Effects of long-term dietary supplementation with clinoptilolite on incidence of parturient paresis and serum concentrations of total calcium, phosphate, magnesium, potassium, and sodium in dairy cows

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Objective—To determine whether dietary supplementation with clinoptilolite affects the incidence of parturient paresis and serum concentrations of total calcium (tCa), inorganic phosphorus (PO_4^{2-}), magnesium (Mg^{2+}), potassium (K^+), and sodium (Na^+) in dairy cattle.

Animals—52 dairy cows.

Procedure—Cows were placed into 3 groups. The first 2 groups (group A [n = 17] and group B [17]) were offered a concentrate supplemented with 1.25% and 2.5% clinoptilolite, respectively. The third (group C [n = 18]) served as a control and was offered the concentrate alone. The experiment started 1 month before parturition and lasted until the beginning of the next nonlactating period. Around the time of calving, all cows were monitored for the development of parturient paresis. Blood samples were taken at the commencement of the experiment, on the day of calving, and thereafter at monthly intervals to measure serum tCa, PO_4^{2-}, Mg^{2+}, K^+, and Na^+ concentrations.

Results—The incidence of parturient paresis in group B cows was significantly lower, compared with group C cows. However, serum concentrations of tCa, PO_4^{2-}, Mg^{2+}, K^+, and Na^+ were not significantly affected by long-term supplementation with clinoptilolite.

Conclusions and Clinical Relevance—In the context of this experiment, clinoptilolite supplementation at 2.5% appeared to have reduced the incidence of parturient paresis in dairy cows, suggesting that its effectiveness depends on the amount incorporated in the ration of cows. Addition of clinoptilolite in the concentrate of dairy cows during the nonlactating period could be used as a cost-effective preventive treatment for parturient paresis. (*Am J Vet Res* 2005;66:2081–2085)

In practice, most dairy cows have some degree of subclinical hypocalcemia during the periparturient period. In some cows, the serum calcium concentrations become too low to support nerve and muscle function, resulting in a clinical disease commonly known as milk fever or parturient paresis.[1] Evidence in the literature suggests that known predisposing factors for parturient paresis in cows are age, milk yield, breed, body condition, length of the nonlactating period, and diet.[2] Results of numerous studies[3-7] support the idea that reducing dietary calcium intake during the nonlactating period will improve the resistance of cows to parturient paresis. However, the formulation of rations with low calcium is difficult with ordinary feedstuffs.[8] Hence, research efforts have been focused on the reduction of dietary calcium availability in the gastrointestinal tract by use of materials that act as calcium binders. Jorgensen et al,[9] Thilsing-Hansen and Jorgensen,[10] and Thilsing-Hansen et al[11] demonstrated that the administration of synthetic zeolite A to dairy cows during the nonlactating period prevents the abrupt reduction of serum calcium concentrations around the time of calving and the incidence of parturient paresis. These findings were attributable to the activation of the calcium homeostatic mechanisms prior to parturition.

The existing literature does not provide a consistent framework to account for the function between zeolite material and calcium availability in dairy cows. Moreover, results of an earlier study by Mumpton and Fishman[12] indicate that natural and synthesized zeolites have common ion exchange properties. Zeolites have been used in cow nutrition since the 1960s, mainly to improve performance and protect against mycotoxins.[13] One of the major issues regarding the use of natural zeolites in the nutrition of ruminants is their potential adsorbent and binding properties for certain macroelements, such as **total calcium (tCa)**, **inorganic phosphorus (PO_4^{2-})**, **magnesium (Mg^{2+})**, **potassium (K^+)**, and **sodium (Na^+)**.[14-16]

The purpose of the study reported here was to investigate whether dietary supplementation with a natural zeolite (clinoptilolite) at various concentrations in concentrate affects the incidence of parturient paresis in dairy cows. A complementary objective was to evaluate the effect of the percentage of clinoptilolite provided on the incidence of parturient paresis and serum concentrations of tCa, PO_4^{2-}, Mg^{2+}, K^+, and Na^+ when fed for a prolonged period (ie, beginning 1 month before the expected day of calving until the onset of the following nonlactating period).

