses in plasma calcium and parathyroid hormone level were only significant after two days of milking. The lack of effect on parathyroid hormone at parturition in Paper II and the delay in parathyroid hormone level increase at prepartum milking in Paper IV (although the concentration of CTx increased rapidly at both occasions) indicates that factors other than increased parathyroid hormone level affect calcium mobilisation from bones as monitored by CTx level. Administration of parathyroid hormone increases the release of CTx in humans (Schafer *et al.*, 2011). However, as osteoclasts and the bone resorption they mediate are also affected by oestrogens, at least in birds (Oursler *et al.*, 1991), they may be the reason for an increase in CTx level at calving, when the level of oestrogen drops markedly (Kurosaki *et al.*, 2007). Results from Hollis *et al.* (1981) indicate that oestrogen may have an impact on the development of milk fever, with high levels of oestrogen increasing the risk of milk fever. van de Braak *et al.* (1984) found that cows in late gestation responded less to an artificially induced decrease in plasma calcium level than lactating cows. This may have been the reason why plasma calcium level decreased and remained low when cows were milked prepartum in Paper IV, compared with the transient decrease occurring at parturition. It has also been shown that the effect of increased parathyroid hormone level is less pronounced in cows just before calving compared with after calving (Martig and Mayer, 1973). However, the increase in the concentration of CTx at prepartum milking in Paper IV indicates that there was an increased release of calcium from bones, starting even before the decrease in plasma calcium level was significant.

Parathyroid hormone is the main hormone regulating the homeostasis of calcium and has been shown to respond rapidly to changes in plasma calcium level. In spite of this, no effect on the concentration of parathyroid hormone could be seen at parturition in Paper II, even though plasma calcium level did decrease rapidly. In Paper IV, parathyroid hormone level increased at the same time as the concentration of plasma calcium decreased when prepartum milking was started, and for control cows that were not milked until after calving, parathyroid hormone level increased after calving. The level in prepartum milked cows remained the same as on the day prior to calving, but was elevated compared with pre-milking levels. A transient increase in parathyroid hormone level occurring at calving is often reported (Thilsing *et al.*, 2007; Shappell *et al.*, 1987). The reason for the absence of a significant increase in Paper II is unclear, but the levels of parathyroid hormone found in the analyses were low compared with the standards used for calibrating the standard curve and this, together with a high variation between cows, might have resulted in difficulties in detecting a significant increase. However, the samples from Paper IV were analysed in the same way and there it was possible to find a significant increase, despite the smaller number of animals. The measured levels of parathyroid hormone were similar in the two experiments. The lack of significant effect on the level of parathyroid hormone at parturition in Paper II and the delay in parathyroid hormone level increase at prepartum milking in Paper IV may indicate that there are factors other than increased level of parathyroid hormone affecting calcium mobilisation from bone as monitored by the levels of CTx. The osteoclasts express the same kind of calcium-sensing receptors as the parathyroid gland, and there are indications from studies with rabbit osteoclasts that the resorption of bone can be regulated directly by the calcium concentration, without the influence of parathyroid hormone (Kameda *et al.*, 1998). However, the importance of this direct regulation in maintaining calcium homeostasis under a hypocalcaemic challenge like parturition in dairy cows remains to be determined.

6.1.2 Milk fever and hypocalcaemia at calving

Decreasing the calcium intake during the dry period is a method that is used in practice to improve calcium status and decrease the risk of milk fever at calving (Hansen *et al.*, 2007). However, the results from Paper II did not show any effects on the degree of hypocalcaemia when dietary calcium was varied in a range that is achievable with commonly used Swedish feedstuffs, and calcium intake was not different between herds with high or low incidences of milk fever in Paper III. Some previous studies have demonstrated that a decrease in calcium intake reduces the incidence of milk fever and hypocalcaemia (Lean *et al.*, 2006; Shappell *et al.*, 1987; Green *et al.*, 1981). It has been shown in humans that calcium supplementation during the last half of pregnancy results in higher losses of bone minerals during the first year after childbirth, indicating that the higher calcium intake impairs the uptake of calcium (Jarjou *et al.*, 2010). Others have not been able to show any effect of altered calcium intake on hypocalcaemia and milk fever (Kamiya *et al.*, 2005; Goff and Horst, 1997). It has been suggested that the calcium intake must be below 20 g per day to affect the periparturient calcium metabolism in a positive way and decrease the incidence of milk fever (Thilsing-Hansen *et al.*, 2002b), and with the feed stuff used in Sweden, this low level is difficult to achieve.

