Effects of prepartum dietary calcium level on calcium and magnesium metabolism in periparturient dairy cows

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ABSTRACT

The aim of this study was to investigate the effects of dietary Ca level (4.9, 9.3, and 13.6 g/kg of DM) on Ca and Mg homeostasis in dairy cows around parturition. Cows of the Swedish Red breed ($n = 29$) with no previous veterinary treatment for milk fever were divided into 3 groups, and each group was fed one of the different diets during the last 15 to 32 d of gestation. Calcium was added as ground limestone, and the Mg concentration was 1.8 g/kg of DM in all diets. After calving the cows were fed similar diets. Plasma was sampled twice per week until calving, and 6, 12, and 24 h, 2, 4, and 7 d after calving. Spot urine samples were collected twice weekly until calving and creatinine was used as a marker of daily urinary excretion. Fecal samples were collected 2 times per day for 5 d starting 2 wk before expected calving, and acid-insoluble ash was used as an indigestible marker to estimate digestibility. Apparent digestibility of Mg and daily Mg excretion in the urine were lower in the dry period for cows fed the highest Ca level. Plasma Mg concentration was lower on 2, 4, and 7 d after calving in cows fed the highest level of Ca. Treatment groups did not differ in plasma Ca concentration, parathyroid hormone concentration, or bone mobilization, evaluated using crosslinked carboxyterminal telopeptides of type I collagen (CTX) as a marker. Plasma Ca concentration decreased and plasma CTX concentration increased 6 h after calving. The apparent digestibility of Ca during the dry period was not affected by dietary Ca, but the cows fed 4.9 g Ca/kg of DM excreted 1.2 g of Ca/d in the urine, which was higher compared with 0.4 g/d and 0.6 g/d for the cows fed 9.3 g of Ca/kg of DM and 13.6 g of Ca/kg of DM, respectively. The results show that feeding 13.6 g of dietary Ca/kg of DM impaired the Mg absorption during the dry period, and resulted in decreased plasma Mg concentration after calving, but prepartum dietary Ca

level did not affect plasma Ca, parathyroid hormone, or CTX concentrations.

Key words: magnesium absorption, dry period, parturition

INTRODUCTION

Parturition and onset of lactation in dairy cows is followed by a large increase in mineral losses due to the high mineral content of milk and colostrum (Shappell et al., 1987) and is associated with a transient decrease in plasma Ca concentration. Milk fever (i.e., periparturient paresis) is a common condition, especially in multiparous cows. The incidence of milk fever differs among herds and can be affected by the mineral composition of the dry cow diet (Lean et al., 2006).

Decreasing the amount of dietary Ca during the last week of gestation has been shown to decrease the incidence of milk fever (Goings et al., 1974; Green et al., 1981). This is assumed to be an effect of adaptation of the Ca homeostatic mechanisms of the cow and better preparation for increased Ca losses at the onset of lactation. The literature is not consistent regarding in what range of dietary Ca these positive effects occur. A meta-analysis made by Lean et al. (2006) indicated that a gradual change in dietary Ca from 5 to 10 g/kg of DM increased the predicted incidence of milk fever by 327%, and results from Shappell et al. (1987) showed positive effects on plasma Ca concentration at calving by decreasing dietary Ca from 12 to 5 g/kg of DM. However, other studies found no beneficial effect on plasma Ca concentration of a decreased dietary Ca level within that range (Goff and Horst, 1997; Kamiya et al., 2005). Thilsing-Hansen et al. (2002) suggested that the Ca intake had to be below 20 g/d to prevent milk fever, an intake that is difficult to achieve in common dry cow diets.