Materials and Methods

Animals—Fifty-two clinically normal Holstein dairy cows were used. They were randomly assigned to 1 of 3 groups that were similar in age and parity. All cows had completed at least 1 lactation period, and 2 cows in each group

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were about to enter a second lactation. The number of cows per group and the mean ± SD age and parity number for each group as well as the amount of clinoptilolite (percentage of fed concentrate) provided were as follows: group A included 17 cows that were 5.53 ± 1.80 years old, had a parity of 3.71 ± 1.53, and were fed concentrate supplemented with 1.25% clinoptilolite; group B included 17 cows that were 5.29 ± 2.39 years old, had a parity of 3.70 ± 1.99, and were fed concentrate supplemented with 2.5% clinoptilolite; and group C included 18 cows that were 5.05 ± 2.23 years old, had a parity of 3.80 ± 1.89, were fed the basal concentrate, and served as a control group.

Zeolitic material—The zeolitic material used in the experiment had a particle size of < 0.80 mm in diameter and contained approximately 92% clinoptilolite; the admixture was 8% opal ($SiO_2 \times nH_2O$) as determined by x-ray powder diffraction. The exchange capacity of the zeolitic material was 220 mEq/100 g (**Appendix 1**).

Experimental design—All cows began a nonlactating period 1 month before the start of the experiment. At the commencement of the experiment, 3 isonitrogenous and isoenergetic concentrates with the same content in macroelements were formulated and fed to the 3 groups of cows (groups A, B, and C; **Appendices 2 and 3**). During the last month of the nonlactating period, each cow was offered corn silage (20 kg/d), molasses (2 kg/d), and concentrate (2 kg/d), whereas during lactation, cows were offered corn silage (30 kg/d), molasses (2 kg/d), and concentrate (300 g/L of milk produced each day). The amount of concentrate fed was divided into equal portions and offered twice a day during the morning and afternoon milking.

The start of the experiment was set 1 month before the expected day of calving. At that point, a blood sample was obtained from each cow, and immediately after, the cows were assigned to a group. Blood sample collections were then repeated on the day of calving and thereafter at monthly intervals until the beginning of the new nonlactating period. Samples were taken by jugular vein puncture from each cow after the afternoon milking, in vacuum glass tubes with an 18-gauge needle. After clotting, the serum was separated by low-speed centrifugation (1,600 × *g* for 15 minutes), transferred in plastic vials, stored in a refrigerator, and analyzed within 24 hours after blood sample collection.

All experimental cows were monitored around the time of calving for the development of parturient paresis. Diagnosis was based on clinical observations (ie, decreased appetite, decreased rectal temperature, decreased rumination, paresis, and recumbency) and confirmed by determination of serum calcium concentration. Affected cows received the appropriate treatment with IV injection of calcium borogluconate solution[a] and supportive care, if necessary.

The incidence of subclinical hypocalcemia was evaluated from serum calcium concentrations on the day of calving. Cows with subclinical hypocalcemia were defined as cows that had serum calcium concentrations of < 2 mmol/L, as suggested by DesCoteaux et al,[17] without clinical signs of parturient paresis.

Chemical analyses—All blood samples were analyzed for serum tCa and Mg^{2+} concentrations by means of flame atomic absorption spectrophotometry with an analyst instrument.[18,b] The evaluation of serum concentrations of PO_4^{2-} was done by use of the heteropoly acid-blue method,[19] whereas serum K^+ and Na^+ concentrations were measured with the aid of flame atomic emission spectrophotometry in a flame photometer.[c]

Statistical analysis—Data were analyzed by use of a software program.[d] Normality of data was tested with the Kolmogorov-Smirnov test and the homogeneity of variances with the Levene test. The long-term effect of the clinoptilolite supplementation was evaluated by use of repeated-measures ANOVA. Post hoc analysis was done by use of the Tukey multiple range test. Also, the χ^2 test was used to determine whether the incidence of parturient paresis and subclinical hypocalcemia was significant among groups. A value of $P < 0.05$ was considered significant.

