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Calcium chloride gel treatment of parturient dairy cows:

Effect on hypocalcemia and parturient diseases

Garrett R. Oetzel, DVM, MS

From the Food Animal Production Medicine Section, Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, 2015 Linden Drive West, Madison, WI 53706.

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Summary: *Holstein cows (n = 204) were paired according to parity, previous incidence of milk fever, and calving date. Cows in the treatment group received doses of calcium chloride in a gel formulation 12 hours prior to expected calving and at 0, 12, and 24 hours after calving. Cows in the control group received no calcium chloride gel treatment. Serum calcium concentrations were significantly higher for cows in the treatment group on days 1 and 2 post-calving. Increase in serum calcium concentration due to treatment was greatest in older cows. The treatment group also had significantly reduced incidence rates of parturient paresis, parturient hypocalcemia, and displaced abomasum. Odds ratios for reduced risk of these diseases were 3.9, 7.2, and 10.7, respectively. Pre-calving dosing of the calcium chloride gel reduced the incidence rate of parturient paresis compared to treatment group cows who did not receive their first dose of gel until after they had calved. Dosing of the calcium chloride gel starting immediately after calving (without the pre-calving dose) was sufficient to increase serum calcium concentrations and to reduce relative risk for parturient hypocalcemia and displaced abomasum.*

Milk fever is a metabolic disease of parturient dairy cows that is evoked by a temporary imbalance between calcium availability and high calcium demand associated with the onset of lactation. This imbalance results in hypocalcemia, which may cause impaired muscle contractility, flaccid paralysis, recumbency, and/or death.[1] Recent epidemiological surveys report milk fever incidence rates of 6.3%[2] and 7.2%.[3] Readily observed economic losses associated with milk fever include death losses and treatment costs for affected cows. Less apparent, but more economically important, are losses due to increased risk of other periparturient disorders[4] and impaired milk production in the subsequent lactation.[5]

Not all cows that become hypocalcemic around the time of calving (parturient hypocalcemia) exhibit clinical signs of milk fever (parturient paresis). Parturient hypocalcemia has been defined as serum ionized calcium concentrations less that 4.0 mg/dl on the day of parturition with or without clinical signs of paresis.[6] Parturient hypocalcemia occurred in 67% of control cows in one study,[6] but only 25% of the cows with hypocalcemia exhibited clinical signs of milk fever. Because calcium is necessary for motility of the gastro-intestinal tract,[7] health and productivity of dairy cows may be impaired by hypocalcemia even when clinical signs of paresis are not present. Hypocalcemic cows are at 4.8 times greater risk for left displacement of the abomasum (LDA) than normocalcemic cows.[8] The proportion of cows with hypocalcemia that later develop LDA is not affected by the presence or absence of clinical signs of paresis.[8] The impact of subclinical hypocalcemia in parturient dairy cattle may be nearly as great as that of clinical milk fever.[9]

Feeding strategies for preventing milk fever include dietary calcium restriction[10] and feeding acidogenic diets[11] during the prepartum period. Feeding anionic salts creates acidogenic diets[12] which decrease the risk of milk fever via improved 1,25-dihydroxyvitamin D response to hypocalcemia.[13]

An alternative approach to adding anionic salts to the prepartum diet is to administer an anionic salt such as calcium chloride, in repeated oral doses, around the time of parturition. Prophylactic treatment with four doses of calcium chloride in a gel carrier (each dose contained 150 g calcium chloride, which supplied 54 g elemental calcium) significantly reduced the incidence rate of milk fever in a Swedish study.[14] Other studies have documented significant and rapid increases in blood calcium concentration following oral administration of calcium

chloride at varying doses and with varying carriers.[15-17] Oral administration of calcium chloride improves blood calcium concentrations apparently because calcium chloride is acidogenic[18] and because it is a highly available source of calcium.[17]

Purposes of this study were to determine the effect of prophylactic treatments with a calcium chloride gel on 1) serum calcium concentrations in the immediate post-partum period; 2) relative risk of parturient paresis and parturient hypocalcemia; and 3) relative risk of other periparturient diseases associated with hypocalcemia.