Parathyroid hormone (PTH) is the main calcium-providing hormone and it increases the mobilization of Ca from bone tissue, the kidney reabsorption of Ca, and, through vitamin D, the intestinal uptake of Ca from the diet (Ramberg et al., 1976). Goings et al. (1974) found that cows fed low amounts of dietary Ca

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had a higher PTH concentration in plasma compared with that of control cows. Several studies have shown that PTH increases at calving as a response to the decrease in plasma Ca concentration (Shappell et al., 1987; Kamiya et al., 2005). This results in an increase in Ca mobilization from bone, which is important to re-establish the Ca level in the blood (Yarrington et al., 1976). It has been suggested that at least 48 h of PTH stimulation is needed to increase mobilization of bone Ca (Goff et al., 1986), and Kamiya et al. (2005) found no significant increase in bone mobilization 3 d after calving. This is assumed to be an effect of the low activity of the osteoclasts during the dry period, when Ca requirements are well covered by absorption from the diet (Ramberg et al., 1970).

Magnesium has long been known to be important for both the formation of, and the tissue response to, PTH (Anast et al., 1972; Johannesson and Raisz, 1983). Thus, hypomagnesemia decreases the ability to adapt to Ca deficiency. In adult ruminants, the main site for Mg absorption is in the rumen, and Mg is involved in several essential processes in the body, such as energy metabolism, cell proliferation, and muscle contractions. Magnesium is mainly an intracellular ion and only a small fraction is found in the plasma (Malon et al., 2004). It has been shown that high levels of dietary Ca interfere with the ruminal absorption of Mg in sheep (Care et al., 1984). The effect of Ca levels in dry cow diets on the Mg homeostasis around parturition is inconsistent among studies. Kamiya et al. (2005) found no effect of dietary Ca level on plasma Mg concentration during the dry period or after calving, whereas Verdaris and Evans (1976) observed lower plasma Mg concentration at calving in cows fed 21 g of Ca/kg of DM compared with that in cows fed 2 g of Ca/kg of DM, even though the cows fed the high-Ca diet had a higher Mg intake. In several studies comparing different Ca intakes, the Mg concentration was not similar among diets (Shappell et al., 1987; Goff and Horst, 1997), which makes it difficult to distinguish between effects of dietary Ca and dietary Mg on responses in plasma Mg. Green et al. (1981) found no effect of Ca level on periparturient plasma Mg concentration in cows fed 2 different levels of Mg.

The knowledge is limited and, to some extent, contradictory about the levels of dietary Ca needed to improve Ca homeostasis at calving, as well as how the periparturient plasma Mg concentration is affected by dry cow diets with different Ca levels. Therefore, the aims of this study were to investigate the effects on periparturient Ca and Mg homeostasis, PTH, and bone resorption of feeding varying levels of prepartum dietary Ca in the range of common dry cow diets, maintaining a constant level of Mg.

MATERIALS AND METHODS

The study was performed at Kungsängen Research Centre in Uppsala, Sweden, and was approved by the Uppsala Local Ethics Committee.

Animals and Experimental Design

Thirty dry cows of the Swedish Red breed were used. The breed has a slightly lower incidence of milk fever than the Swedish Holstein breed (i.e., 2.9 and 3.5%, respectively; Swedish Dairy Association, 2009). The cows had an average weight of 624 kg (ranging from 488 to 803 kg) before the experiment and were divided according to expected calving date into 5 blocks. The cows in each block were divided according to parity number, with cows about to enter their second lactation ($n = 13$) and cows about to enter their third or more lactation ($n = 17$) in 2 separate groups. The 2 different parity groups were evenly spread over time, resulting in all blocks but one containing 3 cows from each parity group. The cows in each block and parity group were randomly assigned to 3 different Ca treatments: 0, 4.5, and 9 g of additional Ca/kg of DM, aiming at diets with 4.5, 9, and 13.5 g of Ca/kg of DM, and all with 1.6 g of Mg/kg of DM, according to NRC recommendations (NRC, 2001). The average production in the herd from September 2007 to August 2008 was 9,498 kg ECM. None of the cows had previously been treated for milk fever; they were all pregnant and about to enter their second to sixth lactation. The expected calving dates were from October 2007 to May 2008. The cows were housed indoors in individual tie stalls with sawdust bedding on rubber mats during the dry period and had access to water and salt licks during the entire experiment. When the cows showed signs of approaching parturition, they were moved to single-cow calving pens with straw as bedding on a rubber mat. After calving, the cows were kept in the pen with the calf for some hours up to 1 d. They were then moved to individual tie stalls with chopped straw as bedding on rubber mats, where they were kept during the remainder of the experiment. All cows were milked twice daily after calving, at approximately 0630 and 1530 h. One of the multiparous cows calved before receiving the experimental diet and was excluded from the experiment.

