The Effect of Zeolite A Supplementation in the Dry Period on Periparturient Calcium, Phosphorus, and Magnesium Homeostasis

T. Thilising-Hansen,* R. J. Jørgensen,* J. M. D. Enemark,*
and T. Larsen†

*The Royal Veterinary and Agricultural University
Department of Clinical Studies,
Cattle Production Medicine Research Group,
Dyrhedevej 88, DK-1870 Frederiksberg C Denmark

†Dept. of Animal Health and Welfare,
Danish Institute of Agricultural Sciences,
P. O. Box 50, DK-8830 Tjele, Denmark

ABSTRACT

One potential way of preventing parturient hypocalcemia in the dairy cow is to feed dry cow rations very low in calcium (<20 g/d); but, because it is difficult to formulate rations sufficiently low in calcium, this principle has been almost abandoned. Recent studies have shown, however, that it is possible to prevent milk fever, as well as subclinical hypocalcemia, by supplementing the dry cow ration with sodium aluminium silicate (zeolite A), which has the capacity to bind calcium. The aim of this study was to further evaluate the effect, if any, of such supplementation on other blood constituents, feed intake, and milk production in the subsequent lactation. A total of 31 pregnant dry cows about to enter their third or later lactation were assigned as experimental or control cows according to parity and expected date of calving. The experimental cows received 1.4 kg of zeolite pellets per d (0.7 kg of pure zeolite A) for the last 2 wk of pregnancy. Blood samples were drawn from all cows 1 wk before the expected date of calving, at calving, at d 1 and 2 after calving, and 1 wk after calving. Additionally, a urine sample was drawn 1 wk before the expected date of calving. Zeolite supplementation significantly increased the plasma calcium level on the day of calving, whereas plasma magnesium as well as inorganic phosphate was suppressed. Serum 1,25(OH)₂D was significantly increased 1 wk before the expected date of calving among the experimental cows, whereas there was no difference in the urinary excretion of the bone metabolite deoxypyridinoline between the two groups. Feed intake was decreased among the zeolite-treated cows during the last 2 wk of pregnancy. No effect was observed on milk yield, milk fat, and milk protein in the subsequent lactation. The mechanisms

and interactions involved in zeolite supplementation are discussed in relation to the observed improvement in parturient calcium homeostasis and to the observed depression in blood magnesium and inorganic phosphate.

(Key words: dairy cow, parturient hypocalcemia, zeolite A, vitamin D)

Abbreviation key: DPD = deoxypyridinoline, PTH = parathyroid hormone, 1,25(OH)₂D = 1,25-dihydroxy vitamin D.

INTRODUCTION

Milk fever prevention has been the subject of intensive research during the last 50 yr. Among the different preventive principles tested, the administration of rations very low in dietary calcium in the dry period has had excellent results (Boda and Cole, 1954; Goings et al., 1974; Kichura et al., 1982; Wiggers et al., 1974). Feeding dry cow rations low in calcium leads to an activation of the calcium homeostatic mechanisms before calving rendering the cow ready for the massive draw on blood calcium for final stages of prenatal growth and colostrum production. The effect is explained as follows. When dietary calcium availability is decreased below calcium requirements, the cow is brought into a state of negative calcium balance. A drop in serum calcium leads to a secretion of parathyroid hormone (PTH) from the parathyroid glands, which in turn increases renal reabsorption of calcium (within minutes), stimulates calcium resorption from the bone (within hours to days) and stimulates renal vitamin D metabolism toward production of 1,25-dihydroxyvitamin D (1,25(OH)₂D) (within hours to days) (Goff et al., 1991). The 1,25(OH)₂D stimulates the active transport of calcium across the intestinal epithelial cells (Horst et al., 1994). During bone resorption, urinary excretion of pyridinoline and deoxypyridinoline, derived from collagen breakdown, is increased. In humans pyridinoline

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Corresponding author: T. Thilising-Hansen; e-mail: trh@kvl.dk.

is present in bone, cartilage, and many soft tissues, whereas deoxyypyridinoline (DPD) is located primarily in bone collagen (Robins et al., 1994). Accordingly, studies (Liesegang et al., 1998) have shown urinary DPD determinations to be a very useful tool in following the course of degradation of bone collagen in dairy cows.