Although increases in the potassium concentration did not seem to have any negative effect on magnesium uptake at high potassium levels in Paper I, there was an association between high potassium intakes and the incidence of milk fever in Paper III. This supports the conclusion of Lean *et al.* (2006) and Goff and Horst (1997) that the increase in milk fever incidence found at high dietary potassium concentrations is not due to a negative effect on the magnesium status of the animal. In Paper III, the potassium concentrations were in the range tested by Goff and Horst (1997), where differences in potassium intake did not result in any differences in plasma magnesium around calving, so the effect on milk fever incidence can be assumed to originate from the effect on the acid-base status of the cow. However, results from Schonewille *et al.* (1999) and Jittakhot *et al.* (2004a) indicate that dietary potassium at high levels has a negative influence on magnesium absorption in dry cows. The effect of potassium intake on the risk of high milk fever incidence in Paper III was linear, at least over the range 118 to 304 g per day, corresponding to concentrations of 11 to 30 g per kg of dry matter. Potassium is one of the minerals that affects the acid-base status of the cow to a large extent (Rérat *et al.*, 2009), and it is thus possible that the difference in potassium concentration corresponds to similar differences in the acid-base status of the cows. This is supported by the results from lactating cows, where an increase in potassium concentration in the diet increased the urine pH, indicating a more alkalotic state of the cow (unpublished data from Study I). However, there were no differences in urine pH between the medium and the high potassium concentration, indicating that the effect on the acid-base status of the cow was maximal already at a potassium concentration of 28 g per kg of dry matter. Assuming that the strong ion theory is correct, this is in accordance with studies on dry cows, lambs and steers, where increased potassium concentrations or increases in dietary cation- anion difference only affected the acid-base balance up to a certain point (Luebbe *et al.*, 2011; Goff and Horst, 1997). Based on the diets in the case-control study (Paper III) and with normal sodium, sulphur and cloride composition of Swedish feed stuffs (Spörndly, 2003; Pehrson *et al.*, 1999), the strong cation-anion difference is usually above the level suggested in the diet to transition cows to prevent them contracting milk fever (McNeill *et al.*, 2002). However, others have found a linear effect of increasing the strong cation-anion difference on the incidence of milk fever (Lean *et al.*, 2006), which supports the findings in Paper III that potassium intake was linearly related to milk fever incidence, even at high levels of potassium.

Prepartum milking did not affect postparturient levels of plasma calcium, parathyroid hormone or bone mobilisation measured as the concentration of CTx in Paper IV. However, the cows that were prepartum milked had lower concentration of plasma calcium prior to calving, so the decrease in plasma calcium level was less pronounced, even if it decreased to the same level as in control cows after calving. It can be speculated that the extent of the drop in plasma calcium level is more important than the actual level. The drop was relatively smaller in prepartum milked cows compared with control cows, which may reduce the risk of developing milk fever. However, Larsen *et al.* (2001) found that the degree of paresis was more severe in cows with low plasma calcium level compared with cows with higher levels of plasma calcium. Furthermore, Jørgensen *et al.* (1999) reviewed different studies and concluded that the level of ionised calcium was similar when cows developed signs of hypocalcaemia after EDTA infusion. The decrease in the level of plasma calcium at calving was rapid and was evident shortly after parturition. In contrast, when prepartum milking was started, no significant decrease in plasma calcium level was seen until 2 days after milking began. This indicates that the removal of milk is not the only determinant of plasma calcium level at calving and thus is not of exclusive importance in the development of hypocalcaemia and milk fever. Nevertheless, removal of milk prepartum caused a decrease in the level of plasma calcium, which in a milk fever-prone cow may aggravate the hypocalcaemia occurring at parturition, and perhaps increase the risk of development of clinical hypocalcaemia. However, Smith *et al.* (1948) investigated the incidence of milk fever and the degree of hypocalcaemia in cows that were milked restrictedly after calving and cows that were completely milked and found no positive effects of restricted milking. To my knowledge, there are no more recent studies that have evaluated the effects of restricted milking after calving on milk fever or hypocalcaemia, although it is a measure that practitioners believe can decrease the risk of milk fever (Sørensen *et al.*, 2002).

In Paper II, high intakes of calcium decreased the uptake of magnesium during the dry period, and this was associated with decreased excretion of magnesium in the urine, indicating impaired magnesium status in the cows fed high levels of calcium. Magnesium status of the cow may be important for the capacity to cope with a challenge to calcium homeostasis (van de Braak *et al.*, 1986). Furthermore, Lean *et al.* (2006) found that dietary magnesium concentration was linearly and negatively related to the incidence of milk fever, *i.e.* with increasing predicted milk fever incidence when magnesium concentration decreased. In Paper III, herds feeding low amounts of magnesium during the dry period also had an increased risk of having a high milk fever incidence. Thus, there may be a more indirect effect of calcium intake, mediated by changes in magnesium uptake, on the risk of milk fever.