Materials and Methods

Experimental Animals — Holstein cows (n = 204) from the United States Dairy Forage Research Center herd were included in the trial. Cows were paired according to parity, previous incidence of milk fever, and calving date. First, second, and third or greater parity animals were paired together. For pairs of third and greater parity, a maximum difference in parity of two was allowed. The maximum allowed difference in calving date within a pair was 35 days. All cows calved in a ten-month period from December, 1991 to September, 1992. Mature equivalent milk production from the previous lactation, adjusted to 305-day lactation length, was recorded for all cows entering in their second or later lactation.

Treatment Protocol — One cow within each pair was randomly selected, using a random numbers generator, to receive prophylactic dosing with a commercial calcium chloride gel product in a thick gel preparation.[a] According to the product label, each tube (355 g) provided a minimum of 54 g elemental calcium, about 70% of which came from calcium chloride. Each tube also provided phosphorus (8.2 g) and a small amount of magnesium (1.5 g). The other cow in the pair was designated as the control cow and received no calcium chloride gel treatments. Different pre-calving diets were used during the study; however, both cows within a pair always received the same diet prior to calving and for the first two days after calving. Diets received by the cows were not controlled after two days post-calving.

Prophylactic treatments of the calcium chloride gel were administered to treated cows at approximately 12 hours prior to expected calving, immediately after calving, 12 hours after calving, and 24 hours after calving. Timing of the administration of the pre-calving dose was subjectively determined by the herdsmen who administered the treatments. If the precalving dose was given to a cow and the cow did not calve within the next 12 hours, the treatment was repeated at 12-hour intervals until calving occurred. The remaining three doses were then administered according to protocol.

Serum Calcium: Collection and Analysis — Whole blood for serum calcium analysis was collected from the coccygeal vein of cows. The first sample was taken 18 to 24 hours after calving. Subsequent samples were taken daily (in the afternoon) for days 2 through 7 post-calving. The pre-treatment serum calcium sample was used in place of the daily sample if an episode of paresis requiring intravenous treatment occurred near the time that the daily sample would have been taken.

Blood was collected into serum separation tubes,[b] allowed to clot at room temperature, and centrifuged to allow separation of the serum from the clot. The tubes were then refrigerated at 0°C on the farm. Blood tubes were collected weekly from the farm and brought to the laboratory, where the tube was centrifuged again and the serum removed. A pilot study[c] using three lactating Holstein cows showed no effect ($P > 0.75$) of days in storage at 0°C on calcium concentration of blood collected and handled according to this protocol.

After the serum was removed from the tubes in the laboratory, it was frozen in borosilicate

glass vials at -20°C. Serum was later thawed and analyzed in batches. Serum total calcium concentration was determined by a dry reagent chemistry analyzer[d] using the Arsenazo III dye colorimetric method.

Disease Outcome Recording — Parturient paresis (clinical milk fever) was recorded for cows who exhibited clinical signs of paresis in the time period starting 12 hours before calving and ending seven days after calving. Each clinical case was treated with an intravenous calcium solution and the diagnosis of parturient paresis was confirmed by pre-treatment serum calcium concentration less than 8.0 mg/dl.[6,8] Cows assigned to the treatment group that developed parturient paresis prior to their first treatment with the calcium chloride gel were disqualified from the study, along with their cohort.

Parturient hypocalcemia was defined as serum calcium concentration less than 8.0 mg/dl on any day post-calving, with or without clinical signs of paresis.[6,8] Serum total calcium concentration of 8.0 mg/dl is approximately equivalent to blood ionized calcium concentration of 4.0 mg/dl.[8,19] Retained fetal membranes was defined as failure to expel fetal membranes within 24 hours of parturition and was recorded by the herdsmen. Displaced abomasum was diagnosed and recorded by the research herd's attending veterinarian. Diagnosis of displaced abomasum (left or right-sided) was made by simultaneous auscultation and percussion of the paralumbar regions and was recorded only for cows less than 21 days in milk.

