Calcium homeostasis at calving in cows milked prepartum

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Sabine Ferneborg

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Handledare: Cecilia Kronqvist
Examinator: Kjell Holtenius

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Abstract

At calving, and the initiation of milking, dairy cows suffer a great loss of calcium to milk and a difficulty in maintaining calcium homeostasis. Often, this is too large of a challenge and the cow suffers a condition mostly known as milk fever, or parturient paresis. Clinical parturient paresis can cause paralysis, coma and even death, and the subclinical condition predisposes the cow to other diseases. There are many methods of prevention of the condition, one of them is prepartum milking.

The aim of the present study was to investigate the effects of prepartum milking on calcium homeostasis around calving and to study the effects of a sudden loss of calcium via milk on calcium homeostasis independent of calving. It also included analysis of milk composition to compare the milk before and after calving. In total, fourteen multiparous cows of the breed Swedish Red were used, whereof nine were milked prepartum and five were used as controls. Blood and urine samples were collected from 20 days prepartum to 7 days postpartum. Milk samples were collected at all milkings and milk yield was registered. Blood samples were analyzed for calcium content, CTx (C-terminal telopeptide of collagen, used as a marker of bone resorption) and PTH (Parathyroid hormone). Urine samples were analyzed for pH and calcium content and milk samples were analyzed for fat, protein, density, lactose, IgG (immunoglobulin G), calcium content and somatic cell count.

The results indicate that prepartum milking resulted in lower calcium excretion in milk in early lactation, but also lower calcium levels in blood, elevated PTH levels and increased bone resorption on the day of calving. Bone resorption was not affected until after calving in prepartum milked cows. The milk excreted before calving had higher density and higher concentration of most components than the milk after calving, and prepartum milking resulted in a lower initial milk yield. It was also concluded that a sudden loss of calcium to milk before calving resulted in lower calcium levels in blood during the first seven days of milking. It seems that despite substantial milk yield prepartum, no positive effects were found that could overshadow the effect of calving on calcium homeostasis and milk production.

Sammanfattning

I samband med kalvning genomgår en mjölkko stora förändringar. Hon går från att vara dräktig till att producera stora mängder mjölk. På grund av allt kalcium som utsöndras i mjölken får kon svårt att bibehålla kalciumbalansen i kroppen. Om hon misslyckas kan hon drabbas av så kallad kalvningsförlamning, eller pares. Både den kliniska och den subkliniska svårighetsgraden av sjukdomen kan ha allvarliga konsekvenser. Klinisk pares kan leda till förlamning, koma och ibland även död, och subklinisk hypokalcemi gör kon mer mottaglig för andra sjukdomar. Många metoder finns för att försöka hindra för grav kalciumbrist, en av de mindre vanliga är mjölkning före kalvning.

Syftet med studien var att undersöka effekterna av mjölkning före kalvning på kalciumbalansen och att studera effekterna av en plötslig kalciumförlust via mjölk på kalciumbalansen oberoende av när kalvningen inträffade. Totalt användes 14 kor av rasen SRB, varav nio mjölkades före kalvning och fem användes som kontroller. Blod- och urinprov samlades in från 20 dagar före beräknad kalvning till 7 dagar efter kalvning. Vid varje mjölkning mättes mjölkmängden och mjölkprov togs. Blodproven analyserades för kalciuminnehåll, CTx (C-terminal telopeptid, använd som markör för benresorption) och PTH (Parathormon). Urinprov analyserades för pH och kalciuminnehåll och mjölkprov analyserades för fett, protein, densitet, laktos, IgG (Immunoglobulin G), kalciuminnehåll och celltal.

Resultaten tyder på att mjölkning före kalvning gav lägre kalciumutsöndring i mjölk i början av laktationen, men också lägre kalciumhalter i blod, förhöjda halter av PTH och ökad benresorption vid kalvning. Benresorptionen påverkades inte förrän efter kalvning hos kor mjölkade före kalvning. Mjölken som utsöndrades före kalvning hade högre densitet och högre koncentration av de flesta näringsämnen än mjölken som utsöndrades efter kalvning, och mjölkning före kalvning gav en lägre mjölkavkastning initialt. Vi kunde också dra slutsatsen att en plötslig kalciumförlust till mjölk före kalvning gav lägre kalciumnivåer i blod under de första sju dagarna av laktationen.

Introduction

It seems impossible to discuss calcium homeostasis in dairy cows without mentioning, and to some extent focusing on, parturient paresis. The condition is also known as milk fever, and is characterized as clinical hypocalcaemia leading to paralysis and in severe cases coma. The incidence of the condition varies largely between farms and years, but can rise up to 90% or more. Though, 95 % of cows that receive medical treatment are fully cured (Underwood & Suttle, 2001).

It is well known that the calcium level in the blood of cows decreases at calving when the milk production starts. Before calving, the cow has a requirement for maintenance and an added requirement for fetal growth. After calving, the addition for pregnancy is exchanged for an addition for the milk produced. This increases the total need of calcium by approximately 100 %. The loss of calcium is compensated by increased uptake from the feed, reduced excretion in urine and increased mobilization from the skeleton. If the loss of calcium is not compensated for properly, the cow is at risk of periparturient paresis, since calcium is crucial for nerve and muscle function. In less severe cases of calcium deficiencies reduced feed intake, poor rumen and intestine motility, poor productivity and increased susceptibility to other diseases can be seen.

The incidence of hypocalcaemia and parturient paresis is affected by many factors, among others the balance of other minerals in the body, feeding, age, milk yield and lactation number, and the methods of improving calcium homeostasis are numerous. Most of these methods are focused on feeding, recommending either a high or a low intake of calcium and other minerals, and some are more focused on injections, oral drenching or milking. Prepartum milking is a method that aims to stimulate the cow to activate the calcium homeostatic mechanisms before calving, preparing the cow for the challenge of milk production in time. Milk production is often considerably lower prepartum than postpartum, which makes the strain on the homeostatic mechanisms lower at this time. The results of prepartum milking have been varying between studies, as some have found it successful and some have seen no effect. Most studies have focused on milking cows a certain number of days before calving rather than on trying to get a high quantity of milk at the first milking. A higher milk yield imitates the postpartum initiation of milking, and causes a sudden loss of calcium similar to what is seen around calving. However, it is uncertain how the milk composition is affected.

Aim and Hypothesis

The aim of this study was to investigate the effects on milking prepartum on calcium homeostasis around calving in dairy cows. We also wanted to study the effect of a sudden loss of calcium to milk on calcium in blood independent of calving, and to investigate differences in milk composition between prepartum milked and control cows.

The hypothesis was that cows milked prepartum would have improved calcium homeostasis at calving and thereby reduced risk of hypocalcaemia. We also expected that the milk from prepartum milked cows would have a colostral character until a few days after calving.

Literature survey

Calcium

Calcium ions play a very important role in many fundamental biological processes in the body, such as muscle contraction, blood coagulation and hormone release, and are important structural components of the skeleton (Rosol & Capen, 1997). Therefore it is of major importance that the plasma concentration is kept within a narrow range. Most of the calcium (99%) in the body is stored in bone, and only a minor part (0.1%) is present in the extracellular fluid. The remainder is isolated in the plasma membrane and in the endoplasmic reticulum.

In cows' milk, the concentration of free calcium (Ca^{2+}) is approximately 4 mmol/l, and the concentration of total calcium, free and bound, is approximately 30 mmol/l (Sjaastad *et al.*, 2003). In colostrum, the calcium concentration is higher, approximately 42-58 mmol/l (Goff, 2000). The total concentration of calcium in blood plasma is maintained at 2.1-2.5 mmol/l (Goff, 2008) in the adult cow. The lowest calcium concentration in blood can be expected 12 to 24 hours after calving.

To maintain calcium homeostasis after calving, at the start of lactation, the cow will increase reabsorption in the kidneys, increase absorption in the intestine and withdraw calcium from bone (Goff, 2008). In the first month of lactation, the cow is expected to lose 9-13 % of her bone calcium in attempt of restoring calcium homeostasis. Bone calcium mobilization and renal reabsorption are both induced by the secretion of parathyroid hormone (PTH) which is produced when a decline in blood calcium appears. The increased intestinal absorption is induced by 1,25-dihydroxyvitamin D, which is produced in the kidneys in response to increased PTH levels. The urinary excretion of calcium is normally low and therefore only small amounts can be saved from the urine.

Feeding requirements of calcium

In the periparturient period, the need of calcium from feed changes to a large degree in dairy cows. From late pregnancy to 20 kg production of ECM (energy corrected milk), the requirement of calcium can be doubled (NRC, 2001; Spörndly, 2003). For example, a cow with 500 kg body weight requires 43 g calcium per day the last month of pregnancy, and 80 g calcium per day when producing 20 kg ECM. According to Swedish feeding recommendations, 2.6 g of calcium should be added per kg ECM (Spörndly, 2003). American recommendations claim an increased uptake of 2.1 g per kg FCM (fat corrected milk) for colostrum and 1.22 – 1.45 g calcium per kg FCM depending on breed, where the Jersey breed requires most (NRC, 2001).