Despite the effectiveness of feeding dry cow rations low in calcium in preventing milk fever, this method of prevention has been almost abandoned because of difficulties in keeping the calcium intake sufficiently low (<20 g/d) when using commonly available feeds. Recent studies have shown, however, that it is possible to prevent milk fever by adding a substance to the feed capable of binding dietary calcium thereby making it unavailable for absorption (Thilsing-Hansen and Jørgensen, 2001; Wilson, 2001).

The purpose of this study was to investigate whether the effect could be attributed to activation of active calcium absorption from the intestine alone or if increased bone resorption was involved as well. For this purpose, measurements of 1,25(OH)₂D and deoxyypyridinoline were used. In addition, we investigated whether the chosen calcium binding substance had any effect on other blood constituents normally linked to calcium homeostasis (phosphorus and magnesium), on feed intake during the dry period, and on milk production in the subsequent lactation.

MATERIALS AND METHODS

Cows and Experimental Protocol

A total of 31 dry cows were included in this trial. All cows were about to enter their third or later lactation. The cows were assigned as either experimental (15 cows) or control (16 cows), and they were evenly distributed according to parity and expected date of calving. After calving, the mean parity was 4.2 (SEM = 0.31) in the experimental group and 3.9 (SEM = 0.27) in the control group.

During the last 3 wk before the expected date of calving, the cows were kept in a tie-stall barn and fed individually. All cows were fed a TMR consisting of grass and clover silage (24% DM), beet pulp silage (24% DM), beet molasses (75% DM), barley straw (85% DM), grain mixture (45% wheat, 25% distiller's grains, 20% oat, and 10% barley; 87% DM) and concentrate (UNIK 50, Svenska Lantmännen, SE-20503 Malmö, Sweden; 89% DM). Each cow daily received 31.5 to 33.5 kg of this TMR. The requirements for energy, proteins, and minerals were met according to NRC (1989). The mineral composition of the TMR is shown in Table 1. The TMR daily contributed: 66 g of calcium, 33 g of phosphorus, 19 g of magnesium, 178 g of potassium, and 24 g of sodium per cow.

Table 1. Mineral composition of the TMR.

Mineral	% of DM
Calcium	0.60%
Phosphorus	0.30%
Magnesium	0.17%
Potassium	1.62%
Sodium	0.22%

In addition to the TMR, the cows in the experimental group received zeolite A in the form of feed pellets with a high zeolite A content. The pellets were mixed into the daily ration. The composition of these pellets was as follows: 50.0% synthetic zeolite A (sodium-aluminium silicate, Dedima 100, Degussa-Hüls AG, D-60287 Frankfurt an Main, Germany), 8.0% wheat, 26.0% beet pellets, 4.0% rapeseed oil, and 12.0% beet molasses. According to the zeolite producer, the calcium binding capacity of the zeolite was >110 mg of Ca/g zeolite at pH 11. The exact binding capacity at pH ≤ 7 is unknown.

The third week before the expected date (wk -3) of calving served as a stepping-up period in which the administration of zeolite pellets to the experimental cows was increased from 0 to 1.4 kg/d. During the last 2 wk before calving (wk -2 and -1), each experimental cow received 1.4 kg of the zeolite pellets per day corresponding to 700 g of zeolite A. The 1.4 kg of zeolite pellets contributed 2 g of calcium, 0.7 g of phosphorus, 0.5 g of magnesium, 10 g of potassium, and 3 g of sodium (based on table values).

The cows were fed twice daily (morning and evening) and, before each morning feeding, the refusals from each cow were weighed and recorded.

At calving, the zeolite supplementation was stopped. To ensure that the calcium requirements of all cows (experimental and control) were met around calving, 250 g of calcium carbonate were given in the form of feed-grade chalk. The chalk was mixed into the daily ration on the day of calving and d 1 and 2 after calving. Each dose contained 90 g of calcium.

Therapeutic Treatments

An oral calcium drench (Recovin Calciumpasta, Kruuse, DK-5290 Marslev, Denmark) was given by the herdsman when a periparturient cow was judged to be borderline hypocalcemic (depression, muscle shivering, decreased appetite). A blood sample was drawn before drenching.