Statistical Analysis — Mean serum calcium concentrations for days 1 through 7 post-calving were calculated and plotted with standard error values. The difference in serum calcium concentration (calcium chloride gel treated value minus control value) was calculated for each pair of cows. Mean values for the difference were compared using a paired *t* test.[20]

Analysis of variance[20,21] was used to test for significant effects of covariates on the difference in serum calcium concentration within pairs. Covariates used in the analysis were parity, previous milk fever, pre-calving diet, calving month, and pre-calving dose status (not all cows in the treatment group received the pre-calving dose of calcium chloride gel). Analysis of variance was performed only on data from days in which the treatment effect was significant (days 1 and 2 post-calving). Interactions between covariates were not included in the model because of high correlations between many of the covariates. Assumptions of normal distribution of errors and constant variance were validated by checking them graphically. Parity was the only covariate that significantly contributed to the model; least squares (LS) means by parity were then calculated and compared.

Incidence rates of disease outcomes were calculated for the treatment and control groups. Effects due to treatment and covariates were evaluated using multiple logistic techniques with maximum likelihood analysis.[21,22] All five main covariates were included in the final logistic regression models, regardless of significance, except when their inclusion created redundant parameters. Because no cases of parturient paresis occurred in parity 1 cows, only parity 2 and greater cows were included in the regression analysis for parturient paresis. Odds ratios (with 95% confidence intervals) for the effect of treatment on reducing the risk of disease outcomes were calculated.[22] Significance of the likelihood ratios[21,22] were calculated and used to evaluate the goodness of fit of the models. Odds ratios for parturient hypocalcemia were also calculated by parity, since this covariate significantly contributed to the model for parturient hypocalcemia.

Effect of the pre-calving dose of calcium chloride gel on the incidence rate of parturient paresis was evaluated qualitatively only. Statistical evaluation of this outcome could not be conducted because the determination of which cows received the pre-calving dose was not

random and because the incidence rate of parturient paresis was 0% in the group that received the pre-calving treatment.

Significance was declared at $P < 0.05$ unless otherwise noted.

Results and Discussion

Descriptive statistics of the experimental cows are presented in Table 1. Both young and mature cows were included in the study. Mean values for mature equivalent milk production from the previous lactation and parity were very similar for the treatment and control groups.

Only 39 of the 102 cows in the treatment group (38.2%) received one or more pre-calving doses of the calcium chloride gel. This indicated that the herdsmen were able to identify impending parturition in a rather low percentage of the cows. Inaccurate prediction of parturition has been reported in other studies of milk fever prevention.[23,24] The experimental herd had three herdsmen and housed cows in groups in free stalls. Herds with a single herdsman and cows housed in individual stalls may have less difficulty in predicting impending parturition and correctly administering a pre-calving dose of calcium chloride gel.

Serum Calcium Concentrations — Prophylactic treatment with the calcium chloride gel increased ($P < 0.01$) serum calcium concentrations for days 1 and 2 post-calving (Table 2, Fig 1) but not on days 3 through 7. Mean increase in serum calcium concentration on day 1 was .72 mg/dl, which was similar to that reported by Goff and Horst[17] using a single tube of the same calcium chloride gel product. Queen et al.[16] reported greater increases in serum calcium concentrations following administration of two tubes together (approximately 102 total grams of elemental calcium) of a different calcium chloride gel product. In both studies,[16,17] blood calcium concentrations peaked 30 minutes after one dose of a calcium chloride gel. In this study, serum calcium concentrations were first measured, on average, 8.7 hours from previous administration (usually the 12-hour post-calving dose) of the calcium chloride gel. Thus, the calcium concentrations measured in this study would be expected to be lower than the peak calcium concentrations measured in the other studies.[15,16]

Analysis of variance indicated that parity significantly affected differences in serum calcium concentrations on days 1 and 2 post-calving. The LS means were significantly higher for third or greater parity cows than for first parity cows (Table 3). Increases in serum calcium concentrations were small or negative with calcium chloride gel treatment for parity 1 and 2 cows. These results were consistent with the known relationship of increased risk of milk fever with increased parity.[25]

Disease Outcomes — Seventeen cases of parturient paresis (8.3% overall incidence rate) were recorded. Mean pre-treatment serum calcium concentrations in these cases was 4.5 mg/dl, with a range of 2.7 to 7.1 mg/dl. Mean interval from calving to onset of clinical signs was 22.3 hours, with a range of -6 to 151 hours.