Hypocalcaemia

If the calcium level decreases to below the normal concentration, the cow is suffering from subclinical (blood calcium 1.4-2.0 mmol/l) or clinical (blood calcium <1.4 mmol/l) hypocalcaemia, also known as milk fever or parturient paresis (DeGaris & Lean, 2007).

Ender et al (1971) divided the symptoms of hypocalcaemia into three categories depending on the severity of the condition. Slight symptoms include reduced appetite, dullness, relaxation

and an increased preference for lying down. Borderline symptoms also include cold ears and horns, depression, paralysis of the hind quarters, and an abnormal reclining position. When clinical symptoms are shown, the surface temperature of the animal is lowered, the respiration is groaning and there is a risk of coma. Also, rumino-intestinal stasis is seen, and the animal is unable to rise.

It has been shown that hypocalcaemia during the periparturient period is an effect of the calcium drain to milk (Goff *et al.*, 2002; Kume *et al.*, 2003). Mastectomy completely eliminates the risk of hypocalcaemia around parturition if only the intake of calcium is sufficient (Goff *et al.*, 2002). When understanding the cause of hypocalcaemia, contributing factors can be identified. The two most important factors that may impair the calcium homeostasic mechanisms to a level where hypocalcaemia is induced are metabolic alkalosis and hypomagnesaemia (Goff, 2006).

Both subclinical and clinical hypocalcaemia can have secondary effects on cows' health. Kimura *et al.* (2006) showed that parturition and hypocalcaemia leads to an immunosuppression through blunting of the calcium signals in immune cells. Due to the lowered immune function, the risk of mastitis and metritis is increased and reproduction is affected negatively (Mulligan *et al.*, 2006). The decrease in muscle function due to hypocalcaemia also reduces the motility of the rumen and gastro-intestinal tract, and thereby the feed intake, which increases the risk of displaced abomasum, ketosis and fatty liver, reduces the milk yield and affects reproduction.

Influence of age and parity

Several studies have shown an influence of age and parity on calcium homeostasis around calving (Kume *et al.*, 2003; Špakauskas & Klimienė, 2007; Quiroz-Rocha *et al.*, 2009). Cows in higher lactations have lower plasma calcium levels both pre and post partum (Quiroz-Rocha *et al.*, 2009), and in some cases higher plasma PTH (Kume *et al.*, 2003). Along with an increase in age, the number of active osteoclasts is decreased, giving fewer cells that can respond to an increase in PTH and thereby a lowered ability to mobilize calcium from bone (Goff, 2000). Other contributing factors to the effect of ageing might be the increased milk production that comes with advancing age, reduced active transport of calcium in the intestine and impaired production of 1,25-dihydroxyvitamin D.

Control principles

Several methods have been used to prevent and treat milk fever. Most of these are principles that are applied during the dry period and late pregnancy, and some are applied after calving. A review by Thilsing-Hansen *et al.* (2002) listed the used control principles for prevention as follows:

1. Oral drenching with calcium around calving
2. Acidifying diets
3. Diets low in calcium
4. Administration of vitamin D, vitamin D metabolites and analogues before calving
5. Control of dietary magnesium
6. Control of body condition
7. Control of carbohydrate intake
8. Shortening of the dry period
9. Prepartum milking

10. Reduced milking in early lactation

Where number 1-3 seems to be the most commonly used in Scandinavia.

Oral drenching of calcium around calving

Oral drenching of calcium can be used around calving as a preventative measure or as a supplement to intravenous injections for cows already affected by parturient paresis (Thilsing-Hansen *et al.*, 2002). It is supposed to maintain calcium homeostasis until the homeostatic mechanisms have been activated and is in full function. Used as a prophylactic, the drenching is performed during the high risk periods, often including four doses. If used as a supplement, one or two doses is recommended. The bolus, gel, paste or liquid often contains easily absorbed calcium salts, and should provide free ionized calcium. Free ionized calcium is easily absorbed in the rumen and abomasum, but bound calcium is absorbed in the small intestine, and is dependent on functioning homeostatic mechanisms for increased absorption. The method has been shown to be successful and to have few drawbacks.

Hypocalcaemia and other minerals

The incidence of hypocalcaemia has been shown to be influenced by other minerals, and the feeds content of these in the pre partal period. The effect of other minerals is generally indirect, by affecting the alkaline or acidic effect of the diet, measured by the dietary cation-anion difference (DCAD). Some minerals, like magnesium, affect the DCAD but also have a direct effect on hypocalcaemia. Therefore several equations have been used to calculate the DCAD, some involving Mg, P and Ca, and some excluding one or several of these minerals. The risk of hypocalcaemia is increased by a positive DCAD (alkalinity) and reduced by a negative DCAD (acidity) (Thilsing-Hansen *et al.*, 2002). Lean *et al.* (2006) showed that the only statistically significant equation for calculating DCAD does not contain either Mg, P or Ca, but only Na, K, Cl and S, which are minerals that do not have a direct effect on calcium homeostasis. A linear relationship between DCAD and hypocalcaemia was found, and Lean *et al.* (2006) developed an equation for predicting the risk of milk fever from the feed composition. The two logit transformations (LT) are statistically equivalent and biologically plausible.

$$LT\,1 = -5.76 + 5.48\,(Ca) - 5.05\,(Mg) + 1.85\,(P) + 0.02\,(DCAD) - 2.03\,(Ca^2) + 0.03\,(Exposure\,time)$$

Where DCAD = (Na + K) - (Cl + S) in meq/100 g DM
Ca, Mg, P, K, Cl and S are expressed as percentages of DM
Exposure time = exposure in days

$$LT\ 2 = -5.17 + 5.74\,(Ca) - 8.66\,(Mg) + 2.30\,(P) + 0.78\,(K) - 2.16(Ca^2) + 0.04\,(Exposuretime)$$

$$Milk\ Fever\% = \frac{e^{LT}}{(1+e^{LT})} \times 100$$

Here, Ca, Mg and P are included but with special regard to their direct effect on the calcium homeostasis of the animal. Also, an effect of the exposure time to a certain diet is included. As reference breed, the Holstein-Friesian was used, but different coefficients can be used to include the effect of breed in the equation.

Using these formulas, the increased risk of milk fever due to a change in feeding can be predicted. For example, an increase in calcium concentration in the feed from 0.5 to 0.6 % while maintaining all other variables will increase the calculated risk of milk fever by 37 %, and an increase from 0.5 to 1.0 % will increase the risk by 327 %. This indicates that a high calcium concentration in feed will affect the milk fever incidence independent of the acidity of the diet. In these equations, the impact of other minerals than calcium is also included. While maintaining all other variables, both the magnesium and phosphorus content will affect the milk fever risk. An increase in Mg will decrease the risk, while an increase in P will increase the risk of milk fever. Contrary to Lean *et al.* (2006), Ender *et al.* (1971) recommended a high calcium concentration in the feed prepartum, as the incidence of milk fever was slightly lower in cows fed supplemental $CaCO_3$ in combination with both alkaline and acidifying diets.

Metabolic alkalosis and hypocalcaemia

Already in 1947, Craige and Stoll showed that metabolic alkalosis has an effect on parturient paresis, and concluded that hypocalcaemia and alkalosis are closely connected. Ender *et al.* (1971) found that among cows fed an alkaline diet, 60 % were considered clinical cases of hypocalcaemia, 10 % were borderline and 20 % showed slight symptoms. Only 10 % were considered healthy. Among cows fed an acidifying diet 85 % were considered healthy, while 15 % showed slight symptoms. None of these cows were considered borderline or clinical.

Metabolic alkalosis is due to a high cation-anion balance in the diet, which increases pH in the blood. As pH rises, the PTH receptor is believed to undergo a conformation change (Goff, 2008), rendering the blunting of the tissue response to PTH that can be seen in these cases (Gaynor *et al.* 1989). The desensitizing in bone prevents bone resorption, and in the kidneys the reabsorption is reduced (Goff 2008). Following this, the kidneys fail to produce 1,25-dihydroxyvitamin D, which prevents the increase of intestinal absorption of calcium. In the end, the metabolic alkalosis hinders all important systems for restoring calcium homeostasis, and thereby the cow is less able to reverse hypocalcaemia on her own.

Hypomagnesaemia and hypocalcaemia

Hypomagnesaemia has an effect on calcium homeostasis by reducing the effect of PTH on target tissues, rendering a reduced production of adenylate cyclase and cyclic-AMP, which acts as a second messenger and also requires magnesium for full activity (Goff, 2008). It also seems that hypomagnaesemia can reduce the secretion of PTH due to hypocalcaemia (Littledike *et al.*, 1981).