Most herds in Paper III used oral calcium supplements at calving to prevent hypocalcaemia. This is to provide the cow with a high amount of easily absorbable calcium ions to compensate for the drop in plasma calcium concentration at calving, and has been shown to be efficient (Goff *et al.*, 1996). There was no difference in the use of prophylactic treatment between case and control herds, and prophylaxis was not used more restrictedly in case herds, according to statements given by the farmers. Hansen *et al.* (2007) found that farmers applied preventive measures against milk fever irrespective of whether they perceived it as a problem in the herd or not. Due to the beneficial effect of oral calcium supplements around calving, the use of prophylactic treatments in Paper III may have prevented milk fever, but as there was a large difference in milk fever incidence between case and control herds, either the routines for the application differed, or the use of prophylaxis cannot overcome suboptimal feeding or management. Interestingly, there were no differences in the proportion of cows in the third lactation or older between case and control herds. Old cows have been shown to be more susceptible to parturient hypocalcaemia (Reinhardt *et al.*, 2011) and milk fever (Roche and Berry, 2006), but the results from Paper III indicate that the difference in milk fever incidence in this study was not related to differences in age structure of the herds. Breed composition of the herds in Paper III was also not found to differ between case and control herds, although it is known that some breeds are more susceptible to milk fever (Roche and Berry, 2006). The main breeds in the 52 herds studied in Paper III were Swedish Holsteins and Swedish Reds, and the difference in milk fever incidence between those breeds is not as pronounced as the difference between *e.g.* Holsteins and Jerseys (Swedish Dairy Association, 2010).

The extent of subclinical hypocalcaemia postpartum among the experimental cows in Papers II and IV was high. Assessing hypocalcaemia after calving in all cows from Papers II and IV according to the cut-off point used by Reinhardt *et al.* (2011), 35 of 44 cows (80 %) had concentrations of plasma calcium less than 2.0 mM in at least one sample during the week after calving, and 21 cows (48 %) had plasma calcium levels below 1.88 mM, which is the limit suggested by Goff *et al.* (1996). Although six cows showed plasma calcium levels below 1.5 mM, which is the level at which Yarrington *et al.* (1976) found that cows started to show signs of milk fever, only one cow showed clinical signs of hypocalcaemia. These levels are associated with a decrease in rumen motility (Daniel, 1983), and Hansen *et al.* (2003) found that subclinical hypocalcaemia resulted in a decrease in feed intake. During the first week after calving, cows are commonly in a negative energy balance (Tamminga *et al.*, 1997), which increases the risk of metabolic diseases (Ospina *et al.*, 2010). Milk fever has been associated with other periparturient diseases such as ketosis (Correa *et al.*, 1990), and subclinical hypocalcaemia may thus also be a risk factor. The incidence of subclinical hypocalcaemia among the cows at calving in Papers II and IV was higher than the level found by Bigras-Poulin and Tremblay (1998) and Reinhardt *et al.* (2011). A reason for this, apart from differences in the populations studied, may be differences in sampling intensity between those studies, when the cows were sampled once after calving, and Papers II and IV, where there were several samplings after calving. The lowest plasma calcium level in cows from both Papers II and IV was found 24 h after calving, and therefore, the extent of subclinical hypocalcaemia could be quantified by plasma sampling 24 h after calving.

6.2 Magnesium

Magnesium absorption from the rumen to the blood is regulated by more mechanistic means and is not as well regulated as *e.g.* hormonal control of calcium absorption. This can be seen in the plasma magnesium levels in Papers II and IV, where there was a larger variation in plasma magnesium levels in dry cows compared with plasma calcium levels, with different levels in the two experiments. If magnesium absorption from the diet is insufficient, the cow has no possibilities to mobilise the magnesium stored in the bones and adequate absorption is thus necessary to maintain the magnesium levels in the cells and in the extracellular fluid.

