Effects of intravenous administration of two volumes of calcium solution on plasma ionized calcium concentration and recovery from naturally occurring hypocalcemia in lactating dairy cows

Johanna G. Doze, DVM; Rogier Donders, PhD; Johannes H. van der Kolk, DVM, PhD

Objective—To compare the effects of administration of 2 volumes of a calcium solution (calcium oxide and calcium gluconate) on plasma ionized calcium concentration (PICaC) and clinical recovery from naturally occurring hypocalcemia (NOHC; milk fever) in lactating dairy cows.

Animals—123 cows with NOHC (PICaC < 0.95 mmol/L [3.81 mg/dL]) and 20 clinically normal control cows.

Procedures—Affected cows were treated IV once or repeatedly with 450 (n = 56) or 750 mL (67) of calcium solution (1.65 g of calcium/100 mL) until clinical recovery was achieved. The PICaC was assessed 48 hours after the first treatment or after the treatment that achieved clinical recovery. Biochemical recovery was defined as PICaC ≥ 0.95 mmol/L. Plasma from control cows was used for PICaC reference range determination. Plasma samples from both groups were assessed after storage for 20 days at 20°C.

Results—The PICaC reference range derived from blood collected in tubes containing lithium heparin was 1.02 to 1.29 mmol/L (4.09 to 5.17 mg/dL). Following storage, plasma samples were suitable for PICaC assessment. All cows treated with ≥ 1 volume of 450 and 750 mL of calcium solution recovered clinically; however, 31 of 83 (37%) evaluated cows were not biochemically recovered at 48 hours following treatment. Only cows with PICaC < 0.48 mmol/L (1.92 mg/dL) before the first treatment had to be treated ≥ 3 times.

Conclusions and Clinical Relevance—Results did not support the need to increase the administered volume of calcium solution from 450 to 750 mL for treatment of NOHC in dairy cows. (Am J Vet Res 2008;69:1346–1350)

Hypocalcemia, also called milk fever or paresis puerperalis, is a common disease in dairy cows. 1 It is a multifactorial disease that has been described since 1925. 2 Results of epidemiologic studies 3,4 in the United Kingdom, Norway, and other countries indicated that the prevalence of hypocalcemia was 5% to 10%. The importance of this disease is reflected by the financial consequences. Research has been done in the United Kingdom into the financial implications associated with reduced milk production as a result of hypocalcemia; most cows with hypocalcemia can be treated by the farmer, and the mean costs per cow are estimated at $118, attributed to decreased milk production and costs of medicine. When hypocalcemia is fatal, the costs are estimated at $4,224 (including veterinary costs, loss of production, and costs of cow replacement). 5 In cows, hypocalcaemia develops when rapid onset of milk production results in acute depletion of circulating ICa. An acute-to-peracute flaccid paralysis or somnolence develops, typically within 72 hours of parturition. The condition most often develops in high-yield cows. If treatment is not initiated, the disease can be fatal. 6,7

In mammals, calcium plays an integral role in many physiologic phenomena, such as neuronal excitability, muscle contraction, cell membrane structural integrity and permeability, bone formation, and enzyme activities. In an animal’s body, the amount of intracellular calcium is minute, compared with the amount of calcium located in extracellular spaces. Extracellularly, calcium exists in 2 forms: nondiffusible (protein bound) and diffusible. 8 Diffusible calcium can be either complexed or ionized. Physiologically, ICa is the most

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<th>Abbreviations</th>
<th>Meaning</th>
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<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>ICa</td>
<td>Ionized calcium</td>
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<tr>
<td>TCa</td>
<td>Total calcium</td>
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The most important part of the treatment of hypocalcemia in cattle is the administration of calcium. In 1925, IV infusion of calcium chloride was initially used to treat this disease; in 1930, calcium gluconate was introduced in an attempt to decrease treatment-associated toxic effects. Presently, calcium gluconate and calcium borogluconate are most commonly used. The purpose of the study reported here was to compare the effects of administration of 2 volumes of a calcium solution (a combination of calcium oxide and calcium gluconate) on plasma ICa concentration and clinical recovery from naturally occurring hypocalcemia (milk fever) in lactating dairy cows.