Urine pH as an indicator

Urine pH is negatively correlated to the urinary calcium concentration (Constable *et al.* 2009) and reflects the acid-base status of an animal (Seifi *et al.*, 2004). Therefore it has been shown to be a good indicator of the risk for hypocalcaemia. When sampling cows within 48 hours prior to parturition, the difference in urine pH and serum calcium is significant between healthy cows and cows that later develop hypocalcaemia. The same difference is found for cows sampled 1-7 days before calving. During the last 48 hours prior to parturition, a negative correlation between urine pH and serum calcium has been found. Also, serum calcium is lower and urine pH higher during 48 hours prior to parturition in cows that develop hypocalcaemia compared to 7-2 days before calving. As a cut off point for urine pH, 8.25 is suggested. Using this cut off point, the cows with highest risk of developing hypocalcaemia is found, with a small risk of false positives.

High vs. low feeding of calcium

Ender *et al.* (1971) claim that the content of calcium in feed is of less importance than DCAD on calcium homeostasis around calving, but a low calcium feeding level is often recommended to prevent hypocalcaemia. The theory behind this is that the cow is forced to activate her calcium mobilizing systems before calving, making it easier for her to cope with the challenge of lactation after calving (Goff 2008; Kichura *et al.*, 1982). Contrary to this, a high calcium feeding level may also be beneficial (Oetzel *et al.*, 1988). The idea behind this is that the calcium stores should be increased via passive absorption and protect against hypocalcaemia in combination with a high feeding level of calcium post calving (DeGaris & Lean, 2009).

In the meta-analysis by Lean *et al.* (2006) the milk fever risk, as calculated by above mentioned equations, forms a bell curve with reduced risk for diets containing high or low levels of calcium (see Fig. 1). In agreement with this, Oetzel (1991) found the highest risk of milk fever at calcium feeding levels of 1.16 % of DM. Though, few studies have been performed on high calcium feeding levels, which increases the uncertainty.

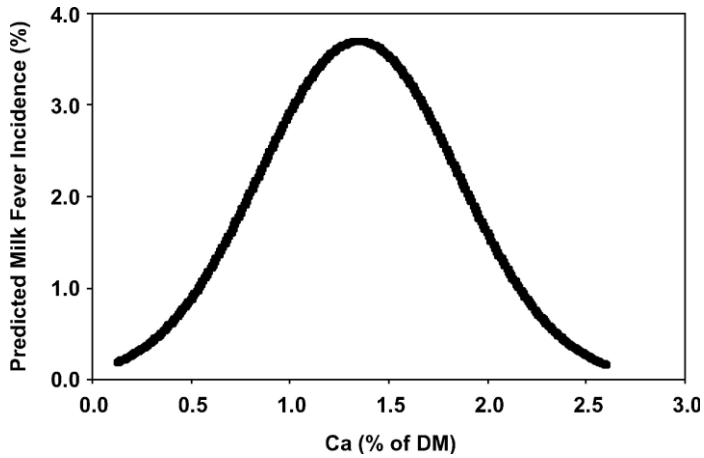

Figure 1. Predicted milk fever risk with different feeding levels of calcium (Lean *et al.*, 2006).

Along with the effect of calcium feeding on the milk fever risk, there is also an interaction with exposure time. DeGaris & Lean (2009) found that short exposure to a pre-calving diet high in dietary calcium increases the risk of milk fever markedly. Also, long exposure to a pre-calving diet low in calcium increases the risk. The highest risk of milk fever was found with 1.1-1.3% calcium in the diet together with a long exposure time.

Vitamin D and its metabolites

The major sources of vitamin D are feed and absorption in the skin (Horst, 1984). The main circulating form of vitamin D is as 25-hydroxyvitamin D, which can be converted to 1,25-dihydroxyvitamin D in the kidney. Together with PTH, 1,25-dihydroxyvitamin D regulates calcium and phosphorus homeostasis in the body through increasing the intestinal absorptions of Ca and P, and is also important for the function of PTH on bone (Capen & Rosol, 1989).

Injections or implantations of vitamin D, its metabolites or analogues have been proved efficient in preventing milk fever (Goff, 2008). In a study performed by Goff and Horst (1990), pellets with 24F-1,25-dihydroxyvitamin D were implanted in cows 7 days before expected parturition to investigate the effect on milk fever. Cows with implants showed an

incidence of milk fever of 9 %, compared to control cows, which showed an incidence of 80 %. Comparing the plasma calcium concentration around calving, there was a significant difference between the two groups. Among the cows with implants, the mean plasma calcium concentration was higher before as well as after calving.

Though both injections and implants of vitamin D has been proven efficient, the method is associated with some problems; timing, toxicity and delayed milk fever (Goff & Horst, 1990; Thilsing-Hansen *et al.*, 2002). The timing is crucial for the prevention of milk fever as the substance must be administered at least 24 hours, but maximum 6 days prepartum to ensure protection. Since calving doesn't always occur on the expected date this can be a major problem. In case of delayed calvings, several injections can be administered, with the risk of the cow suffering from hypercalcaemia due to the sudden increase in plasma 1,25-dihydroxyvitamin D that is seen 48-72 hours after the injection.

Since the dose of vitamin D that is required for prevention of milk fever is close to the toxic dose, toxicity is another problem related to injections of vitamin D (Goff & Horst, 1990; Thilsing-Hansen *et al.*, 2002). The risk could possibly be reduced using an implant instead of injections, which could excrete vitamin D at a slow, constant rate before calving. Delayed milk fever, occurring 1 to 2 weeks after calving, is also a problem associated to administration of vitamin D (Goff & Horst, 1990; Thilsing-Hansen *et al.*, 2002). In some cows, the endogenous production of 1,25-dihydroxyvitamin D is inhibited due to exogenous treatment with vitamin D metabolites, which can be predisposing to milk fever when the exogenous 1,25-dihydroxyvitamin D is cleared from the body. A solution for this might be a gradual decrease in exogenous 1,25-dihydroxyvitamin D.

Parathyroid Hormone (PTH)

The effects of PTH can be divided into acute and long term effects. The acute effects include increased calcium release from bone by stimulation of calcium releasing bone cells (osteoblasts and osteoclasts), increased reabsorption of calcium in the kidneys and thus reduced excretion in urine. The long term effects include increased bone resorption by stimulation of osteoclasts and increased intestinal absorption of calcium and phosphate by increased synthesis of vitamin D. During a Ca^{2+} concentration of 1,0-1,25 mmol/l in extracellular fluid, PTH is secreted constantly at a moderate rate (Sjaastad *et al.*, 2003). Below the mentioned interval, PTH secretion increases rapidly, reaching a maximum at a Ca^{2+} concentration of 0,5-0.7 mmol/l. At Ca^{2+} concentrations of 1.25 mmol/l or more, PTH secretion is reduced to a minimum.

The serum levels of PTH for healthy cows and cows with hypocalcaemia have been found to not differ significantly, except for severe cases (Kurek & Stec, 2005). The level of PTH in serum for healthy cows was 19.84 pg/ml compared to 19.61, 13.35, 25.09 and 6.84 for cows with increasing severity of the disease. The groups with the lowest PTH-levels (13.35 and 6.84) consisted of cows that fell into coma and cows that did not respond to treatment. In the cows that showed less severe symptoms, the cows' own systems for regaining calcium balance was activated and functioning, as indicated by the PTH levels. The cows with the lowest PTH-levels were unable to keep calcium at a level where clinical symptoms are prevented, and were therefore more severely affected. This could be due to an overfeeding of calcium prepartum, which stimulates the release of calcitonin and thereby inhibits PTH, or an effect of disturbances in the body that results in lower secretion of the hormone. The blood calcium levels differed between cows, and did not seem to be correlated to the PTH levels.

The lowest calcium levels were found in cows with less severe symptoms of hypocalcaemia, while the highest levels were found in healthy cows and cows that suffered severe symptoms.

C-terminal telopeptide (CTx)

C-terminal telopeptide (CTx) is released as a result of degradation of type 1 collagen and can be used as a marker for bone resorption (Christensson, 1997). Holtenius & Ekelund (2004) showed initial CTx levels in plasma of 40-50 nmol/l after calving, which was followed by a steady decrease for the coming 33 weeks and continuously low levels up until next calving. Interestingly, the decrease did not follow the lactation curve, indicating that the bone turnover is not controlled by calcium loss through milk alone, but may to a larger extent be affected by estrogens.

Serum CTx has been found to increase significantly from 14 days prepartum to 10 days post partum (Stojević *et al.* 2009), and to be negatively correlated to oestradiol (Filipović *et al.*, 2008). The negative correlation is indicative of the role of oestradiol on bone metabolism around calving.

Starič *et al.* (2008) found that primiparous cows in peak lactation had higher CTx values (1.01 versus 0.78 ng/ml) than fourth or higher parity cows in peak lactation. This indicates a more intensive bone metabolism and an ability to more effectively release calcium in the younger animals.

Milking prepartum

Prepartum milking and hypocalcaemia

Prepartum milking has in some studies been shown to decrease the incidence of clinical hypocalcaemia (Greene *et al.*, 1988), but in others to not affect the incidence at all (Smith & Blosser, 1947; Eaton *et al.*, 1949). Greene *et al.* (1988) showed a hypocalcaemia incidence of 6.1 % in prepartum milked cows compared to 11.4 % in postpartum milked cows. In the same study, 31 % of cows milked prepartum yielded more than 9 kg/day, 33 % 4.5-9 kg/day and 36 % less than 4.5 kg/day on the day before calving. Cows that yielded more the day before calving tended to have a higher peak yield and a higher milk yield postpartum than cows that yielded less, but overall no effect of prepartum milking on lactation milk yield was found (Greene, 1987). Smith & Blosser (1947) found that cows that developed hypocalcaemia had lower mean milk yields the day before calving.