Results

The mean ± SD duration of the nonlactating period was 67.17 ± 8.35 days, 65.44 ± 9.48 days, and 68.90 ± 8.92 days for group A, B, and C cows, respectively, and was not significantly different among the experimental groups. Parturient paresis was observed in 7 of 18 group C cows (control group), in 3 of 17 group A cows, and in 1 of 17 group B cows.

The difference in the incidence of parturient paresis was significant between group B and group C (control group) cows. All cows that had parturient paresis after parturition also had serum calcium concentrations of < 1.5 mmol/L. The group B cow with parturient paresis successfully recovered after a single dose of 500 mL of calcium borogluconate solution. One of 3 affected group A cows relapsed and required a second IV injection of calcium borogluconate solution after approximately 24 hours, whereas 4 of 7 affected group C cows relapsed, and 1 of those cows recovered after 3 IV injections of calcium borogluconate solution given at 24-hour intervals.

On the day of calving, 9 of 17 group A cows, 5 of 17 group B cows, and 9 of 18 group C cows had sub-

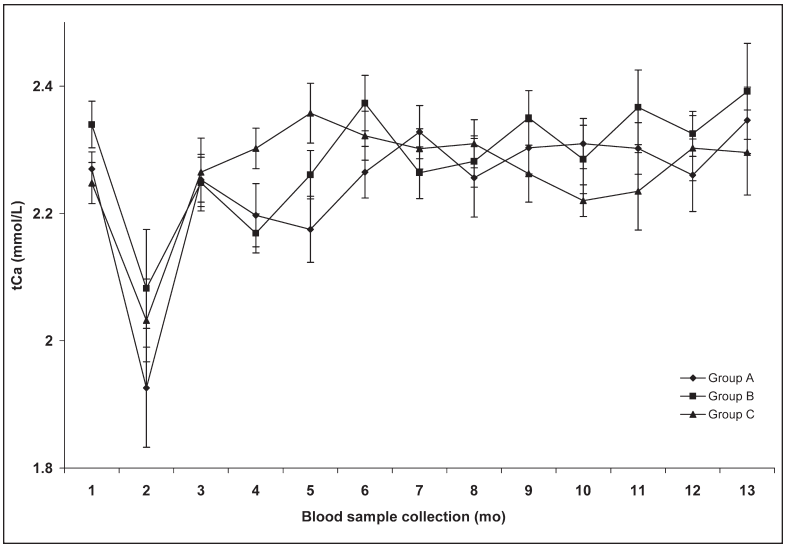

Figure 1—Mean ± SE serum total calcium (tCa) concentrations (mmol/L) in blood samples of 3 groups of cows that were obtained 4 weeks before the expected calving date (blood sample collection 1), on the day of calving (blood sample collection 2), and subsequently at monthly intervals during lactation and until the start of the next nonlactating period (blood sample collections 3 through 13, respectively). Cows were offered concentrates that were supplemented with either 1.25% (group A) or 2.5% (group B) clinoptilolite, whereas group C cows were fed the unsupplemented concentrate and served as controls.