Feeding

During the dry period, the cows were fed 2 times per day (at approximately 0600 and 1700 h) with equal amounts of late-cut, high-DM grass silage, and 3 times per day (at approximately 0600, 1200, and 1700 h) with

Table 1. Nutrient concentration of forage and concentrate, per kg of DM unless otherwise stated

Item	Dry cow feed		Lactating cow feed	
	Silage	Concentrate	Silage	Concentrate
DM, % of feed	70	89	39	88
ME, MJ	8.7	14.0	11.5	13.2
CP, g	52	340	155	187
Ash, g	62	69	88	60
Ca, g	2.7	9.7	4.5	8.3
P, g	1.4	8.9	2.6	5.2
Mg, g	1.0	3.8	1.4	4.5
K, g	16.0	15.6	28.7	8.4
Na, g	<0.1	0.1	<0.1	2.8
S, g	1.2	3.9	2.4	2.2
Cl, g	3.7	0.6	9.5	4.9
DCAD, mEq ¹	234	142	320	61

¹Calculated as (Na + K) – (Cl + S) in mEq/kg of DM.

equal amounts of pelleted concentrates. For chemical composition and calculated value of ME in the feedstuffs, see Table 1. The feed was manually weighed and fed. Starting 3 wk before expected calving, additional Ca (0, 103, or 205 g/d) was added to the diets as ground limestone (Nordkalk, Malmö, Sweden) that was manually mixed in the concentrates at morning and evening feeding. The mineral concentration of the limestone was 38.4% of Ca, 0.3% of Mg, 0.3% of S, and 0.1% of K. The ingredients of the concentrates are listed in Table 2. The diets were kept unchanged, with 6.1 kg of DM forage and 2.7 kg of DM concentrates fed daily until calving. After calving, all cows were fed 4 times daily using automatic feed wagons with a chopped grass and clover silage ad libitum, increasing amounts of pelleted concentrate and 100 g/d of a mineral supplement suited for lactating cows. Crude protein, ME, and mineral values of the feeds are found in Table 1. The postpartum diets were prepared according to the Swedish nutrient recommendations for lactating cows (Spörndly, 2003) with no differences in mineral content between treatments. Feed residues were collected once daily and weighed.

Sampling

The sampling period started 5 wk before expected calving. Blood samples were drawn from the tail or jugular vein into heparinized 10-mL vacuum tubes (Venoject, Terumo Europe N.V., Leuven, Belgium) twice per week until calving, and approximately 6, 12, and 24 h, 2, 4, and 7 d after calving. All blood samples were collected between 0730 and 0830 h, except for the samples collected 6, 12, and 24 h after calving. The blood was centrifuged (Jouan 4.11, Saint-Nazaire, France) within 1 h at $1800 \times g$ for 10 min at 20°C, and the plasma was stored at –20°C until analyzed. Spot samples of urine

were collected twice per week until calving at voluntary urination or by manual stimulation. Immediately after collection, pH was measured with a pH meter (MP 125 Mettler Toledo, Schwerzenbach, Switzerland) and the urine was stored at –20°C until analyzed. Starting about 2 wk before expected calving, equally sized grab samples of feces were collected twice per day for 5 subsequent days and stored at –20°C. After the collection period, all samples from each cow were thawed, pooled, and mixed. The samples were frozen to –80°C, freeze-dried, ground in a hammer mill using a 1-mm sieve (Kamas, Malmö, Sweden) and stored until analysis. Samples of the forage were collected regularly during

Table 2. Composition (% of DM) of the concentrates used for dry and lactating cows