Eaton *et al.* (1949) and Smith and Blosser (1947) agree that other factors than lactation seem to be involved in the changes in serum calcium and hypocalcaemia incidence at calving, while Goff *et al.* (2002) claims that the major cause of hypocalcaemia is the calcium drain due to lactation.

Factors affecting milk production prepartum

During late pregnancy, the level of progesterone in blood is high, which reduces the binding of prolactin to the receptors that are located on the mammary epithelial cells (Sjaastad *et al.*, 2003). At parturition, progesterone levels falls to near zero, increasing the ability of prolactin to bind to receptors. Also, the levels of prolactin increases by 5-10 times. The increased levels of prolactin increase the production of α-lactalbumin, which increases milk production.

When prepartum milked, cows can be divided into responsive and non-responsive based on yield before calving (Rowland *et al.*, 1953; Malven *et al.*, 1987; Greene *et al.*, 1988). This has also been seen in heifers (Walsh & Downey, 1971). In most studies, around 50 % of cows are classified as responsive to prepartum milking (Rowland *et al.*, 1953; Malven *et al.*, 1987). In responsive cows, lactogenesis is initiated by the prepartum milking, while in non-responsive cows, lactogenesis is not initiated until after parturition (Malven *et al.*, 1987). This gives a difference in milk yield, as the responsive cows show a significantly larger increase in milk yield prepartum. Also, the prepartum secretion is in all cows colostral at first, but in responsive cows the composition reaches that of normal milk faster (Rowland *et al.*, 1953). In non-responsive cows, the milk remains colostral until after calving.

Milk composition

The composition of the postpartum excreted milk has been shown to differ significantly between prepartum milked and control cows (Rowland *et al.* 1953; Zeliger *et al.*, 1972). The concentration of solids-non-fat, protein, ash, phosphorus, chloride, casein, albumin, globulin and proteose-peptone was lower and the concentration of lactose higher in the milk of prepartum milked cows. During the prepartum period, the actual yield of the constituents mentioned above increased as the yields increased, with the exception for globulin.

The immunoglobulin content of milk is important because of the passive transfer of antibodies from mother to young. Zeliger *et al.* (1972) investigated the effect of prepartum milking on the yield and content of immunoglobulins. The prepartum milk secretion contained high levels of immunoglobulins until calving, when it decreased. 14 days prepartum, 60% of the total protein consisted of immunoglobulins. The content then decreased to 25% at the day of calving, and two days after calving the proportion had decreased to 3.3%. Despite the decrease in concentration, the total amount of immunoglobulins did not differ from that of control cows, since the milk yield was significantly higher, and all prepartum milked cows yielded about 1.6 kg of immunoglobulins before calving.

Materials and Methods

In total, fourteen multiparous cows of the breed Swedish Red were studied in the weeks prior to and immediately after calving. The cows were divided into two groups;

- PPM: cows milked prepartum (9 cows)
- Control: control cows (5 cows)

All cows were fed grass silage or a mix of corn and grass silage combined with pelleted commercial concentrate and housed in a traditional tied-up stall. During the last three weeks prior to calving, blood and urine samples were collected twice a week on all cows. Blood samples were collected at 6, 12 and 24 hours after calving, and in the morning on day 2, 4 and 7. All blood samples were collected from the tail vein in heparin tubes, and centrifuged for 10 minutes at 1800×g before the plasma was separated and frozen until further analysis. On PPM cows, blood samples were collected at 6 and 24 hours after the initiation of milking, and thereafter in the morning every other day up until calving, whereafter the same pattern of sampling was used as for cows in the control group.

Urine samples were collected 24 hours after calving, and thereafter in the morning on day 2, 4 and 7. All urine samples were centrifuged (1 min, 1000 rpm) before frozen down until further analysis, also, pH was measured immediately after collection. For PPM cows, urine samples were collected at 24 hours after the initiation of milking, and thereafter in the morning every other day up until calving. After calving, the same pattern of sampling as for cows in the control group was used.

All cows were dried off five weeks prior to the beginning of the sampling period. Starting one week before expected calving, udder fill was controlled daily. When the udder fill was sufficient, some milk was extracted by hand to investigate the viscosity of the secretion. If milk-like, milking was initiated, if not, milking was postponed until the viscosity was reduced. After initiation of milking, all cows were milked twice daily (at 6.30 and 15.30) with regular milking units, and samples were taken from all milkings up until the fourteenth milking after calving. Milk samples were taken in duplicates; one sample was frozen down until further analysis for IgG and calcium concentration and one conservated with bronopol and stored at 4^{o}C.

The experimental design and animal handling procedures were approved by the local ethics committee.

Analytical methods

Blood samples were analyzed for calcium concentration, CTx and PTH. Urine samples were analyzed for calcium content and milk samples were analyzed for fat, protein, density, lactose, IgG (immunoglobulin G), calcium content and somatic cell count. Analysis of PTH, CTx and IgG was made with ELISA kits. IgG analysis was only performed on milk samples collected before calving and at the first 6 milkings after calving. All calcium analyses were made with spectrophotometry using commercial kits (Randox Laboratories Ltd.). All milk samples were diluted with distilled water to 1:20.

Analysis of milk composition was performed using mid-infrared spectroscopy (MilcoScan FT 120), and of somatic cell count using electronic fluorescence based cell counting (Fossomatic

5000). Milk yield was estimated by weighing of the milk or by the machines (DeLaval DelPro™ MU480) milk meter.

Statistical analysis

The data was analysed using PROC MIXED procedures in SAS (SAS institute, 1999). A compound symmetry covariance structure was used for samples within cow. Two different models were used, each with two different time variables. The time variables for plasma/urine parameters were divided into periods. Somatic cell counts were log transformated for better distribution. Due to the three different roughages fed, cows were divided into feed groups. A significance level of 95% was used for all models.

The model used for milk parameters was:

$$Y_{ijklmn} = \mu + (ppm)_i + (milking)_j + (age)_k + (yield)_l + (ppm*milking)_{ij} + (ppm*age)_{ik} + (feed)_m + \varepsilon_{ijklmn}$$

The model used for plasma and urine parameters was:

$$Y_{ijklmn} = \mu + (ppm)_i + (age)_i + (time)_k + (milk)_l + (age*ppm)_{ik} + (age*milk)_{il} + (time*ppm)_{jk} + (ppm*milk)_{kl} + (feed)_m + \varepsilon_{ijklmn}$$

Where

μ = mean value of all observations
ppm = fixed effect of prepartum milking, 2 levels
milking = fixed effect of milking relative to calving or milking number
age = fixed effect of age, 2 levels
yield = fixed effect of milk yield
time = fixed effect of time relative to calving (in periods, 8 levels) or time relative to first milking (in periods, 8-15 levels depending on group)
milk = fixed effect of milking, 2 levels
feed = random effect of feed
ε = random error

Non significant interactions were eliminated from the models.

Results

In average, PPM cows were milked 5 times before calving, ranging between 2 and 14 milkings. Milking was successful and none of the cows in the study developed parturient paresis. The average milk yield the day before calving was 13.4 l, ranging between 6.3 and 25.8 l.

Plasma and urine parameters

Calcium in plasma

The calcium level in plasma differed significantly ($p<0.05$) between control cows and cows milked prepartum (PPM) during the first day of milking and on day 2, 3, 4, 6 and 7 in lactation (see Fig. 2.). Control cows had higher calcium levels in plasma than did PPM cows, except for the first and second day of milking. Also, the lowest level of plasma calcium was seen in PPM cows on day 2 relative to first milking. For calcium level in plasma relative to calving, no differences were found between groups (see Fig. 3).

Younger cows (age = 3 years) had higher levels of calcium in plasma both before ($p=0.044$) and after ($p=0.003$) initiation of milking.