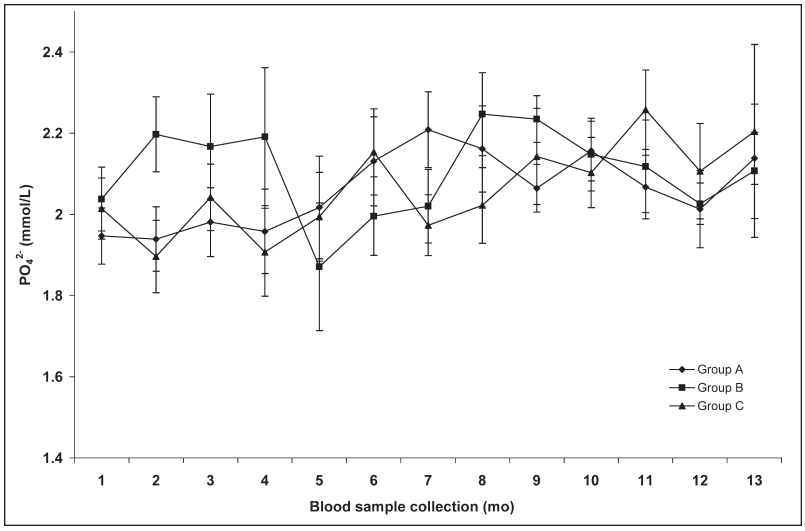

Figure 2—Mean ± SE of serum phosphate (PO_4^{2-}) concentrations (mmol/L) in blood samples of 3 groups of cows that were obtained 4 weeks before the expected calving date (blood sample collection 1), on the day of calving (blood sample collection 2), and subsequently at monthly intervals during lactation and until the start of the next nonlactating period (blood sample collections 3 through 13, respectively). Cows were offered concentrates that were supplemented with either 1.25% (group A) or 2.5% (group B) clinoptilolite, whereas group C cows were fed the unsupplemented concentrate and served as controls.

clinical hypocalcemia. No significant difference in serum calcium concentration was recorded among groups.

Serum tCa concentration was not significantly affected by the addition of clinoptilolite to the concentrate throughout the experiment (mean ± SE, 2.251 ± 0.017 mmol/L, 2.282 ± 0.017 mmol/L, and 2.269 ± 0.017 mmol/L for group A, B, and C cows, respectively). Mean serum tCa concentration for group B cows was significantly lower, compared with group C cows (control group), at the fourth blood sample collection time, whereas the mean serum tCa concentration for group A cows was significantly lower, compared with that for group C cows, at the fifth blood sample collection time (**Figure 1**).

On a long-term basis, mean ± SE serum PO_4^{2-} concentration was not significantly affected by feeding treatment (2.064 ± 0.045 mmol/L, 2.106 ± 0.045 mmol/L, and 2.058 ± 0.045 mmol/L for group A, B, and C cows, respectively). On the day of calving, serum PO_4^{2-} concentration in group B cows was significantly higher, compared with control group cows, whereas no significant differences were found among groups at the other sample collection times (**Figure 2**).

Mean ± SE serum Mg^{2+} concentrations were not significantly affected by the long-term dietary supplementation with clinoptilolite (1.018 ± 0.011 mmol/L, 1.026 ± 0.011 mmol/L, and 1.003 ± 0.011 mmol/L for group A, B, and C cows, respectively). Furthermore, no significant differences were found among the experimental groups at any sample collection time.

Mean ± SE serum K^+ and Na^+ concentrations were not significantly affected by the treatment throughout the experiment. Mean ± SE serum concentrations of K^+ were 4.261 ± 0.027 mmol/L, 4.250 ± 0.027 mmol/L, and 2.279 ± 0.027 mmol/L for group A, B, and C cows, respectively, and mean serum concentrations of Na^+ were 142.365 ± 0.376 mmol/L, 142.165 ± 0.376 mmol/L, and 142.010 ± 0.376 mmol/L for group A, B, and C cows, respectively. No significant difference was found among groups at any sample collection time.