Ingredient	Dry cow concentrate	Lactating cow concentrate
Soybean meal	43	
Peas	14	20
Oats		23
Barley		23
Expro00 ¹	20	
Wheat	18	
Rapeseed cake		13
Rapeseed		3
Sugar beet pulp		9
Wheat bran		7
Acofeed ²	2	
Maize gluten meal	1	
Premix ³	0.8	0.1
CaCO ₃	0.5	1.3
CaPO ₄	1.0	
NaCl		0.6
MgO		0.4

¹Heat-treated rapeseed meal (AarhusKarlshamn Sweden AB, Karlshamn, Sweden).

²Vegetable feed fat; >96% fat (AarhusKarlshamn Sweden AB).

³Contains per kilogram: 243 g of Ca, 18 g of Fe, 8 g of Zn, 5 g of Mn, 2.9 g of Mg (MgO), 1.4 g of Cu, 0.3 g of S, 0.1 g of P, 298 mg of I, 100 mg of Co, and 80 mg of Se.

the experiment, dried at 60°C overnight and ground, as for the fecal samples. Samples of the concentrate were collected 3 times during the experiment, dried, and ground, as described for the forage samples. One sample of the limestone was collected.

Analyses

All feed samples were analyzed for CP content by the Kjeldahl method (Nordic Committee on Food Analysis, 1976) using a 2020 Digestor and a 2400 Kjeltec analyzer unit (Foss Analytical A/S, Hillerød, Denmark). Dry matter content was determined by overnight drying at 105°C, and the ash content was determined by heating the samples to 550°C for 3 h. The metabolizable energy value of the forage was calculated using the *in vitro* digestibility (Lindgren, 1979), with the modified regression used as standard in Sweden (Lindgren, 1983). The metabolizable energy values of the concentrates were calculated according to Spörndly (2003). Mineral content of feed, limestone, and feces was analyzed by inductively coupled plasma emission spectrometry (Spectro Analytical Instruments GmbH & Co., Kleve, Germany) using HNO₃ according to the method described by Balsberg-Påhlsson (1990), except for chloride in feeds, which was extracted with HNO₃ and determined by titration with AgNO₃. Feed, limestone, and feces samples were analyzed for acid-insoluble ash using the method described by van Keulen and Young (1977). The urine was analyzed for creatinine with an autoanalyzer (Technicon, Dublin, Ireland), using the Jaffee reaction, a colorimetric method with picric acid (Technicon, 1974). Plasma and urine were analyzed for Ca using a colorimetric method with O-cresolphthalein (CA 590, Randox, Antrim, UK) and for Mg with a colorimetric method with xylydyl blue (MG 531, Randox), both using a spectrophotometer (Ultrospec K, Boule Nordic, Huddinge, Sweden). Before analysis of Mg, the urine was acidified to pH 3 to 4 with 12 M HCl, with most samples requiring between 1 to 2% HCl. Plasma was analyzed for crosslinked carboxyterminal telopeptides of type I collagen (CTx) as a marker of bone resorption with a commercial ELISA kit (Serum Cross-Laps ELISA, Nordic Bioscience, Herlev, Denmark) using a plate reader (Labsystems Multiscan RC, Helsinki, Finland), and for PTH with a commercially available ELISA kit validated for bovine PTH (Bovine Intact PTH ELISA Kit, Immotopics, San Clemente, CA), using a combined shaker and plate reader (Multiscan FC, Thermo Fisher Scientific, Vantaa, Finland).

Calculations and Statistics

Acid-insoluble ash was used as an indigestible marker for calculation of the apparent digestibility of Ca and

Mg. Creatinine was used for calculating the amount of urine excreted each day, assuming a creatinine excretion in urine for all cows of 29 mg/kg of BW × d⁻¹ (Valadares et al., 1999). To avoid problems with few observations on any single day, due to the fact that the cows were sampled only twice per week until calving, time relative to calving was defined as the number of 4-d periods the cow had left to calving at each sampling time before calving, but as 6, 12, and 24 h, 2, 4, and 7 d after calving.