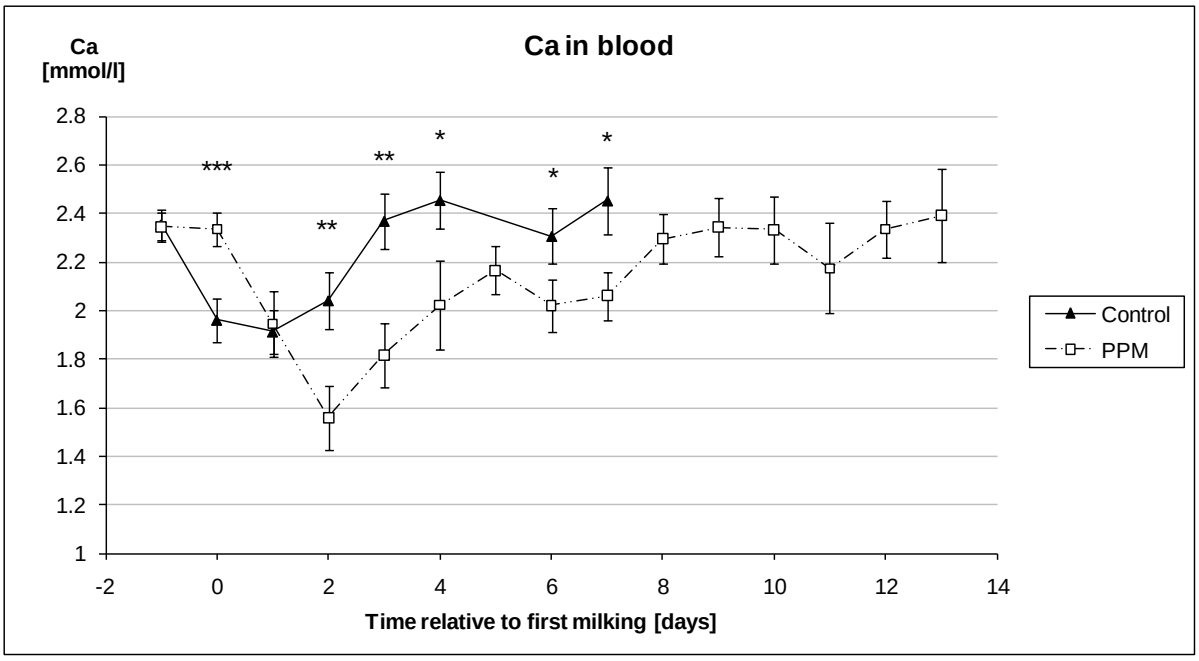

Fig. 2. Calcium levels in plasma for cows milked prepartum (PPM) and control cows (control) in days relative to first milking (LSMeans, error bars display the standard error of the mean. * $p<0.05$; ** $p<0.01$; *** $p<0.001$).

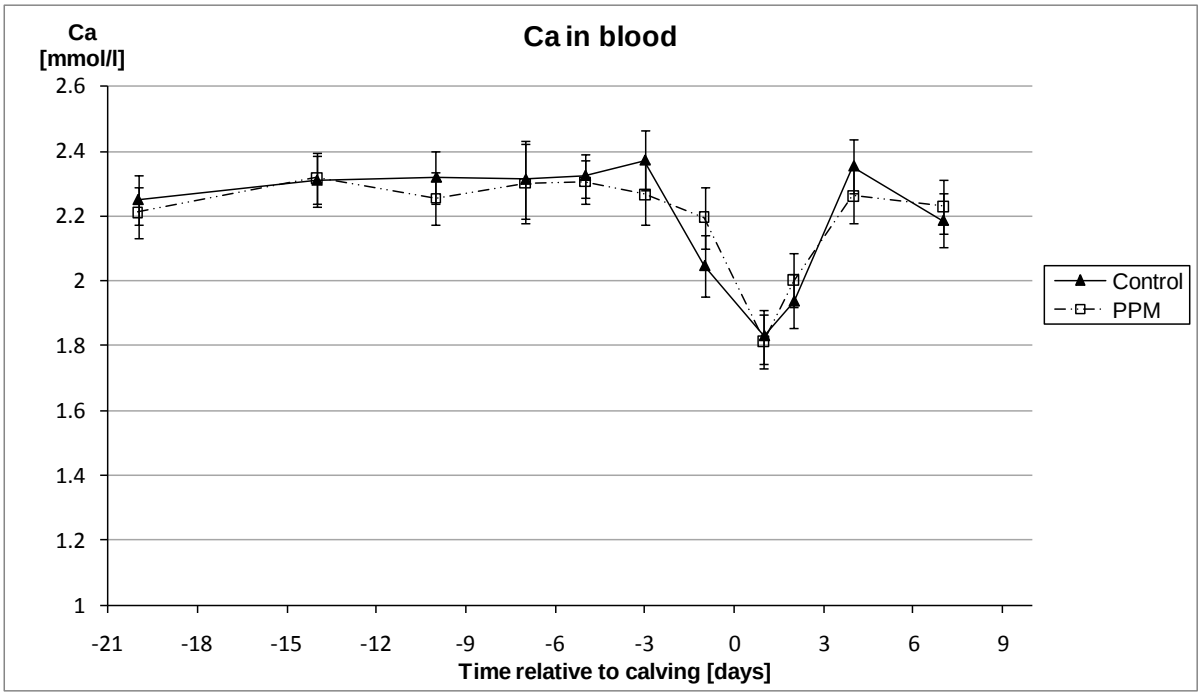

Fig. 3. Calcium levels in plasma for cows milked prepartum (PPM) and control cows (control) in days relative to calving (LSMeans, error bars display the standard error of the mean.)

PTH

Control cows showed a peak in PTH levels at the day of calving and first milking ($p<0.001$) and thereafter an immediate decrease (see Fig. 4). PPM cows showed a slower increase, peaking at day 3 after first milking, which was followed by a decrease. Relative to calving, PPM cows started to increase in PTH levels at the day of calving, reaching a small peak at day 2 followed by a slow decrease. Control cows reached a nadir in day 4 after calving, where there was a significant difference between the groups ($p=0.01$). Thereafter, PPM cows had continuously higher levels than control cows until the end of the trial. There was also an age effect; older cows had higher levels of PTH ($p=0.03$).

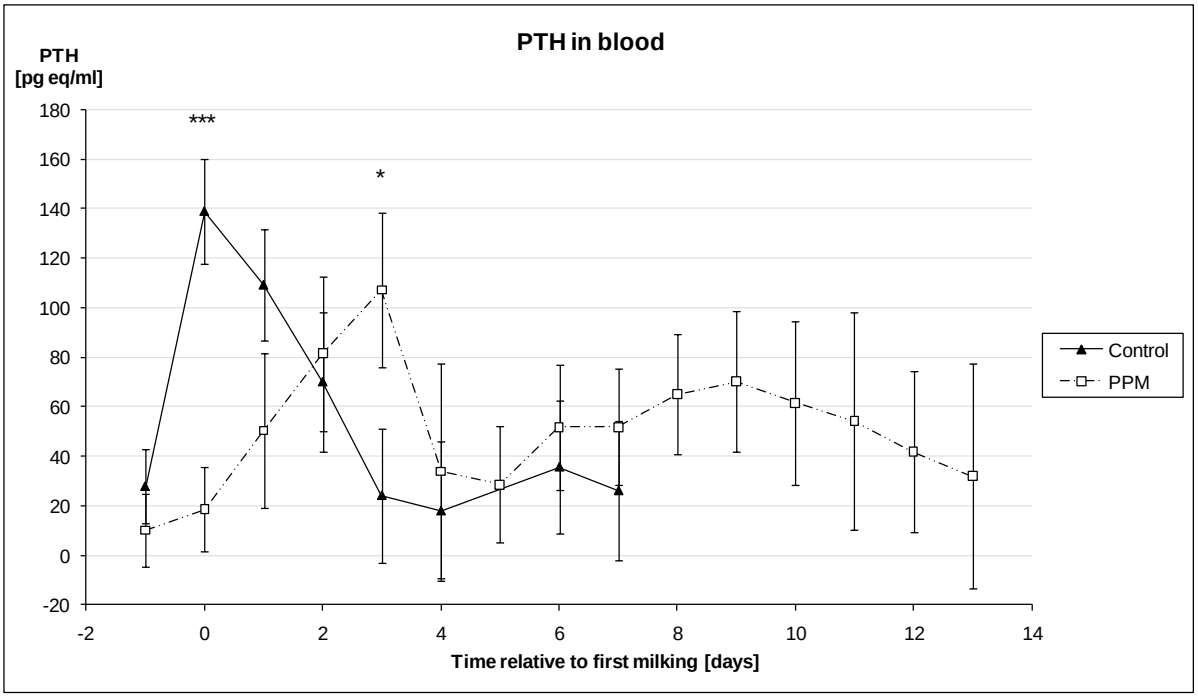

Fig. 4. PTH in plasma from prepartum milked (PPM) and control cows (LSMeans, error bars display the standard error of the mean. * p<0.05; ** p<0.01; *** p<0.001).

CTx

Before calving, the levels of CTx were similar in both groups, but the day after calving, PPM cows displayed a peak (p<0.001) in CTx levels when control cows showed only a small increase (see Fig. 5). There was a low variation in plasma CTx relative to milking in both groups until day 6 and 7 in milk, where PPM cows had a higher elevation in levels than control cows. At day 8 in milk, the levels had decreased to levels similar to those before the increase. There was also a significant effect of age on CTx, younger cows had higher levels (p<0.01).