Discussion

The objective of our study was to investigate the effect of long-term dietary clinoptilolite supplementation of dairy cows at amounts of 1.25% and 2.5% of the concentrate on the peripartum incidence of parturient paresis and on serum concentrations of tCa, PO_4^{2-}, Mg^{2+}, K^+, and Na^+. It is apparent from the literature that to maintain serum tCa concentrations within reference range limits around the time of calving, homeostatic mechanisms are activated and the negative tCa balance is corrected by increased resorption of bone calcium stores and increased absorption of calcium from the intestine. Parathyroid hormone and 1,25-dihydroxycholecalciferol stimulate bone calcium mobilization, whereas active transport of calcium across the intestinal epithelial cells is controlled by 1,25-dihydroxycholecalciferol alone.[2,20] During the nonlactating period, these mechanisms for replenishing serum tCa concentration are relatively inactive. Thus, nearly all dairy cows have some degree of hypocalcemia during the first days after parturition. Approximately 24 hours of 1,25-dihydroxycholecalciferol stimulation is required before intestinal calcium transport is increased substantially, whereas bone calcium resorption is not increased until after approximately 48 hours of parathyroid hormone stimulation.[2] The findings of Thilsing-Hansen and Jorgensen[10] and Thilsing-Hansen et al[11] revealed a smaller decrease in tCa on the day of calving when the synthetic zeolite A was provided at 1 kg/d for the last 4 weeks of the nonlactating period or at 700 g/d for the last 2 weeks of the nonlactating period, respectively. According to those authors, the smaller decrease in serum tCa concentration was a result of the activation of the calcium homeostatic mechanism before parturition, as increased amounts of 1,25-dihydroxycholecalciferol were found in the serum of the experimental cows.[11]

In our study, a significantly lower incidence of parturient paresis was found in group B cows, compared with control group cows. It is possible that clinoptilolite may have an effect that is similar to that of zeolite A[10,11] in activating calcium homeostatic mechanisms prior to parturition. As a consequence, group B cows responded faster and more efficiently to the decrease in serum tCa concentration observed on the day of calving and did not have clinical signs of parturient paresis in the days that followed. However, the exact mechanism for this positive effect of dietary supplementation with clinoptilolite is currently unknown.

On the day of calving, indicators of subclinical hypocalcemia in our study did not differ among the 3

groups of cows. This finding was expected, as on the day of calving, some degree of hypocalcemia was observed in dairy cows as a result of the onset of lactation.[1] However, according to Thilsing-Hansen and Jorgensen[10] and Thilsing-Hansen et al,[11] cows receiving synthetic zeolite A did not have subclinical hypocalcemia, a fact attributed to the oral administration of calcium preparations on the day of calving.

In our study, monitoring of serum tCa concentration at the end of the second (fourth blood sample collection time) and third (fifth blood sample collection time) months of lactation revealed reduced serum concentrations in group B and group A cows, which differed significantly, compared with those of control group cows. However, none of the observed values were < 2 mmol/L, which is indicative of hypocalcemia[17]; decreases in serum tCa concentrations were not observed at later blood sample collection times. After the third month of lactation, no significant differences were found in serum tCa concentrations among groups. Similarly, Hemken et al[14] and Bosi et al[16] reported that when dairy cows were fed a diet containing 6% and 1% clinoptilolite following the peak of lactation and for a period of 28 or 76 days, respectively, serum tCa concentrations were unaffected. Nevertheless, no information appears to be available about the effect of dietary supplementation with clinoptilolite during the entire lactation period.

The significantly higher serum PO_4^{2-} concentration on the day of calving in group B cows, compared with that of control group cows, was likely the result of the earlier stimulation of the calcium homeostatic mechanism, given that 1,25-dihydroxycholecalciferol increases intestinal absorption and serum PO_4^{2-} concentration.[21] In contrast, Thilsing-Hansen et al[11] showed that although mean serum 1,25-dihydroxycholecalciferol concentrations were significantly higher in zeolite-treated cows, serum PO_4^{2-} concentration was significantly lower on the day of calving. The latter was attributed to a reduced availability of PO_4^{2-} following the hydrolysis of zeolite A caused by the acidic pH of the gastrointestinal tract and the formation of insoluble aluminium phosphate complexes. In contrast, clinoptilolite appears to be stable in response to the acidic pH of the gastrointestinal tract, and such effects are not expected. Pond et al[22] have provided evidence that indicates that the administration of synthetic zeolite A increases the silicon concentration in urine as a result of partial breakdown of zeolite, something that is not observed when clinoptilolite is used.