Due to differences between actual calving date and expected calving date, only samples from the last 3 wk of gestation and the first week after calving were used in the multivariable analysis, and samples from the pretreatment period and the first 4 d of treatment were also not included.

The effect of treatment on plasma concentrations of Ca, Mg, CTx, and PTH, and on urinary pH, and daily excretion of Ca and Mg was analyzed using separate repeated measures models as implemented in the MIXED procedure of SAS (SAS 9.1, SAS Institute, 2002). A spatial power covariance structure, with cow as the repeated subject and day relative to calving as the coordinate for the distance between observations, was used in the models to account for the repeated observations within cows, except in the model for analysis of differences in PTH levels, in which a first-order autoregressive covariance structure was used because the model did not converge with the spatial power structure. The fixed effects included in the models were treatment group (Tr, 3 levels), parity group (2 levels), block (5 levels), time relative to calving (TC, 12 levels), and the interaction Tr × TC, and cow was included as a random effect. One primiparous cow fed 13.6 g of Ca/kg of DM during the dry period was removed from the statistical analysis of the CTx data due to abnormal values with no physiological explanation and high influence on the estimates. The effect of treatment on digestibility was analyzed using a similar model, but the effects of TC, the interaction, and the repeated observations structure were excluded because only one observation per cow was collected. The fixed effects were considered significant at $P < 0.05$. Results from the statistical analyses are presented as least squares means. Pairwise comparisons of the least squares means for Tr at certain time points were only made when there was an overall significant effect. Differences among the groups before receiving the treatments were evaluated with *t*-tests.

RESULTS

The resulting Ca and Mg concentrations of the experimental diets were higher than planned; 4.9 g of Ca/kg of DM ($n = 10$ cows), 9.3 g of Ca/kg of DM ($n = 9$

Table 3. Body weight, parity group composition, treatment period, dry period, gestation length, average dry matter intake 7 d after calving and disease incidence in the 3 treatment groups (SD shown in parentheses)

Item	Treatment, g of Ca/kg of DM		
	4.9	9.3	13.6
BW, kg	616 (58)	631 (107)	625 (44)
Parity group 1 and 2 ¹	5 + 5	4 + 5	4 + 6
Gestation length, d	280 (5)	282 (4)	281 (5)
Treatment period, ² d	—	23 (6)	24 (5)
Treatment period, ² range in d	—	16–32	15–32
Dry period, wk	9 (3)	10 (4)	9 (2)
DMI 1 wk after calving, kg	16.8 (2.2)	17.4 (2.5)	15.9 (2.5)
Mastitis, ³ n	0	0	1
Retained placenta, ⁴ n	1	0	4

¹Parity group 1 = cows calving for the second time; parity group 2 = cows calving for the third or more time.

²Cows fed 4.9 g of Ca/kg of DM were fed this diet during the whole experimental period.

³Clinical mastitis occurring up to 7 d after calving.

⁴Fetal membranes not expelled 24 h after calving.

cows), and 13.6 g of Ca/kg of DM ($n = 10$ cows). All groups were fed 1.8 g of Mg/kg of DM. The DCAD values of the diets, calculated as $(\text{Na} + \text{K}) - (\text{Cl} + \text{S})$, were 206, 202, and 198 mEq/kg of DM for cows fed 4.9, 9.3, and 13.6 g of Ca/kg of DM, respectively. No feed residues were left from any of the cows during the dry period. None of the cows were paretic or showed clinical signs of milk fever during the experiment. After calving, the intakes of silage and concentrates were gradually increased, and 1 wk after calving the cows were, on average, fed 11.6 kg of DM of silage/d, 5.2 kg of DM of concentrates/d, and 0.1 kg of mineral supplement/d, resulting in concentrations of 5.8 g of Ca, 3.8 g of P, 2.7 g of Mg, and 22.1 g of K/kg of DM in the total diet. The treatment groups did not differ in terms of postpartum DMI and mineral concentrations, $P > 0.20$. Body weight, treatment length, gestation length, dry period length, parity group composition, DMI after calving, and disease incidence in the treatment groups are shown in Table 3. None of the measured variables differed during the pretreatment period.