6.2.1 Improving uptake

Because the magnesium supply is solely dependent on the uptake of magnesium from the diet, and because the factors regulating magnesium absorption are most often diet related, many studies have been made of dietary factors affecting magnesium absorption in ruminants (Jittakhot *et al.*, 2004a; Jittakhot *et al.*, 2004b; Care *et al.*, 1984). In Paper I, with lactating cows, increasing the intake of magnesium decreased the digestibility, although in absolute figures the cows fed higher amounts of magnesium still absorbed more magnesium, resulting in increased outputs of magnesium in the urine but also a more positive magnesium balance. This is in agreement with results from other studies in sheep (Ram *et al.*, 1998). However, in dry cows, increased magnesium intake has been reported to result in increased uptake of magnesium (Jittakhot *et al.*, 2004a). This is explained by the different mechanisms of magnesium uptake from the rumen, with one transport mechanism that is effective at low concentrations of magnesium, and another occurring when the magnesium concentration gradient is large enough. The magnesium concentrations in the diets fed in Paper I were much lower than the concentrations fed by Jittakhot *et al.* (2004a), and this may be the reason for the differences in results, as the rumen concentration of magnesium was probably different. High intakes of magnesium may result in diarrhoea in cattle (Chester-Jones *et al.*, 1990). This was supported by the finding that the faeces of the lactating cows in Paper I were judged to be wetter when the cows were fed a higher level of magnesium. This can be one of the factors limiting the level of dietary magnesium, especially in lactating cows.

In Paper II, the cows fed high amounts of calcium had a decreased uptake of magnesium during the dry period, while the decreased urinary magnesium excretion indicates that the absorption of magnesium was also impaired. In normal feeding, the majority of the magnesium absorption is from the rumen, while calcium is absorbed mainly from the intestines. However, when high levels of calcium are fed, a significant net absorption of calcium occurs in the rumen (Khorasani *et al.*, 1997). In rats, it has been shown that high intake of calcium decreases the absorption of magnesium (Behar, 1975), and this has been suggested to result from competition for the transport mechanism that can be utilised for both calcium and magnesium (Li *et al.*, 2007b; Shachter and

Rosen, 1959). The same transport mechanisms can be found in the rumen epithelial cells (Schweigel *et al.*, 2008), and thus competition from calcium ions may be the cause of the decreased uptake of magnesium. The results from Paper II support findings by Care *et al.* (1984) that magnesium absorption decreases in sheep when dietary calcium is increased.

The lack of effect of increasing potassium concentration on the apparent absorption of magnesium in Paper I is contradictory to the conclusions of Jittakhot *et al.* (2004b), Weiss (2004) and Schonewille *et al.* (2008). However, the potassium concentration in the rumen seems to be important only up to a certain point (Jittakhot *et al.*, 2004b), and this may be the reason for the lack of effect of potassium. Swedish forages contain on average 21 g potassium per kg of dry matter but may vary up to double that concentration (Eriksson, 2005), and may constitute a large proportion of the diet, especially during late lactation and the dry period. There may be a possibility that the potassium concentration in most diets used in practice is at the level where alterations in potassium concentration have no further effect on magnesium absorption. However, with the increasing use of maize silage, which is harvested at a much more mature stage compared with grass and clover silages, the potassium concentration in the dairy diets will decrease, perhaps reaching the levels where the potassium concentration will have an effect on magnesium absorption.

6.2.2 Magnesium supply

The low excretion of magnesium during the end of the dry period in Paper II was mainly detected in cows on the high calcium diet but cows on all diets were represented. This indicates that the cows had a limited supply of magnesium, although they were fed magnesium slightly above the recommendations (Nielsen and Volden, 2011; Spörndly, 2003). It is possible that the potassium concentration in grass silage, which constitutes a large part of the dry cow diet in Sweden, may result in decreased magnesium absorption on average, and thus increased requirements for dietary magnesium. However, the potassium concentration in the forage used in that experiment was not very high, resulting in a total dietary concentration of potassium of 16 g per kg of dry matter. The current Swedish recommendations for magnesium during the dry period (Nielsen and Volden, 2011) are in accordance with the National Research Council (NRC) recommendations adapted to meet the requirements, but only 50 % of the levels suggested by NRC to provide optimal absorption of magnesium independent of the diet composition (NRC, 2001). Based on the finding in Paper III that dietary magnesium fed at almost twice the recommended level was associated with a decrease in milk fever frequency, there may be reasons to exceed the minimum recommendations of magnesium to dry cows, at least during the last weeks of gestation. It remains to be determined whether this beneficial effect of extra magnesium is due to compensation for decreased absorption of magnesium when the forage proportion in the diet is high, underestimation of the magnesium requirement during the last part of the pregnancy, or some other reason.