Cows judged by the herdsman and the veterinarian to be cases of milk fever based on clinical observations (subnormal rectal temperature, decreased rumination, paresis, decreased surface temperature) received a standard intravenous calcium treatment for milk fever

(Calphon vet., Bayer, SE-40224 Göteborg, Sweden). A blood sample was drawn before the infusion.

Milk Yield

Accumulated milk yield as well as the milk fat and protein content of each cow were recorded weekly through out the lactation.

Blood and Urine Sampling

Two blood samples were drawn from the tail vein into Vacutainer tubes (Vacutainer, no additive, silicone coated, and sodium heparin) 1 wk before the expected date of calving, as soon after calving as possible, approximately 24 and 48 h after calving, and 1 wk after calving. Serum and plasma was separated by centrifugation ($1500 \times g$ for 10 min) within 30 min after collection.

Urine was collected as midstream samples of spontaneously deposited urine. All urine samples were collected 1 wk before the expected date of calving in the morning before the a.m. feeding. Serum, plasma, and urine were stored at -23°C until the analyses were performed.

Laboratory Analyses

Serum samples were analyzed for $1,25(\text{OH})_2\text{D}$, plasma samples were analyzed for calcium, inorganic phosphate, and magnesium, and urine samples were analyzed for deoxypyridinoline and creatinine.

Plasma Determinations of Calcium, Inorganic Phosphate, and Magnesium

Plasma calcium and magnesium were determined by atomic absorption spectrophotometry (Perkin-Elmer 5000, Analytical Instruments, Perkin-Elmer Corp., Norwalk, CT). Plasma inorganic phosphate was determined by spectrophotometry (ADVIA 1650 Chemistry System, Bayer A/S, Lyngby, Denmark).

Serum Determinations of $1,25(\text{OH})_2\text{D}$

Serum concentrations of $1,25(\text{OH})_2\text{D}$ were determined by a modified version of the method described by Lund et al. (1979). Briefly, the analysis includes extraction of the metabolite from serum using diethyl ether (48 h), purification on Waters C-18 columns, separation of vitamin D components on Waters silica columns, assay determinations by radioimmunoassay using calf thymus as receptor binding protein, and quantification (LKB 1214 rack Beta Liquid scintillation counter, Turku, Finland).

Urine Determinations of Deoxypyridinoline and Creatinine

Urinary deoxypyridinoline concentrations were measured by competitive enzyme immunoassay with a commercial available kit (Pyrilinks-D-test kit; Metra Biosystems, Inc., Mountain View, CA) validated for cattle urine. The intraassay variation was 8.4% (CV) for samples in triplicate. Urinary creatinine was measured in triplicate by colorimetry (Jaffe, 1986) using an OpeRA Chemistry system autoanalyzer (Bayer Corp. Tarrytown, NY). Intraassay variation of analyses was 2.4%.

Statistical Analysis

All data are presented as means ($\pm$ SEM). The blood level of calcium, inorganic phosphate, and magnesium in experimental and control cows were compared using a two-sample *t*-test or the Wilcoxon Signed Rank Sum Test in SAS (Schlotzhauer and Littell, 1987). The differences were considered statistically significant if $P \leq 0.05$.

RESULTS

The average number of days the experimental cows received the full amount of zeolite pellets was 13.7 (range = 4 to 21; SD = 4.6; SEM = 1.2). The first blood sample and the urine sample were drawn on average 9.5 (range = 1 to 14; SEM = 1.4) and 7.2 (range = 1 to 17; SEM = 1.5) d before the actual date of calving in the control and experimental groups, respectively. In both groups, two cows calved within 1 to 3 d after the samples were taken.

Feed Intake

During the last week before calving, a total of 12% of the feed (mixed with zeolite pellets) fed to the experimental cows and 0.4% of the feed fed to the control cows were left uneaten. Twelve zeolite cows and three control cows had reduced feed intake during this period. Detailed information about feed intake during the last 2 wk of pregnancy is shown in Table 2.