Plasma Changes at Parturition

Plasma concentrations of Ca, Mg, CTx, and PTH did not change during the dry period (data not shown), and the values during the last week before calving were, thus, representative for the whole period. A drop in Ca levels in plasma was evident in all cows at 6 h postpartum (Figure 1). Plasma Ca values were normalized 4 d after calving. The decrease in plasma Ca concentration was concurrent with an increase in plasma Mg and CTx concentrations (Figure 1). Plasma Mg concentration had returned to prepartum levels 24 h after calving,

and decreased further in the cows fed 13.6 g of Ca/kg of DM, resulting in lower plasma Mg concentration 2, 4, and 7 d after calving compared with that of the other treatments. Cows fed 13.6 g of Ca/kg of DM also had lower plasma Mg concentration 12 h after calving compared with that of cows fed 9.3 g of Ca/kg of DM. Plasma CTx remained elevated during the first week after calving ($P < 0.01$). The PTH concentration in plasma was not significantly affected by TC, $P = 0.06$ (Figure 1). No effects of treatment group on plasma Ca, plasma CTx, or plasma PTH concentrations were found. Plasma CTx was 0.65 ng/mL higher in cows calving for the second time compared with that of cows calving for the third time or more, $P = 0.033$.

Urinary Excretion

Time relative to calving did not affect daily urinary excretion of Ca during the prepartum period. The cows fed 4.9 g of Ca/kg of DM excreted more Ca in the urine, 1.2 ± 0.1 g/d (LSM $\pm$ SEM) compared with 0.4 ± 0.1 and 0.6 ± 0.1 g/d for the cows fed 9.3 and 13.6 g of Ca/kg of DM, respectively ($P = 0.003$). Urinary excretion of Mg was affected by time ($P = 0.001$) and treatment ($P = 0.001$, Figure 2). The cows fed 13.6 g of Ca/kg of DM excreted lower amounts of Mg than did the cows fed 4.9 or 9.3 g of Ca, and the excretion decreased during the last 3 wk of gestation. No differences existed in urinary pH over time ($P = 0.406$) or among treatments ($P = 0.152$); the pH values were 8.0 ± 0.05 , 8.1 ± 0.05 , and 8.2 ± 0.05 for the cows fed 4.9, 9.3, and 13.6 g of Ca/kg of DM, respectively. The concentration of creatinine did not differ among treatment groups ($P = 0.621$), least squares means $\pm$ standard error of the

Figure 1. Plasma Ca, Mg, crosslinked carboxyterminal telopeptides of type I collagen (CTx), and parathyroid hormone (PTH; LSM $\pm$ SEM) 1 wk before calving to 1 wk after calving in periparturient cows fed different amounts of Ca during the last 3 wk of the dry period (Δ = 4.9 g of Ca/kg of DM; $\square$ = 9.3 g of Ca/kg of DM; $\circ$ = 13.6 g of Ca/kg of DM). The standard error of the means in the figure is between 0.06 and 0.07 mM for Ca, 0.03 mM for Mg, between 0.31 and 0.34 ng/mL for CTx, and between 56 and 64 pg Eq/mL for PTH.

means were 1.4 ± 0.17 , 1.2 ± 0.18 , and 1.4 ± 0.17 g/L for the cows fed 4.9, 9.3, and 13.6 g of Ca/kg of DM, respectively.

Digestibility of Ca and Mg

The different Ca intakes did not affect the calculated apparent digestibility of Ca 2 wk before expected calving ($P = 0.153$); the least squares means values $\pm$ standard error of the means were 0.19 ± 0.04 , 0.09 ± 0.04 , and 0.17 ± 0.04 for the cows fed 4.9, 9.3, and 13.6 g of Ca/kg of DM, respectively. The apparent digestibility of Mg was higher in the cows fed the lowest amount of Ca ($P = 0.006$), 0.17 ± 0.04 , compared with 0.05 ± 0.04 and -0.04 ± 0.04 (LSM $\pm$ SEM) for the medium and highest level of dietary Ca, respectively.