Materials and Methods

Animals—The control group consisted of 20 clinically normal lactating Holstein-Friesian cows from the Utrecht University herd. The mean ± SD age of the cows was 3.8 ± 1.8 years, and the mean duration of lactation was 21 g for maintenance with supplementation of 2.4 g/kg of milk yield. Water was provided ad libitum. The diet of all cows (experimental and control) was based on daily requirements for maintenance and milk yield according to the Dutch standard, which recommends that a 650-kg cow should receive 36 MJ net energy of lactation for maintenance with supplementation of 3.2 MJ/kg of milk yield. In addition, on the basis of that standard, the calcium requirement of a 650-kg cow was 21 g for maintenance with supplementation of 2.4 g/kg of milk yield. Water was provided ad libitum.

On the basis of physical examination findings in the cows of the control group, a tentative diagnosis of hypocalcemia was made in ill cows if at least 6 of the following clinical signs were present: anorexia; heart rate < 50 beats/min; irregularity of cardiac rhythm; rectal temperature < 38.0 °C; decreased temperature of extremities, compared with rump surface; abnormal mentation; recumbency; inability to rise despite humane encouragement; diminished rumen motility (≤ 1 rumen roll/minute); constipation; and a dry nose. In addition to detection of ≥ 6 of these clinical signs, plasma ICa concentration had to be < 0.95 mmol/L (3.81 mg/dL) for initial inclusion of a sick cow in the study. This plasma ICa concentration was regarded as the gold standard in the diagnosis of hypocalcemia.

Experimental design—The reference range of plasma ICa concentration was determined via analysis of blood samples obtained from the healthy control cows. Blood samples (8 mL each) were collected into a plain evacuated tube (for analysis of serum analytes), and a portion of each sample (2 mL) was removed by use of a heparinized syringe. The samples in the heparinized syringes were used to determine the reference range. In addition, a blood sample (8 mL each) was also collected into an evacuated tube containing lithium heparin as an anticoagulant; a reference range was similarly determined from these samples. The intent was to assess the equivalence of the 2 reference ranges because the latter method of blood sample collection is more suitable under field conditions. Furthermore, additional validation studies with these blood samples were done to assess the effects of storage conditions (20-day storage period at −20°C) on plasma TCa and ICa concentrations, pH, and total protein concentration. The differences between various values obtained from both samples were < 5%.

Dairy cows with naturally occurring hypocalcemia were randomly assigned to 1 of 2 treatment groups on the basis of the instruction in a closed envelope (1 envelope/cow). The cows were treated IV with either 450 or 750 mL of calcium solution9 that contained 1.65 g of calcium/100 mL. The calcium solution (containing 11.7 g of boric acid, 3.4 g of calcium oxide, 53.7 g of calcium gluconate, and 16.7 g of magnesium chloride/450 mL) was administered over a 5-minute period. Just prior to and 10 seconds following completion of treatment, a blood sample (8 mL each) was collected in an evacuated tube containing lithium heparin as an anticoagulant; these samples were designated as the pretreatment and 10-second samples, respectively. The 10-second sample was collected to assess the highest plasma concentration of ICa.

To determine eligibility for inclusion in the study, each cow was examined before treatment by the veterinarian on duty. For each cow, clinical recovery was defined as standing and eating within 6 hours following initial treatment as assessed by the farmer. Following clinical recovery, the cow was reexamined 48 hours after the initial treatment and a blood sample (8 mL) was collected in an evacuated tube containing lithium heparin as an anticoagulant; this sample was designated as the 48-hour sample. If clinical recovery was not achieved, repeated treatment with the same volume of solution (450 or 750 mL) was performed. Clinical recovery remained defined as standing or eating within 6 hours following a subsequent treatment. If clinical recovery was achieved after a subsequent treatment, the cow was reexamined 48 hours after the last treatment and a blood sample (8 mL) was collected. Biochemical recovery was defined as plasma ICa concentration ≥ 0.95 mmol/L.

Analyses—Blood samples were collected from a jugular vein of the 20 control cows via venipuncture into both a plain evacuated tube and an evacuated tube containing lithium heparin. A heparinized syringe was filled with blood from each plain evacuated tube immediately after collection. Samples in the heparinized sy-
ringes were immediately assessed for ICa concentration and associated venous blood pH by use of an automated ICa analyzer. Blood collected into the plain vacuum tubes was allowed to clot for the determination of serum total protein concentration by use of an automated computerized analyzer. The evacuated tubes containing lithium heparin were centrifuged at 6,000 × g for 10 minutes to separate plasma and erythrocyte fractions by a gel within the tube. Each plasma sample obtained from these tubes was immediately assessed for ICa concentration and plasma pH by use of the automated ICa analyzer as well as for TCa and total protein concentrations by means of another automated analyzer. The intratube gel facilitated storage of each heparinized tube at −20°C without transferring the plasma fraction into another tube. After 20 days of storage at −20°C, assessments of the plasma pH and TCa, ICa, and total protein concentrations were repeated.