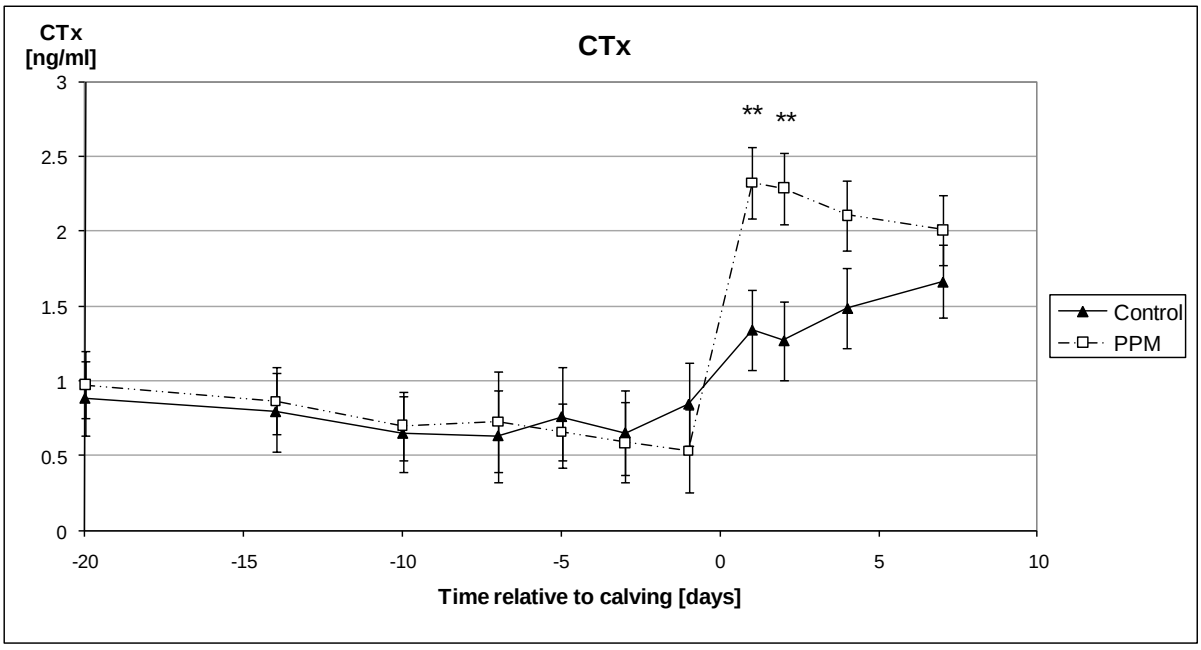

Fig. 5. CTx in plasma from prepartum milked (PPM) and control cows (LSMeans, error bars display the standard error of the mean. * p<0.05; ** p<0.01; *** p<0.001).

Calcium in urine and urine pH

The calcium level in urine decreased significantly between the day before first milking and the subsequent two days ($p<0.05$), and did not differ between groups. PPM cows had slightly higher urine pH, 8.0 compared to 7.9 in control cows ($p=0.024$).

Correlations between plasma and urine parameters

The correlations between plasma and urine parameters are shown in Table 1. Significant correlations were found between calcium in plasma and PTH ($p<0.001$), CTx ($p=0.004$) and calcium in urine ($p<0.001$). A significant correlation was also found between calcium in urine and urine pH ($p<0.001$).

Table 1. Correlations between plasma and urine parameters. $p<0.01$, N.S = not significant ($p>0.01$)

	Calcium in plasma	PTH	CTx	Calcium in urine	Urine pH
Calcium in plasma	---	-0.54	-0.23	0.38	N.S
PTH	-0.54	---	N.S	N.S	N.S
CTx	-0.23	N.S	---	N.S	N.S
Calcium in urine	0.38	N.S	N.S	---	-0.35
Urine pH	N.S	N.S	N.S	-0.35	---

Milk parameters

Yield

Control cows had higher milk yield than PPM cows for the first 14 milkings ($p=0.008$). As seen in Fig 6, before calving, the average yield was low for PPM cows, followed by a steady and rapid increase after calving. No significant differences in yield were found between groups relative to calving other than for the first milking after calving, when control cows yielded more than PPM cows.

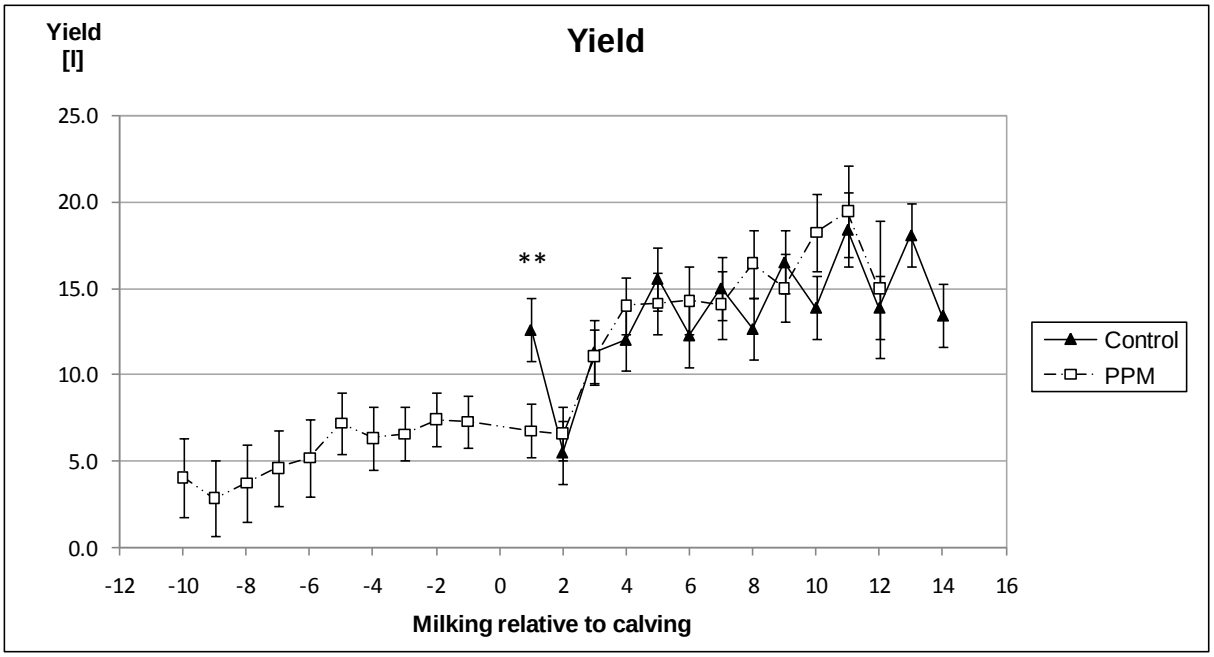

Fig. 6. Milk yield for prepartum milked (PPM) cows and control cows (LSMeans, error bars display the standard error of the mean. * p<0.05; ** p<0.01; *** p<0.001).

Protein

PPM cows had significantly higher initial content of protein in milk (p<0.05), see Fig. 7. After calving, the protein content decreased slowly in both groups.

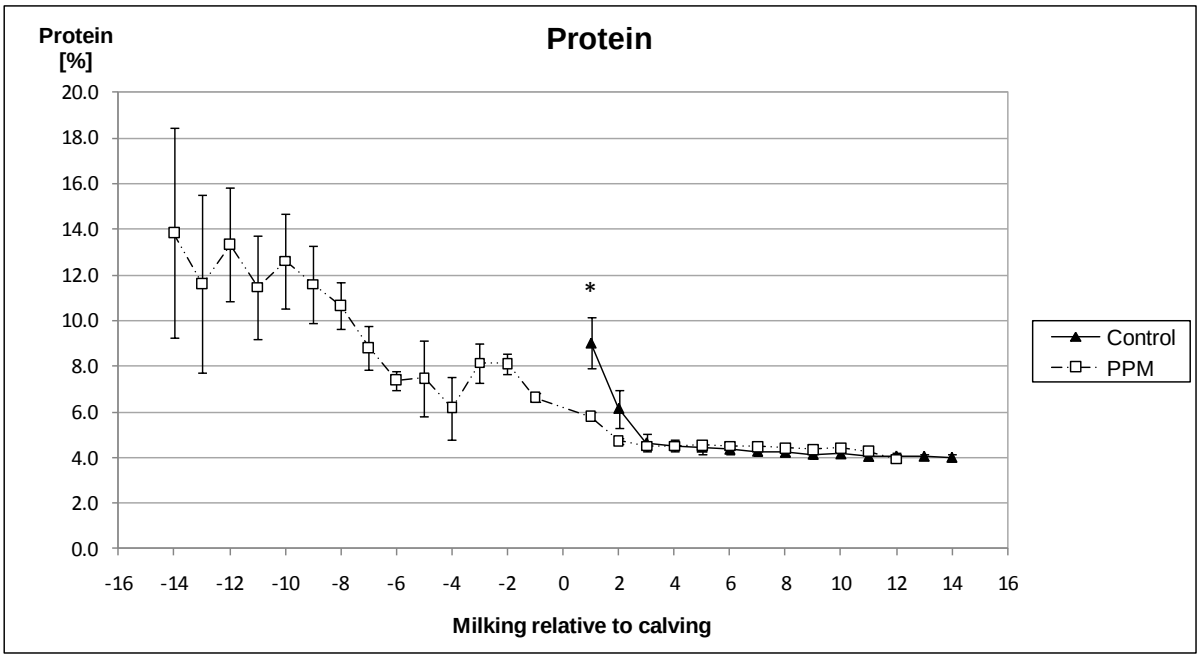

Fig. 7. Protein content in milk from prepartum milked (PPM) and control cows (Means, error bars display the standard error of the mean. * p<0.05; ** p<0.01; *** p<0.001).