DISCUSSION

The cows fed 13.6 g of Ca/kg of DM during the dry period excreted less Mg in the urine than did the cows on the other treatments. This indicates that the uptake of Mg was lowered due to the high level of dietary Ca. This is supported by the lower digestibility of Mg during the fecal sampling period 2 wk before expected calving. The present result is in line with results of Care et al. (1984) showing that increased Ca concentrations decrease Mg uptake. Even if the main site of Ca absorption is in the small intestine, high levels of dietary Ca are associated with significant net absorption in the forestomachs (Khorasani et al., 1997). It is, thus, reasonable to assume that in the present study, ruminal Ca absorption interfered with Mg absorption. This

Figure 2. Calculated daily urinary excretion of Mg from periparturient cows fed different amounts of Ca during the last 3 wk of the dry period (Δ = 4.9 g of Ca/kg of DM; $\square$ = 9.3 g of Ca/kg of DM; $\circ$ = 13.6 g of Ca/kg of DM). Data are presented as least squares means $\pm$ standard error of the means. The standard error of the means ranged between 0.26 and 0.50 g/d.

decreased absorption did not affect plasma Mg during the dry period. However, 2 d after calving, plasma Mg concentration decreased significantly more in the cows that had been fed 13.6 g of Ca/kg of DM, although the dietary mineral concentrations were similar for all cows. A decrease in plasma Mg concentration after calving has been found in previous studies (Verdaris and Evans, 1976). The intracellular pool of Mg is several times larger than the plasma pool and exchangeable with plasma Mg (Malon et al., 2004), and, therefore, plasma Mg concentration is a specific but not very sensitive tool when used to detect Mg deficiency. In fact, a recent study by Schweigel et al. (2009) indicates that the intracellular pool of Mg in cattle could be decreased even when plasma Mg concentration is within the normal range. It is, thus, possible that the reason for the apparent delay in plasma Mg response to the low Mg uptake in cows fed 13.6 g of Ca/kg of DM was a gradual depletion of the intracellular Mg pool, which was only reflected in plasma Mg after calving, when the losses of Mg to the milk increased rapidly. The concentration of Mg in colostrum is about 3 times higher than that in regular milk (Shappell et al., 1987). Although parturition is a natural process, it is associated with pain and stress in the animal (Goff et al., 2002). Stress has been shown to decrease plasma Mg concentration (Ali et al., 2006). This is suggested to be due to a shift of Mg from the extracellular to the intracellular compartment because hormones associated with stress may increase the influx of Mg into the cells (Elliott and Rizack, 1974). The stress associated with calving may, thus, have further decreased plasma Mg concentration after calving.

A plasma Mg level below 0.74 mM may be considered as subclinical hypomagnesemic (Mayland, 1988), and a large fraction of the cows had several samples below that limit.

It has been suggested that a urinary excretion of less than 2.5 g of Mg/d in lactating dairy cows indicates inadequate Mg supply (Mayland, 1988). Urinary excretion of Mg was below 2.5 g/d for one or more days in almost all cows in our experiment. Urinary excretion of Mg is usually a good marker of the Mg status in the ruminant because excess Mg is excreted in urine, and our findings indicate that many of the cows were subclinical hypomagnesemic or close to hypomagnesemic, although they were fed a level of dietary Mg that exceeded the NRC recommendations (NRC, 2001). It is possible that the cut-off value for urinary Mg excretion in dry cows differs from the value for lactating cows, but 8 cows excreted less than 1 g of Mg/d on at least one sampling, which is clearly very low. The cows fed 13.6 g of Ca/kg of DM contributed to most of the low urinary Mg samples, but cows from all treatments were represented. This may indicate that feeding Mg slightly above the recommendations was not enough in our study.