6.2.3 Assessing magnesium supply

In order to prevent production losses due to subclinical magnesium deficiency and to detect cows and herds at risk of developing hypomagnesaemic tetany, there is a need to evaluate whether the dietary magnesium provision is adequate. This is best done by assessing the magnesium status at herd level. The level of magnesium in plasma is considered to be a specific but not very sensitive tool to detect subclinical hypomagnesaemia in humans (Malon *et al.*, 2004), and Schweigel *et al.* (2009) found that cows with plasma magnesium levels considered to be normal still showed indications of depleted intracellular magnesium pools. In Paper II, there were no differences in plasma magnesium concentration in the dry period among cows with different calcium intakes, although the cows fed high calcium had a decreased uptake of magnesium and a lower excretion of magnesium in the urine. However, after calving, cows that had been fed the high calcium diet showed lower plasma magnesium levels compared with cows on the other treatments, although the feeding at that time was the same. It is possible that this was the result of a gradual depletion of the intracellular magnesium stores, which was reflected in plasma only after calving when the output of magnesium increased. The urinary excretion of magnesium was then a more sensitive indicator of inadequate magnesium supply through the diet in the dry period. The possibility of using the intracellular compartment as a magnesium store would perhaps explain parts of the highly positive magnesium balances found in Paper I, and positive magnesium balances have also been found in studies with dry cows (Jittakhot *et al.*, 2004a) and sheep (Ram *et al.*, 1998), although their estimated magnesium retention was numerically much smaller. The different magnesium intakes in Paper I resulted in small but significant differences in plasma magnesium levels. This is in agreement with results from studies with periparturient cows (Shappell *et al.*, 1987), but Jittakhot *et al.* (2004a) did not see any significant effect of magnesium in the diet of dry cows on plasma magnesium levels. Urine sampling seems to be a more reliable way to assess magnesium status than plasma sampling, but the assessment has to be done using an adequate cut-off point. Guéguen *et al.* (1989) suggested a minimum loss of 1.8-2.4 g magnesium per day, while Mayland (1988) suggested excretion of less than 0.1 g magnesium per litre of urine as indicating an insufficient magnesium supply. However, the calculated daily excretion of urine in dry cows from Papers II and IV varied widely, probably due to differences in water excretion, which shows the difficulties in assessing total magnesium output through urine and thus magnesium status only based on the concentration of magnesium in urine. The variability in urine excretion, as measured by variability in creatinine excretion, indicates that several cows should be sampled to give an adequate assessment of the magnesium status in dry cows in a herd, as long as only the magnesium concentration in the urine is measured. However, there was a clear relationship between the total excretion of magnesium and the concentration, especially at low concentrations. Cows excreting less than 0.1 g magnesium per litre, the level suggested by Mayland (1988), were likely to have a total excretion of less than 2.5 g magnesium per day, which was the amount suggested by Kemp and Geurink (1978) and Guéguen *et al.* (1989). As magnesium concentration in a spot sample of urine is easier to determine than the total daily excretion of magnesium, this may be the preferred method, at least to assess magnesium status at herd level. The lactating cows in Paper I had a higher daily excretion of magnesium compared with dry cows at the same concentration of magnesium in urine, which was most evident at high total magnesium excretions. This was caused by a higher amount of urine, and it can be speculated that the efficiency of kidney reabsorption of magnesium may depend more on the concentration of magnesium in the renal filtrate than on the total excretion of magnesium. If so, the concentration of magnesium in the urine is a better marker of the magnesium status than the daily total excretion, as a high volume of urine with low concentration of magnesium can result in high total excretion. Furthermore, samples from the lactating cows with concentrations below 0.1 g per litre corresponded to total amounts below 2.5 g per day, so this concentration could be used to test the magnesium status in both lactating and dry cows.

Due to the impact of magnesium status on the adaptations to hypocalcaemia in dairy cows (van de Braak *et al.*, 1986), and also the high outflow of magnesium after calving caused by the high concentration of magnesium in colostrum, the magnesium status in periparturient cows is of major importance. Goff (2008) suggests that several cows should be sampled within 12 h after parturition to determine the herd magnesium status, and that plasma magnesium levels less than 0.8 mM on that occasion suggest an inadequate magnesium supply.