IgG

The IgG concentration in milk decreased from the first day of milking for PPM cows (see Fig.8). In PPM cows, the content of IgG was high at the first milking, but had decreased to

about a third of the starting value by the time of calving. Control cows had high levels on the day after calving and showed larger variation than PPM cows.

Older cows in the PPM group had the highest IgG content in milk, while older cows in the control group had the lowest content ($p<0.01$). Overall, PPM cows displayed higher contents of IgG in milk ($p=0.002$).

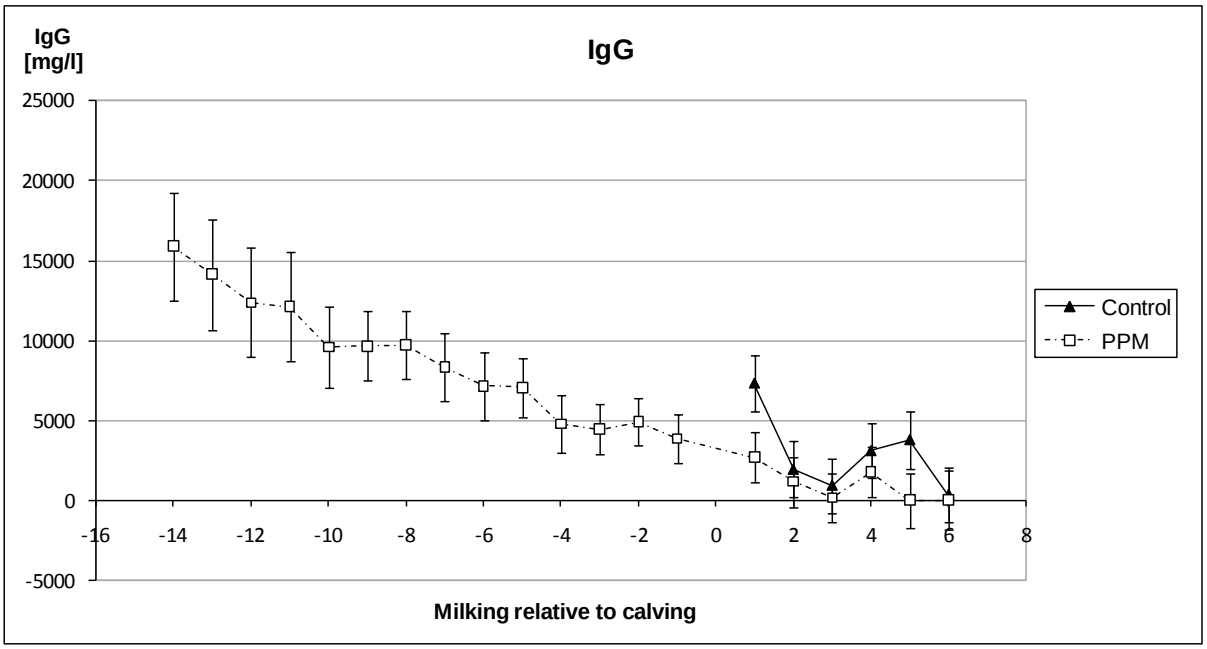

Fig. 8. The IgG content of milk from prepartum milked (PPM) and control cows (LSMeans, error bars display the standard error of the mean).

Density

As seen in Fig. 9, the density of the milk in both groups decreased from the first day of milking, independent of calving. The control group had a lower density of milk on the first milking than the PPM group. After calving, the pattern of decrease in density was similar between the groups.

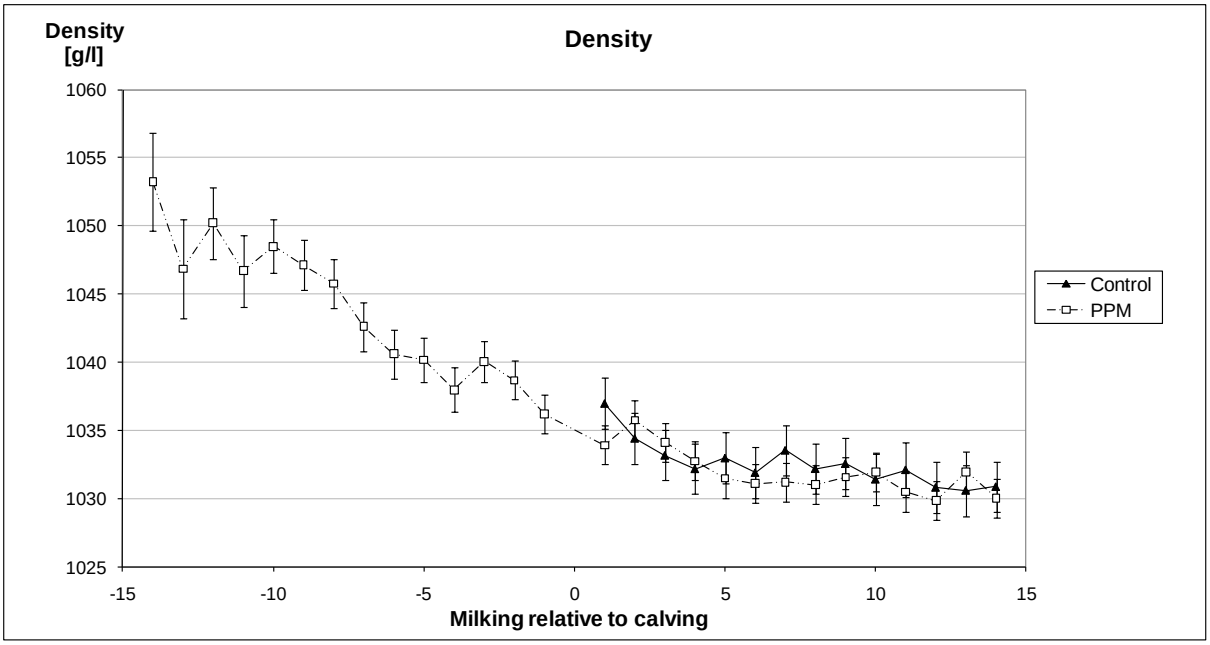

Fig. 9. The density of milk from prepartum milked (PPM) and control cows (LSMeans, error bars display the standard error of the mean).

Lactose

The lactose content of milk was slightly higher for control cows during the first milkings. PPM cows had low initial values, but at the time of calving, the levels were higher than for control cows (p=0.035), see Fig. 10. There was also a significant effect of age, younger cows had higher levels than older (p=0.049).

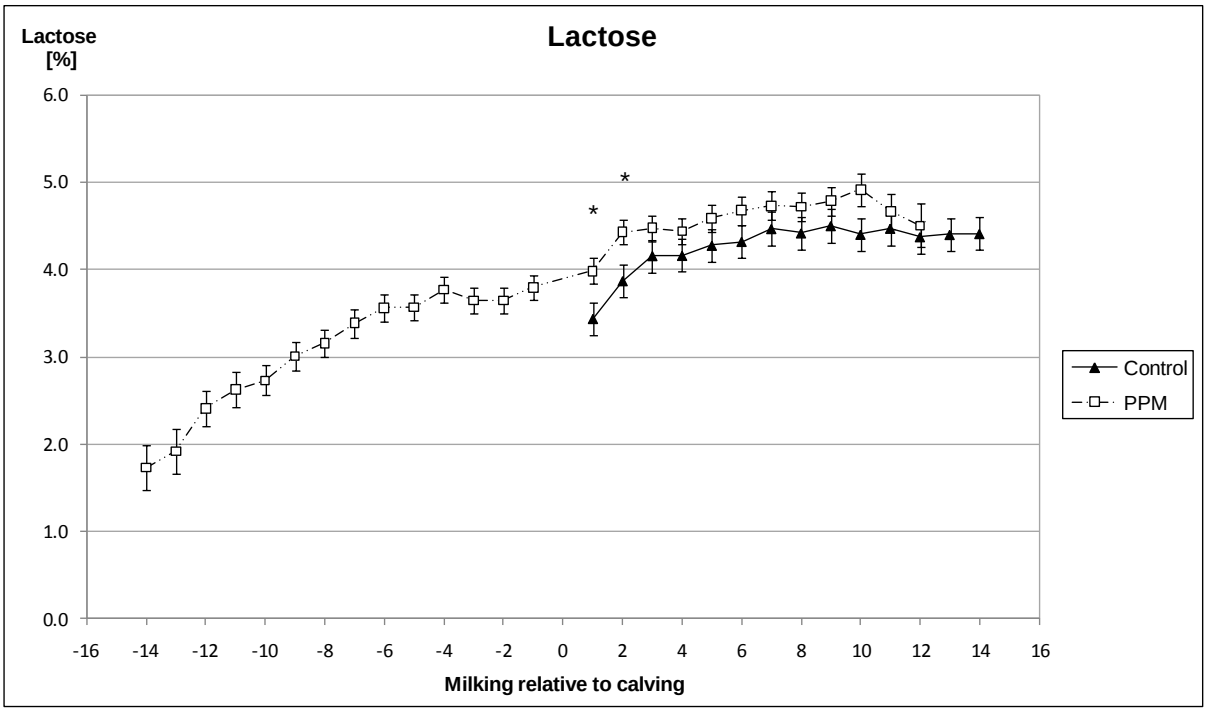

Fig. 10. Lactose content of milk from prepartum milked (PPM) and control cows (LSMeans, error bars display the standard error of the mean. * p<0.05; ** p<0.01; *** p<0.001).