Milk Yield

The mean accumulated milk yield 105 d after calving was 4515.8 kg in the control group and 4345.9 kg in the experimental group. The difference was not significant ($P = 0.448$). Additionally, there was no significant effect of zeolite treatment on total yield of milk fat and protein 105 d after calving ($P = 0.089$ and $P = 0.483$, respectively), although there was a tendency to a

Table 2. Feed consumption during the last two weeks of pregnancy in untreated control cows and zeolite-treated experimental cows.

	d -14 to -8			d -7 to -4			d -3 to -1		
	Number of cows leaving feed	Mean amount of refusals per cow kg $\pm$ SEM	%	Number of cows leaving feed	Mean amount of refusals per cow kg $\pm$ SEM	%	Number of cows leaving feed	Mean amount of refusals per cow kg $\pm$ SEM	%
Control (n = 16)	1	2.4 $\pm$ 0	1%	3	2.7 $\pm$ 0.2	2%	1	3.3 $\pm$ 0	5%
Experimental (n = 15)	7	15.8 $\pm$ 5.5	7%	9	21.1 $\pm$ 3.3	16%	10	13.7 $\pm$ 1.9	20%

slightly lower milk fat yield among the zeolite-treated cows.

Plasma Biochemical Analysis

The results of the plasma calcium, magnesium, and inorganic phosphate analyses are shown in Figures 1 to 3. From looking at Figure 1, it is obvious that zeolite supplementation had a stabilizing effect on blood calcium around calving. This is in accordance with the results obtained in previous experiments (Thilising-Hansen and Jørgensen, 2001). In this herd, the mean plasma calcium level in both groups did, however, stay above the hypocalcemia limit of 2.00 mmol/L suggested by DesCoteaux et al. (1997). Statistical analysis revealed a significant ($P < 0.0001$) difference in plasma calcium level between the two groups on the day of calving, whereas there was no difference in the plasma calcium level 1 wk before calving ($P = 0.27$).

Figure 1. Mean plasma calcium (and SEM) in zeolite-treated cows and untreated control cows. The solid horizontal line indicates the hypocalcemia limit.

From Figure 2, it is observed that the mean magnesium level was significantly lower ($P = 0.0068$) in the experimental group on the day of calving, whereas there was no difference ($P = 0.83$) 1 wk before calving. The mean plasma magnesium level in the experimental group stayed within the reference interval suggested by Kaneko et al. (1997) but increased above the upper limit in the control group on the day of calving and 1 and 2 after calving.

In this herd, mean plasma inorganic phosphate was below the lower limit of the reference interval given by Kaneko et al. (1997) among the untreated control cows 1 wk before calving as well as around calving (Figure 3). Plasma inorganic phosphate in the experimental group was significantly lower on the day of calving ($P = 0.015$) as well as 1 wk before calving ($P = 0.0004$). One week after calving, the phosphate level of the experimental cows increased to within the reference interval.

Serum 1,25(OH)₂D Analysis

Figure 4 shows the mean serum 1,25(OH)₂D level of the control cows (0) and the experimental cows (1) on

Figure 2. Mean plasma magnesium (and SEM) in zeolite-treated cows and untreated control cows. The solid horizontal lines indicate the upper and lower limit of the reference interval.

Figure 3. Mean plasma inorganic phosphate (and SEM) in zeolite-treated cows and untreated control cows. The solid horizontal lines indicate the upper and lower limit of the reference interval.

average 9.5 (range = 1 to 14; SEM = 1.4) and 7.2 (range = 1 to 17; SEM = 1.5) d before calving, respectively. The slight difference in mean sampling time is thought to be insignificant, as the number of samples taken in the critical time period just before calving is the same in both groups. According to Horst et al. (1994), the normal level of circulating $1,25(\text{OH})_2\text{D}$ is 20 to 50 pg/ml in late pregnancy, whereas during parturition and initiation of lactation the $1,25(\text{OH})_2\text{D}$ level rises to values ranging from 100 to >300 pg/ml. In this study, the mean level of serum $1,25(\text{OH})_2\text{D}$ among the zeolite-treated cows and the untreated control cows was 64.7 pg/ml and 43.7

Figure 4. Mean serum $1,25(\text{OH})_2\text{D}$ (and SEM) in zeolite-treated cows (1) and untreated control cows (0) on average 7.2 d (SEM = 1.5) and 9.5 d (SEM = 1.4) before calving, respectively.

pg/ml respectively, the difference being statistically significant ($P = 0.0409$).