In our study, long-term dietary supplementation with clinoptilolite did not affect serum PO_4^{2-} concentration, which remained within reference range limits during the entire experimental period. To our knowledge, no other published data exist about the effect of clinoptilolite on serum PO_4^{2-} concentrations in dairy cows, but similar results were obtained by previous experiments performed on a short-term basis in other ruminants. In steers, 3% dietary supplementation with 3 clinoptilolite ores had no effect on serum PO_4^{2-} concentration,[15] and in growing lambs, no differences in serum PO_4^{2-} concentrations were found following dietary supplementation of 2%[23] or 3% clinoptilolite.[22]

No significant differences were found in serum Mg^{2+} concentrations among the 3 groups of cows; serum Mg^{2+} concentrations were not affected by dietary supplementation with clinoptilolite. This result is in agreement with those of previous studies on lactating dairy cows,[14] steers,[15,24] growing lambs,[22] and sows.[25] However, it is contradictory to the results of the study by Thilsing-Hansen et al,[11] in which serum Mg^{2+} concentration was reduced around the time of calving because of binding by zeolite A and the low concentration of dietary Mg^{2+}, which only just met the requirements.

Long-term dietary supplementation with clinoptilolite does not seem to have any influence on serum K^+ and Na^+ concentrations. In previous studies, some alterations in serum K^+ and Na^+ concentrations have been reported for steers[15,24] but not for dairy cows.[14,16] These alterations probably depend on the amount of excess of those macroelements in the diet and the amount of the binder fed.

The main conclusion of our study is that dietary supplementation with clinoptilolite during the last month of the nonlactating period at 2.5% of the concentrate reduces the peripartum incidence of parturient paresis in dairy cows and could be used as a cost-effective preventive treatment for the disease. Furthermore, long-term dietary supplementation with clinoptilolite at 1.25% or 2.5% of the concentrate does not significantly affect serum concentrations of tCa, PO_4^{2-}, Mg^{2+}, K^+, and Na^+.

a. P-Calcium, Novartis Animal Health Inc, Basel, Switzerland.
b. Perkin-Elmer A Analyst 100, Perkin-Elmer Corp, Norwalk, Conn.
c. Sherwood 410 flame photometer, Sherwood Scientific Ltd, Cambridge, England.
d. SPSS 12, SPSS Inc, Chicago, Ill.

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Appendix 1

Chemical composition (%) of the zeolitic material used as a supplement.

Zeolite composition	Amount (%)
Silicon dioxide (SiO_2)	68.9
Aluminum oxide (Al_2O_3)	11.27
Calcium oxide (CaO)	3.02
Magnesium oxide (MgO)	0.6
Sodium oxide (Na_2O)	0.75
Potassium oxide (K_2O)	2.23
Iron oxide (Fe_2O_3)	0.11
LOI	13.05

LOI = Loss on ignition.

Appendix 2

Composition (%) of the concentrate fed to cows throughout the experiment.

	Cows		
Ingredient (%)	Group A	Group B	Group C
Soybean meal	34.47	35.42	34
Maize grains	20	21	17
Wheat bran	17.69	14.33	22.41
Sunflower meal	8	8	8
Barley grains	8	8	8
Carob fruits (locust bean)	8	8	8
Salt	0.8	0.8	0.8
Limestone	1	1	1
Dicalcium phosphate	0.6	0.76	0.6
Zinc oxide	0.04	0.04	0.04
Vitamins and trace minerals	0.15	0.15	0.15
Clinoptilolite	1.25	2.5	0

Appendix 3

Mean ± SE composition (%) of major minerals in the concentrate fed to cows throughout the experiment.

	Cows		
Mineral (DM %)	Group A	Group B	Group C
Calcium	0.68 ± 0.008	0.70 ± 0.006	0.69 ± 0.003
Phosphate	0.70 ± 0.006	0.69 ± 0.008	0.73 ± 0.006
Magnesium	0.40 ± 0.005	0.43 ± 0.009	0.41 ± 0.005
Potassium	0.12 ± 0.001	0.11 ± 0.002	0.11 ± 0.001
Sodium	0.28 ± 0.003	0.29 ± 0.001	0.29 ± 0.001

DM = Dry matter.