Calcium content in milk

For the calcium content in milk, no significant differences were found between treatment or age groups. The total excretion of calcium in milk on the day after first milking and/or day after calving is displayed in Table 2.

Table 2. Total calcium excretion in milk the day after first milking/calving for cows milked prepartum (PPM) and control cows, based on LSMeans

	Milk Yield [l]	Calcium content [mg/dl]	Total calcium excretion in milk [g]
Control, day after calving/first milking	19.3	123.9	24.0
PPM, day after first milking	10.9	134.4	14.7
PPM, day after calving	13.3	131.3	17.5

Other milk parameters

For fat content no significant differences were found between treatments or ages. For somatic cell count no significant differences were found between treatments, but older cows in the control group had higher counts than younger cows in the same group ($p<0.05$).

Correlations between milk parameters

In Table 3, correlations between milk parameters are shown. Significant correlations ($p<0.01$) were found between most parameters. There were high positive correlations between protein and density and IgG, and a high positive correlation between protein and lactose ($p<0.001$ for all).

Table 3. Correlations between milk parameters. $p<0.01$ N.S = not significant

	Yield	Protein	IgG	Density	Lactose	Calcium	SCC	Fat
Yield	---	-0.56	-0.39	-0.36	0.65	-0.21	-0.38	N.S
Protein	-0.56	---	0.71	0.88	-0.88	0.32	0.46	N.S
IgG	-0.39	0.71	---	0.60	-0.66	0.39	0.38	N.S
Density	-0.36	0.88	0.60	---	-0.60	0.39	N.S	-0.44
Lactose	0.65	-0.88	-0.66	-0.60	---	N.S	-0.67	N.S
Calcium	-0.21	0.32	0.39	0.39	N.S	---	N.S	N.S
SCC	-0.38	0.46	0.38	N.S	-0.67	N.S	---	N.S
Fat	N.S	N.S	N.S	-0.44	N.S	N.S	N.S	---

Discussion

In this study, there was a significant effect of treatment on the calcium level in plasma comparing the groups in regard to time relative to first milking. The pattern of variation on day 2 to 7 is similar between the groups, but the control group has higher values. This implies an effect of milking on calcium in plasma. Though, comparing the groups in regard to time relative to calving, there are no significant differences, which would indicate a major effect of calving. Into account must also be taken the milk yield and excretion of calcium. As can be seen in Table 2, the total excretion of calcium in milk is lower in the PPM group both the day after first milking and the day after calving due to the lower milk yield. Hence, there is a lower demand for calcium in the PPM group, and thereby a lower strain on the homeostatic mechanisms.

Also, we can see that both groups have similar initial levels of calcium in plasma, but that the PPM group more drastically falls in plasma calcium to a level of subclinical hypocalcaemia. The control group never reaches a nadir as low in the sampling period. This would implicate a positive effect of calving or a negative effect of prepartum milking on calcium homeostasis.

A clear effect of calving is seen for CTx and PTH levels. Around calving, both groups display increasing values, but the pattern is somewhat different. PPM cows have a drastic increase in CTx, followed by a slow decrease. Control cows on the other hand have a less drastic increase, but do not start to decrease before the end of the sampling period, when there is no longer any significant difference between the groups. In the control group, PTH drops to a nadir in a few days after calving, while it in the PPM group increases from the day of calving and does not start to decrease before the end of the sampling period (7 days). The elevated levels of PTH together with the lower calcium levels in PPM cows would indicate an impaired calcium homeostasis in this group.

In time relative to calving, both groups quickly restores calcium homeostasis after calving, but the PPM group has higher PTH levels in plasma than do the control group, in which PTH decreases to a minimum in a few days after calving. In the PPM group, it seems that the challenge to maintain calcium homeostasis is larger after calving, which is also supported by CTx. In the control group, the major PTH excretion is before calving, but this is not reflected in the CTx levels. In accordance with Stojević *et al.* (2009) CTx is increased from 14 days prepartum to 7 days postpartum, even though the increase is not constant.

As expected, the variation in urine calcium was low, and there were no differences between the groups. As previously mentioned, the variation in urinal calcium excretion is usually low, and there is not much calcium to be saved from this source. There was a slight difference in urine pH, which might indicate an impaired calcium homeostasis in PPM cows, but none of the groups exceeded the cut-off point suggested by Seifi *et al.* (2004).

There were significant correlations between calcium in plasma and PTH, CTx and calcium in urine. The moderate to high negative correlations to PTH and CTx show the effect of plasma calcium on the two parameters. Despite the effect of PTH on bone metabolism, there was no significant correlation between CTx and PTH. This might be partly due to differences in excretion pattern between the groups and the lack of increase in CTx before calving despite increasing PTH levels. There also seems to have been a difference in baseline values of PTH between treatments, which might have affected the correlation analysis. A significant correlation was also found between calcium in urine and urine pH. The correlation is

negative, showing that increased calcium in urine reduces the pH. This is consistent with the results of Constable *et al.* (2009) and is probably a reflection of both urine calcium and the acid-base status of the animals.

The difference in milk composition between the groups can explain the difference in density. In the PPM group the milk had higher density and a colostral character until calving. After calving, the curves for the two groups are similar, implicating a clear effect of calving. The effect of calving is most apparently seen on the density of the milk. In the first milking of PPM cows, the density was significantly higher than that of the first milking of control cows. Interestingly, with an increase in yield, the density slowly decreases until calving. At the day of calving, the density of the milk from the two groups is not significantly different.

There was an apparent difference in milk yield between the groups from the first to the fourteenth milking. The cause of the difference is most likely the effect of calving. In the control group, all cows had calved at the initiation of milking, while PPM cows calved after 2-14 milkings.

The day before calving, 78 % of PPM cows yielded more than 9 kg, and the remaining 22 % more than 4.5 kg milk. This can be compared to the work by Greene *et al.* (1988), in which only 64 % of the prepartum milked cows yielded more than 4.5 kg the day before calving. Out of these 64 %, only 31 % yielded more than 9 kg. The higher milk yield in the present study can be explained by the general increase in yield over the 20 years since the previous study. Also, the present study involved few cows of a specific breed, which makes it less general.

The decrease in IgG content is consistent with the results of Zeliger *et al.* (1972) who found a significant decrease before calving in prepartum milked cows, and after calving a continued decrease. They also concluded that all cows, both prepartum milked and controls, yielded the same amount of immunoglobulins. This is not found in the present study, as PPM cows seem to have yielded more IgG than control cows.

There was an apparent difference in yield, protein content and IgG content of milk between the first and second milking of control cows. The high IgG content demonstrates the importance of the first colostrum for the calf. The following milkings contain less IgG should therefore not be as beneficial from nutritional or animal health aspects.

There are correlations between almost all milk parameters, and mostly they are moderate to high. The strongest correlations found are between protein and density and lactose, and are explained simply by the dilution of milk. Also, protein is highly correlated to IgG content. This ought to be due to the fact that IgG is included in protein analysis. Due to the osmolaric properties of lactose, there are high correlations between lactose and all other milk parameters measured except calcium content. This because calcium is more affected by other mechanisms than dilution in milk. Calcium content has been shown to be increased in colostrum compared to regular milk (Tsioulpas *et al.,* 2007), but no time effect was seen in the present study.

Conclusions

Prepartum milking resulted in lower calcium levels in plasma, elevated PTH levels and increased bone resorption on the day of calving. Bone resorption, as measured by CTx, was not affected until after calving in prepartum milked cows. The lack of positive effects from prepartum milking indicate a strong effect of calving on calcium homeostasis.

A sudden loss of calcium to milk before calving resulted in lower calcium levels in plasma during the first seven days of milking. The milk excreted before calving had higher density and higher concentration of most components than the milk excreted after calving. After calving, there were no differences in milk composition due to prepartum milking. Prepartum milking resulted in a lower initial milk yield and a lower total excretion of calcium in milk from the day of first milking until after calving.

Due to high yield, protein and IgG contents, the first milking seems to provide the most important colostrum for calves. The following milkings appear to be less important for providing colostral benefits.

The hypothesis that prepartum milked cows would have improved calcium homeostasis was not supported and prepartum milking is therefore not recommended.

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