Urinary Deoxypyridinoline Measurements

The reference interval for the deoxypyridinoline:creatinine ratio is 1.9 to 3.3 nmol/mmol creatinine (Liesegang, 1997). In this study, there was no significant difference in mean deoxypyridinoline:creatinine ratio between the two groups ($P = 0.6123$). The mean deoxypyridinoline:creatinine ratio of the control as well as the experimental cows was, however, slightly above the reference interval [3.59 nmol/mmol (SEM = 0.26) and 3.42 nmol/mmol (SEM = 0.19), respectively].

Therapeutic Treatments

Two experimental cows and one control cow received calcium drenches postpartum. At the time of drenching, all cows were normocalcaemic, showing serum calcium levels of 2.57, 2.34, and 2.39 mmol/L, respectively. No cases of milk fever were recorded, and no cows were treated with calcium solutions intravenously.

DISCUSSION

The effect of zeolite supplementation in the dry period on the plasma calcium level around calving in the present study is in accordance with findings in previous studies (Thilising-Hansen and Jørgensen, 2001). The increased plasma calcium level around calving in the zeolite-treated cows is thought to result from an activation of the calcium homeostatic mechanisms before calving, rendering the cow ready for the sudden and massive draw on blood calcium around calving.

As shown in Figure 2, the plasma magnesium level was significantly lower around calving among the zeolite-treated cows than among the untreated control cows. As plasma magnesium concentration is less well controlled than that of calcium and primarily is a result of balance between ruminal and intestinal absorption and renal excretion (Kaneko et al., 1997), the difference observed here might be attributed to decreased dietary availability caused by binding of magnesium by the zeolite. Furthermore, the addition of zeolite pellets to the TMR may have slightly "diluted" the ration, which only just meets the requirements for magnesium. However, there was no difference in plasma magnesium level between control and experimental cows after 1 wk of zeolite supplementation, and plasma magnesium stayed within the reference interval for magnesium during the entire period among the experimental cows. The hypermagnesemia seen among the control cows around calving may be connected to the concurrent drop

in serum calcium in these cows. The mechanism of hypomagnesemia in cows with hypocalcemia is unknown, but according to Riond et al. (1995), increased renal reabsorption of magnesium induced by PTH could play a role. Regarding the absorption of magnesium, it should further be noted, that since it is very dependent on dietary potassium (Martens et al., 1988), the relatively high level of potassium in the TMR (1.62%) may have negatively affected the magnesium absorption.

The low level of plasma inorganic phosphate seen among the untreated control cows after calving can probably be attributed to the hypophosphatemia normally seen in parturient cows (Kaneko et al., 1997). The depression seen on the plasma phosphate level 1 wk before calving among the zeolite-treated cows may be explained by a higher level of circulating PTH at this point, and thereby an increased salivary and renal excretion of phosphate. However, we believe a decreased bioavailability of dietary phosphorus induced by the zeolite is a more likely explanation. At pH values below 4, part of the zeolite may be hydrolyzed (Degussa-Huls, Germany), in which case the crystal structure is partially destroyed, releasing silicic acid, amorphous aluminium silicates, and aluminium (Cook et al. 1982). Soluble aluminium may interfere with the utilization of several minerals, with phosphorus absorption and metabolism being the most affected (Allen, 1984). This would be in accordance with the finding of Pond and Mumpton (1984), who saw a lower mean plasma phosphate concentration in lambs fed sodium zeolite A. They suggested that this effect was due to a reduced bioavailability of phosphorus due to formation of insoluble aluminium phosphate complexes in the intestinal lumen. Additionally, Valdivia and coworkers (1982) showed a marked decrease in phosphorus absorption when high doses of aluminium chloride ($\text{Al}_2\text{Cl}_3 \cdot 6\text{H}_2\text{O}$) was fed to lambs, and Håglin et al. (1988) obtained a significant drop in serum inorganic phosphate and a significant rise in serum calcium in pigs supplemented with aluminium hydroxide. Together, these studies support the view that the observed effect of zeolite on blood phosphorus may at least in part result from binding of phosphorus to free aluminium in the lumen of the gastrointestinal tract. Interaction between the calcium- and phosphorus homeostasis have been thoroughly investigated in ruminants (Barton et al., 1987; Beardsworth et al., 1989; Breves and Schröder, 1999; Care, 1994; Horst, 1986; Khorasani and Armstrong, 1992; Schröder et al., 1995). In ruminants, unlike nonruminants, hypophosphatemia induced by dietary phosphorus deficiency does not stimulate increased production of $1,25(\text{OH})_2\text{D}$ (Barton et al., 1987; Maunder et al., 1986; Schröder et al., 1995). Instead the efficiency of the intestinal receptor for $1,25(\text{OH})_2\text{D}$ is increased, thus making