Eighty-three cases of parturient hypocalcemia (40.7% overall incidence rate) were recorded. Sixty-six of these cases (80.0%) exhibited no clinical signs of paresis and were not treated intravenously. Mean serum calcium concentration in the cows affected with hypocalcemia but not paresis was 7.0 mg/dl, with a range of 4.2 to 7.9 mg/dl. The nadir of serum calcium concentration occurred on day 1 post-calving for 81.9% of the cows affected with parturient hypocalcemia.

Prophylactic treatment with a calcium chloride gel significantly reduced the incidence of parturient paresis, parturient hypocalcemia, and displaced abomasum (Table 4). Incidence rate of retained fetal membranes tended to be lower in the treatment group; however, the reduction

was not significant (Table 4). Odds ratios for the reduction in relative risk for parturient paresis, parturient hypocalcemia, and displaced abomasum were all positive and significant in the full models (Table 5). Probabilities of the significance of the likelihood ratios for the models were very high ($P > 0.25$), indicating good fit of the models. Parturient hypocalcemia was significantly affected by parity, with treatment causing significant reduction in the relative risk of hypocalcemia for only the third or greater parity cows.

Reductions in incidence rates and relative risk of parturient paresis and parturient hypocalcemia observed in this trial were similar to reductions observed when anionic salts were fed prior to calving.[11] Jonsson and Pehrson[14] also observed a similar reduction in milk fever incidence using a calcium chloride gel given in a similar, four-dose protocol.

The five cases of parturient paresis which occurred in the treatment group all in cows who received no pre-calving dose of calcium chloride gel. The five affected cows received either one or two post-calving doses (mean = 1.4) of the calcium chloride gel before becoming paretic. Incidence rates of parturient paresis were 7.9% (5/63) for cows in the treatment group that did not receive any pre-calving dose and 0% (0/39) for cows that received one or more pre-calving doses. Whether or not cows received the pre-calving dose of calcium chloride gel was not a significant factor in predicting serum calcium concentrations post-calving or any other disease outcome tested.

Displaced abomasum in affected cows occurred an average of 11 days after calving, with a range of 2 to 21 days after calving. Incidence rate of displaced abomasum may have been additionally influenced by post-calving diets, which were not controlled beyond the second day post-calving in this study. Reduction in displaced abomasum with oral calcium supplementation has not been previously documented. Results of this study help substantiate the link between parturient hypocalcemia and displaced abomasum reported by Massey et al.[8]

Conclusions — Results of this study demonstrate that prophylactic use of three or more doses of an oral calcium supplement containing predominantly calcium chloride was highly effective in sustaining serum calcium concentrations the first two days post-calving and in reducing incidence rates of parturient paresis, parturient hypocalcemia, and displaced abomasum. Results suggest that use of the calcium chloride gel product will be most effective when older cows are selected for prophylactic treatment. Results also suggest that inclusion of a pre-calving dose of the calcium chloride gel will improve its ability to prevent clinical milk fever, but that other beneficial effects of the treatment will be gained even if the pre-calving dose is missed and treatment is not started until immediately after calving.

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Footnotes

[a] Balance, Miles Inc., Shawnee Mission, KS.

[b] Vacutainer Brand SST tubes, Becton-Dickinson and Co., Franklin Lakes, NJ.

[c] Oetzel GR: Unpublished data, 1994.

[d] Kodak Ektachem 500, Eastman Kodak Co., Rochester, NY.