6.2.4 Magnesium at calving

In both Papers II and IV, a transient increase in plasma magnesium level was found shortly after calving. This has also been reported in other studies (Goff and Horst, 1997; Green *et al.*, 1981), and results from Thilsing-Hansen *et al.* (2002a) and Goff *et al.* (2002) indicate that the increase in magnesium is related to the decrease in plasma calcium level occurring at calving. The reason for this has been suggested to be the elevated renal threshold for calcium when parathyroid hormone is excreted as a response to the decrease in plasma calcium level, resulting in a similar increase in the renal threshold for magnesium (Goff, 2008). Results from Taylor *et al.* (2008) suggest that there may be an effect of parathyroid hormone levels at calving on the concentration of plasma magnesium. However, in Paper IV, urinary excretion of magnesium was not decreased, even though urinary calcium excretion decreased immediately after calving. As the concentration of magnesium in plasma is very low compared with the concentration of intracellular magnesium, the results from Paper IV may suggest that the peak in magnesium occurring at calving may depend on movements of magnesium between the intracellular and extracellular compartment, rather than on decreased excretion. At 2 to 4 days after calving, the concentration of plasma magnesium was depressed compared with prepartum levels in both Papers II and IV. However, the levels were slightly higher both pre- and postpartum in Paper IV compared to Paper II, probably reflecting a higher magnesium supply through the prepartum diet. A decrease in plasma magnesium concentration after calving has also been seen in other studies (Rérat *et al.*, 2009; Verdaris and Evans, 1976). Urine magnesium excretion was lowest 7 days after parturition in Paper IV, probably aa a result of the decreased plasma magnesium concentration found after calving.

6.3 Mineral losses in milk

The daily calcium and magnesium losses in milk immediately after calving in Paper IV constituted more than half the calcium and magnesium requirement of the cows, based on the maintenance requirements estimated in the Nordic feed recommendations (Nielsen and Volden, 2011). The concentration of calcium and magnesium in milk is much higher than the concentration in plasma, which makes active transport of minerals into milk necessary. A large part of the calcium is associated with the casein micelles and is needed for coagulation (Horne, 1998), and this is also the case for some of the magnesium. There is also a high concentration of ionised minerals in milk. Mineral feeding has been shown not to alter the macro mineral concentration in milk (Salih *et al.*, 1987), and the amount of magnesium had no large impact on the magnesium concentration in milk in Paper I. However, dietary supplementation of minerals could result in increased milk production, thus resulting in an increase in total mineral output in milk, as shown with micro minerals by Rabiee *et al.* (2010). In Paper IV, the calcium and magnesium concentration in milk was higher when the cows were milked prepartum compared with after calving. After calving, the concentration was highest on the first day of milking, and then decreased. This decrease in mineral concentration after calving has also been found in earlier studies (Tsioulpas *et al.*, 2007). However, the main determinant of calcium excretion in milk after calving in Paper IV was the milk yield obtained, and as the milk yield increased, this resulted in an increase in the total excretion of calcium during the first week of lactation.

The daily milk output of magnesium during the first week after calving, both from cows milked prepartum and from control cows in Paper IV, was almost twice the daily output of magnesium in milk from the cows in mid to late lactation in Paper I. This was due to both higher milk yield and much higher magnesium concentration in milk in the first week of lactation. This emphasises the importance of a sufficient magnesium supply during early lactation, and may even explain the decrease in plasma magnesium level found during the first week of lactation in both Papers II and IV. In Paper I, magnesium excreted in milk constituted only up to 50 % of the magnesium apparently absorbed by the cows with low magnesium intake, and a much smaller proportion on high magnesium intake, while the urinary excretion of magnesium increased. This is in agreement with results from Kamiya *et al.* (2010). Calcium in milk often constitutes the major part of the absorbed calcium (Kamiya *et al.*, 2010; Taylor *et al.*, 2009), and this difference between the minerals is probably an effect of the different control of homeostasis, where calcium absorption is under hormonal control while magnesium homeostasis is regulated only by excretion, and may thus be absorbed more in excess. The concentration of calcium in colostrum from cows in Paper IV was lower than the levels found by Tsioulpas *et al.* (2007). However, the excretion of calcium in the first milking after calving in the control cows was almost twice the daily amount of calcium retained in conceptus at the end of gestation (House and Bell, 1993). The excretion of magnesium in the first milking was more than 10-fold the amount required for pregnancy.

7 Main conclusions

- Addition of potassium to diets already high in potassium had no negative effect on magnesium uptake in lactating cows.
- High calcium intake during the last weeks of gestation resulted in decreased magnesium absorption.
- Calcium content in the dry cow diet did not affect calcium homeostasis at parturition. It was also not an explanation for the difference in milk fever incidence among Swedish herds.
- High milk fever incidence in Swedish herds may partly be explained by low levels of dietary magnesium and high amounts of potassium in the forage fed to dry cows.
- Milk removal prior to calving decreased the level of plasma calcium and activated calcium mobilisation, but did not prevent the decrease in plasma calcium level at calving.

8 Implications

To maintain good animal health and production when managing high-yielding dairy cows in intense production systems, mineral supplementation of the cows should be considered. The results presented in this thesis show that there are possibilities to improve both the calcium and magnesium status in the dairy cow, but also demonstrate the importance of diet composition to maintain adequate levels of minerals, especially at a challenge such as parturition and onset of lactation.