Different dietary Ca levels did not significantly affect plasma Ca, PTH, or CTx concentrations. This indicates either that the differences among the dietary concentrations of 4.9, 9.3, and 13.6 g of Ca/kg of DM were too small to affect the Ca homeostatic mechanisms, or that the lowest level was too high to alter the Ca homeostasis. Thilising-Hansen et al. (2002) compared several experiments and suggested that dietary Ca levels had to be below 20 g/d to prevent parturient paresis, which can be compared with the daily amount of 40 g for the cows fed 4.9 g of Ca/kg of DM in the present study. However, the results from the meta-analysis of Lean et al. (2006) indicated a gradually increasing milk fever incidence when dietary Ca increased up to about 13 g/kg of DM. Our results show that cows fed the lowest amount of Ca excreted twice as much Ca in the urine than did cows on the other 2 treatments, which may be a result of an improved Ca absorption and, thereby, an increased need of Ca excretion in the urine. However, the digestibility of Ca was not different among treatments and the total amount of Ca excreted in the urine was small compared with the intake in all cows. Urinary excretion of Ca has been shown to be unaffected by dietary Ca concentration (Verdaris and Evans, 1976), but Ramberg et al. (1976) found a decreased urinary Ca excretion when the cows were fed large amounts of dietary Ca. The acid-base status of the cows seems to have been similar around calving in all treatments because no differences in urinary pH were found.

The decrease in plasma Ca concentration did not seem to affect the concentration of PTH in our experiment,

as no significant effect of time on plasma PTH was evident, although the P -value was close to significant ($P = 0.06$), with higher values the first days after calving. Results from other studies have shown an increase in PTH concentration at calving (Shappell et al., 1987; Peterson et al., 2005). Hypomagnesemia has been shown to impair the production of PTH (Anast et al., 1972), and the lack of response in PTH in our experiment may have been due to impaired Mg status in the cows. However, the level of PTH in our experiment was higher than the levels found in some previous studies (Peterson et al., 2005; Penner et al., 2008), and did not follow the same pattern as did the plasma Mg. The reason for the large variation in PTH concentration is unclear. It has been proposed that a lag of up to some days occurs from parturition until bone resorption is significantly increased (Ramberg et al., 1970; Kamiya et al., 2005). In the present study, plasma CTx was increased already at 6 h after calving, which indicates that the mobilization of Ca from bone tissue is activated rapidly. Because Ca mobilization from bone could be mediated through different pathways (Fuller et al., 2007), the difference in results probably reflects the choice of bone resorption marker, and CTx has been found to respond to hypocalcemia within hours (Zikan and Stepan, 2009). The fact that PTH concentration was not found to be significantly increased at calving indicates that the activation of bone mobilization, observed as an increase in CTx, depended on other factors than regulation by PTH. Estradiol has been shown to inhibit the mobilization of bone, resulting in decreased formation of CTx (Prestwood et al., 2000), and the rapid increase in CTx might, therefore, be a result of the decrease in estradiol occurring at parturition (Goff et al., 2002). Holtenius and Ekelund (2005) suggested that estrogen suppressed bone mobilization during the dry period. This is supported by a study made by Liesegang (2008), in which CTx concentration increased after parturition in sheep even if serum Ca concentration did not change. Cows calving for the second time had higher concentrations of CTx in plasma compared with that of older cows. This is in accordance with results from Ekelund et al. (2006) who found that cows had higher CTx value in the dry period before the studied lactation compared with that in the dry period after.

CONCLUSIONS

Feeding 13.6 g of Ca/kg of DM instead of 4.9 or 9.3 g of Ca/kg of DM for the last 15 to 32 d of gestation decreased the apparent digestibility and daily urinary excretion of Mg. Plasma Mg concentration in these cows decreased after calving. The different levels of dietary Ca did not affect plasma Ca, CTx, or PTH concentra-

tions during the peripartum period, which indicates that restricting the Ca level to 4.9 g/kg of DM was not enough to significantly affect Ca status at parturition.

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