Table 1 — Descriptive statistics of the experimental cows

Item	Control cows		Calcium chloride gel treated cows	
	n	**%**	**n**	**%**
Total cows	102	100.0	102	100.0
First parity	41	40.2	41	40.2
Second parity	25	24.5	25	24.5
Third or greater parity	36	35.3	36	35.3
Cows with previous milk fever	8	7.8	8	7.8
	Mean	**SE**	**Mean**	**SE**
Parity	2.32	.14	2.34	.15
ME milk production,[1] lbs.	17941	308.8	17895	250.5

[1] 305 day mature equivalent milk production in previous lactation.
SE = Standard error of the mean.

Table 2 — Effect of calcium chloride gel treatment on serum calcium concentration within pairs of cows

Day after calving	Difference in serum Ca,[1] mg/dl		*P* value[2]
	Mean	**SE**	
1	.72	.12	.0001
2	.38	.10	.0003
3	-.06	.10	.5541
4	-.11	.10	.2343
5	.09	.08	.2604
6	.11	.10	.2864
7	-.04	.08	.6040

[1] Serum calcium value of the calcium chloride gel-treated cow minus the serum calcium value of the control cow within the same pair.
[2] *P* value from Student's *t* test for testing the hypothesis that the population mean is zero.
SE = Standard error of the mean.

Table 3 — Least-squares means for serum calcium concentrations within pairs of cows for days 1 and 2 after calving by parity

Day after calving	Parity	Difference in serum Ca,[1] mg/dl
		LS mean[2]
1	1	.03[a]
	2	.28[a,b]
	3 and greater	.89[b]
2	1	-.15[a]
	2	-.43[a,b]
	3 and greater	.47[c]

[1] Serum calcium value of the calcium chloride gel-treated cow minus the serum calcium value of the control cow within the same pair.
[2] Least-squares mean value.
[a,b,c] Least-squares means for the same day after calving with different superscripts are different ($P < 0.05$).

Table 4 — Effect of calcium chloride gel treatment on disease incidence rates

Disease	Control cows		Calcium chloride gel treated cows		*P* value[1]
	n	%	n	%	
Parturient paresis[2]	12	11.8	5	4.9	.0055
Parturient hypocalcemia[3]	53	52.0	30	29.4	.0001
Retained fetal membranes	18	17.6	11	10.8	.1495
Displaced abomasum	8	7.8	1	1.0	.0334

[1] *P* value of the treatment effect in the final logistic regression model for each disease outcome.
[2] Clinical signs of paresis with pre-treatment serum calcium concentration <8.0 mg/dl.
[3] Serum calcium concentration <8.0 mg/dl on one or more of days 1-7 post-calving, with or without clinical signs of paresis.

Table 5 — Odds ratios, 95% confidence intervals, and likelihood ratios for effect of treatment with a calcium chloride gel on reducing the relative risk of disease; odds ratios by covariate are shown for covariates which significantly (P<0.05) affected the model

Disease	Odds Ratio[1]	95% CI[2]	Likelihood Ratio[3]
Parturient paresis[4]	3.90	1.03 to 14.77	.4575
Parturient hypocalcemia[5]	7.17	2.66 to 19.33	.2802
for parity 1 only	5.56	.62 to 49.82	—
for parity 2 only	2.67	.85 to 8.37	—
for parity 3 and greater only	8.80	2.28 to 34.03	—
Displaced abomasum	10.65	1.20 to 94.06	.9471

[1] Odds ratio of the effect of calcium chloride gel treatment on disease incidence rates; odds ratio >1 indicates decreased relative risk of disease due to treatment.
[2] 95% confidence interval of the odds ratio.
[3] Likelihood ratios are for the full models only, with all covariates included.
[4] Clinical signs of paresis with pre-treatment serum calcium concentration <8.0 mg/dl.
[5] Serum calcium concentration <8.0 mg/dl on one or more of days 1-7 post-calving, with or without clinical signs of paresis.

Figure 1 — Effect of calcium chloride gel treatment on serum calcium concentrations. Mean values are plotted with standard error of the mean indicated. Treatment group (cows treated prophylactically with a calcium chloride gel) is plotted as ■ and control group (no calcium chloride gel treatment) is plotted as ● .