To improve the calcium availability in periparturient cows, the silage used for dry cows during the last part of gestation should preferably have a low concentration of potassium, which to some extent can be achieved by harvesting the plants at a more mature stage. The decrease in nutritional value that results from later harvesting is often not a problem for dry cows, because their feed consumption capacity is usually high. Furthermore, to prevent milk fever, magnesium supply should be higher than the current recommendations during the last part of gestation. This is more important than trying to reduce the calcium concentration of the diet, at least as long as the calcium level still remains above about 45 g per day. The removal of milk negatively affects plasma calcium levels, but it is unclear whether restricted milking after calving will prevent milk fever, as most of the decrease in plasma calcium levels at calving seems to be unrelated to milk removal, and a decrease in plasma calcium concentrations can be seen within 1 h after calving.

To ensure an adequate magnesium supply during lactation and the periparturient period, magnesium intake should be sufficient. Silages that are early cut and thus have high concentrations of potassium can be fed to lactating cows without adverse effects on magnesium absorption. However, high calcium intakes, at least during the last part of gestation, may lead to insufficient absorption of magnesium and thus result in hypomagnesaemia after calving. To monitor the magnesium status of a herd, urine samples should be preferred over plasma samples due to the observable response of urinary magnesium to increases in magnesium intake and the relative insensitivity of plasma magnesium levels to decreases in absorption.

9 Future research

The studies included in this thesis identified several areas for future research:

- As dietary magnesium at almost twice the recommended level appeared to be preventive in the development of milk fever, the level of magnesium that should be fed to dry cows to prevent milk fever and hypocalcaemia should be investigated.
- Bone mobilisation, as measured by the release of CTx into plasma, increased rapidly at calving as well as at start of milk removal prepartum. It should therefore be investigated whether calcium release from bones increases immediately at calving, and also the factors on which onset of calcium mobilisation depends.
- It should be examined whether the amount of milk that is removed after calving influences the extent of hypocalcaemia and the risk of milk fever.
- Dietary calcium seemed to negatively influence magnesium absorption in dry cows. It is thus of interest to determine the extent to which calcium affects magnesium absorption, and whether this interaction is of importance under practical conditions.
- Further studies on the relationship between intracellular and extracellular magnesium in the periparturient period would be of interest.

10 Svensk sammanfattning

10.1 Bakgrund

Dagens mjölkkor har en hög omsättning av mineraler eftersom de producerar så mycket mjölk. Både kalcium och magnesium är viktiga för många av kroppens funktioner, till exempel muskel- och nervfunktioner, och eftersom de förekommer i hög koncentration i mjölken måste de tillföras via fodret i stor mängd för att täcka kons behov. Kalciumbalansen regleras med hjälp av hormoner, framför allt parathormon, som när det utsöndras leder till ökat kalciumupptag i tarmen, minskad utsöndring av kalcium i urinen och ökad frisättning av kalcium från skelettet. Magnesiumbalansen regleras framför allt via utsöndringen i urinen, medan upptaget i förmagarna bland annat beror på koncentrationen av magnesium och andra joner, till exempel kalium, i våmmen. Akut brist på kalcium är oftast kopplat till kalvningsförlamning, och drabbar varje år över 10 000 svenska kor. Även om en hel del kalcium går åt till fostertillväxten är behovet mycket större hos en lakterande mjölkko än hos en dräktig. Då mjölkproduktionen startar tas kalcium från blodet, och plasmanivån sjunker (hypokalcemi). De kalciummobiliserande mekanismerna aktiveras, men hos vissa kor sjunker kalciumnivån trots det så lågt att muskelfunktionen blir så försämrad att de inte kan resa sig. Akut brist på magnesium beror oftast på ett för dåligt upptag i våmmen, ibland i kombination med stress som leder till att magnesiumhalten i plasma sjunker ytterligare beroende på att magnesium transporteras in i cellerna. En låg magnesiumhalt i plasman kan leda till en sänkt magnesiumhalt i cerebrospinalvätskan, som i sin tur är kopplat till kramper. Även subkliniska brister av både kalcium och magnesium kan ge problem, som dock är svårare att upptäcka. Fodrets innehåll av bl.a. kalcium, magnesium och kalium påverkar risken för att kor ska drabbas av både akut och subklinisk brist av kalcium och magnesium, och syftet med avhandlingen var att studera hur kalcium- och magnesiumomsättningen hos mjölkkor kan påverkas och underlättas.