circulating $1,25(\text{OH})_2\text{D}$ more effective at the gut level (Schröder et al., 1990). Barton et al. (1987) have shown, that feeding a low phosphorus diet prepartum (0.7 times maintenance requirements) significantly increased the plasma phosphate and calcium concentration postpartum, and others (Kichura et al., 1982) found that feeding a low phosphorus diet prepartum (10 g of phosphorus/d, 86 g of calcium/d) resulted in a lower incidence of parturient paresis than feeding a diet high in phosphorus (82 g of phosphorus/d, 86 g of calcium/d). Therefore, it cannot be excluded that at least part of the effect of the zeolite supplementation in this study on the calcium status around calving may be attributed to a hypophosphatemia-induced increased effect of $1,25(\text{OH})_2\text{D}$, and thereby an increased intestinal absorption of calcium. However, the fact that the mean $1,25(\text{OH})_2\text{D}$ concentration of the zeolite supplemented cows was significantly higher than among the control cows indicates that the expected calcium binding effect of the zeolite was probably also involved, since a negative calcium balance expectedly would lead to an increase in circulating $1,25(\text{OH})_2\text{D}$ mediated in part by an increased PTH secretion.

The lack of difference in the level of urine deoxypyridinoline:creatinine between the two groups indicates that bone resorption was apparently not increased during zeolite supplementation. It should be pointed out, however, that these measurements were made 1 wk before the expected date of calving. Samples drawn closer to the actual date of calving may have shown a different result.

The immediate and long-term consequence, if any, of the extra drop in blood inorganic phosphate before and around calving is currently unknown. A long-term phosphorus deprivation may lead to anorexia (Gartner et al. 1982; Call et al. 1986; Wan Zahari et al. 1990), rachitic lesions (Miller et al. 1964), and decreased milk yield (Muschen et al. 1988). In the present study, the drop in plasma phosphate among the zeolite-treated cows was only transient, since the phosphate level had normalized within 1 wk after calving. It is currently unknown whether the observed decrease in feed intake during the zeolite supplementation was due to the concurrent hypophosphatemia or to the palatability of the zeolite pellets. It must be pointed out, however, that the hypophosphatemia and decreased feed intake among the zeolite-treated cows apparently did not reduce the milk yield in the subsequent lactation.

The extent to which aluminium released from the zeolite is absorbed is unknown. Studies on broilers revealed that the addition of sodium zeolite A to the diet might increase serum aluminium (Ingram et al. 1991; Rabon et al. 1991). Measurements of serum aluminium may therefore be included in future studies, although

aluminium, even when ingested in reactive forms, such as AlCl_3 , is low in toxicity (Underwood and Suttle, 1999). Blood analysis of other trace minerals such as copper, zinc, and manganese may also be of interest due to their possible adsorption by ion exchange to sodium aluminumsilicate.

In conclusion, the effect of prepartum zeolite supplementation on the periparturient calcium homeostasis may be explained by direct calcium binding and/or an aluminium derived phosphorus binding capacity of the zeolite. The present study was not designed to distinguish between these, but taking into account the effect on the previously mentioned biochemical markers, the present results were most likely derived from a combination of these two mechanisms.

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