10.2 Sammanfattning av studierna

Avhandlingen är uppbyggd av fyra olika studier. Tre av dem gjordes i försöksbesättningen på Kungsängens forskningscentrum i Uppsala, och en gjordes med hjälp av data från Svensk Mjölk och en enkät utskickad till utvalda svenska besättningar med mer än 45 kor. I den första studien användes 6 kor i laktationsmånad 6 eller senare för att undersöka effekterna av ett högt kaliumintag på magnesiumupptaget hos lakterande kor. Försöket var upplagt med en changeover design, med tre nivåer av kalium (19, 28 och 37 g per kg torrsubstans) och två nivåer av magnesium (1.9 och 4.3 g per kg torrsubstans), och alla kor fick varje behandlingskombination under två veckor. I det andra försöket ingick 29 dräktiga kor under sista delen av sinperioden och fram till en vecka efter kalvning. Effekten av kalciumintag (4.9, 9.3 och 13.6 g kalcium per kg torrsubstans) under sista delen av sinperioden på kalcium- och magnesiumbalansen runt kalvning undersöktes. Den tredje studien gjordes för att hitta samband mellan utfodringen under sinperioden och risken för kalvningsförlamning. Genom en enkät erhölls information om utfodringen under sista delen av sintiden i 30 besättningar med hög andel veterinärbehandlade kalvningsförlamningar och 22 besättningar med låg andel veterinärbehandlade kalvningsförlamningar. I det fjärde försöket mjölkades 9 kor under 1 till 7 dagar före kalvning, och kalciumbalansen jämfördes med den hos 6 kor som bara mjölkades efter kalvning.

10.3 Resultat

Resultaten från studierna tydde på att höga kaliumhalter i foderstaten under laktationen inte medförde sämre upptag av magnesium vid de kaliumhalter som användes. Högre intag av magnesium ledde till ett ökat upptag av magnesium, något som resulterade i en ökad magnesiumutsöndring i urinen. Beräknat som en andel av magnesiumintaget var upptaget dock sämre vid det högre intaget. En sänkt kalciumhalt i foderstaten innan kalvningen minskade inte risken för hypokalcemi och kalvningsförlamning, åtminstone inte när kalciumhalten låg mellan 4 och 13 g per kg torrsubstans. Däremot påverkade höga kalciumhalter magnesiumupptaget negativt, och eftersom magnesium är viktigt för att parathormon ska utsöndras och fungera effektivt kan höga kalciumhalter väntas ha en indirekt påverkan på risken för kalvningsförlamning. Ett högt magnesiumintag, omkring dubbla mängden mot NorFors rekommendation under de sista tre veckorna av sinperioden minskade risken för kalvningsförlamning i besättningen, medan ökande intag av kalium ledde till ökad risk för kalvningsförlamning i besättningen. Kalcium- och magnesiumutsöndringen via mjölken påverkades framför allt av mängden mjölk kon producerade, och den första råmjölken innehöll högre halter av både kalcium och magnesium än mjölk senare i laktationen. De kalciumförluster som uppstod via mjölken när laktationen startade ledde till en påfrestning på kalciumomsättningen, men mjölkning före kalvning förberedde inte kon på kalciumförlusterna vid kalvningen, eftersom kalciumhalten i blodet sjönk till samma nivå som hos kor som inte var mjölkade före kalvningen. Det verkade finnas ytterligare faktorer mer än enbart förlusten av mjölk som påverkade kalciumbalansen runt kalvning eftersom kalciumhalten hade sjunkit betydligt redan en timme efter kalvningen, samtidigt som nedbrytningen av benvävnad, och därigenom förmodligen frisättandet av kalcium, hade ökat markant.

10.4 Slutsatser

De slutsatser som kunde dras var att högt kaliumintag, i intervallet 19 – 37 g per kg torrsubstans, inte spelar någon roll för magnesiumupptaget hos lakterande kor. Däremot bör sinkornas kaliumintag begränsas, i alla fall under den sista delen av sinperioden, för att förebygga problem med kalvningsförlamning. Magnesium bör utfodras på en högre nivå än de gällande rekommendationerna för att förebygga problem med kalvningsförlamning, medan variationer i kalciumnivån i sintidsfoderstaten inte spelar så stor roll vare sig för graden av hypokalcemi vid kalvning eller för risken för kalvningsförlamning. För att underlätta sinkornas upptag av magnesium bör kalciumintaget dock inte vara alltför högt. Att mjölka korna ledde till en påverkan på kalciumbalansen, men eftersom det verkade finnas andra faktorer än urmjölkning som också påverkade plasmahalten av kalcium vid kalvning är det osäkert hur stor påverkan urmjölkningen har på risken för kalvningsförlamning.

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