Blood samples (8 mL each) from the 135 cows for which a tentative diagnosis of hypocalcemia had been made were collected into evacuated tubes containing lithium heparin. After centrifugation of each sample, the plasma pH and ICa concentration were determined within 25 days after storage at −20°C.

Statistical analysis—A reference range for plasma ICa concentration was calculated as the 95% CI for samples of plasma obtained from the heparinized syringes and for samples of plasma obtained from blood collected in tubes containing lithium heparin. To compare differences in clinicopathologic variable between the 2 treatment groups, statistical analysis was performed by use of the Mann-Whitney U (2-sided) test, the McNemar test, or the χ² test. Storage effects were assessed by use of an ANOVA. The strength of the linear relationship between plasma ionized calcium concentration and storage time was assessed by obtaining the correlation coefficient (r) and testing whether it was different from zero by use of the Pearson (2-tailed) test. Results are expressed as mean ± SD values. Values of P < 0.05 were considered significant.

Results

Analysis of control group samples—From all 20 lactating control cows, blood samples were collected in plain tubes (from which a portion of each sample was transferred into heparinized syringes for reference range determination) and in tubes containing lithium heparin. The mean ± SD ICa concentrations in these heparinized blood and plasma samples were 1.11 ± 0.09 mmol/L (4.45 ± 0.36 mg/dL) and 1.16 ± 0.07 mmol/L (4.65 ± 0.28 mg/dL), respectively. The corresponding pH values were 7.401 ± 0.023 and 7.452 ± 0.020, respectively. Mean plasma TCa and serum total protein concentrations in the control cows were 2.39 ± 0.09 mmol/L (10.38 ± 0.36 mg/dL) and 70 ± 7 g/L, respectively.

A reference range (ie, 95% CI) for ICa concentration was calculated for both types of sample. Analysis of the blood in heparinized syringes yielded a reference range for ICa concentration of 0.93 to 1.29 mmol/L (3.73 to 5.17 mg/dL). Analysis of plasma samples yielded a reference range for ICa concentration of 1.02 to 1.29 mmol/L (4.09 to 5.17 mg/dL).

Cows with hypocalcemia—On the basis of clinical signs, 135 cows were suspected of having hypocalcemia. However, 12 cows had plasma ionized calcium concentrations ≥ 0.95 mmol/L (3.81 mg/dL) and were ineligible for inclusion in the study. Of the 123 cows that did meet the inclusion criterion (plasma ionized concentration < 0.95 mmol/L), most were Holstein-Friesian (n = 37), Fries-Hollands (7), and Maas-Rijn-Ijssel (26) breeds. The mean age of the affected cows was 77.4 months (95% CI, 74.0 to 80.9 months) and the mean parity was 4.7 (range, 2 to 10). Among these 123 cows, 11 (9%) were treated for the first time before parturition. Fifty-six affected cows were allocated to the group treated IV with 450 mL of calcium solution, and 67 affected cows were allocated to the group treated IV with 750 mL of calcium solution.

Before treatment, the mean plasma ICa concentration in the 123 cows was 0.53 ± 0.17 mmol/L (2.09 ± 0.68 mg/dL; range, 0.25 to 0.94 mmol/L [1.00 to 3.77 mg/dL]). Among the cows assigned to receive treatment with 450 mL of calcium solution, mean plasma ICa concentration was 0.57 ± 0.17 mmol/L (2.29 ± 0.68 mg/dL). Among cows assigned to receive treatment with 750 mL of calcium solution, mean plasma ICa concentration was 0.54 ± 0.17 mmol/L (2.16 ± 0.68 mg/dL).

Clinical and biochemical assessments of recovery after treatment—All 123 treated cows recovered according to the farmers. Of the 56 cows treated with 450 mL of calcium solution, 34 recovered clinically after a single treatment and 22 required 2 to 5 volumes to achieve recovery. Of the 67 cows treated with 750 mL of calcium solution, 44 recovered clinically after a single treatment and 23 required 2 to 5 volumes to achieve recovery.

The pretreatment, 10-second, and 48-hour blood samples were successfully collected from 83 of 123 cows (36 in the 450-mL treatment group and 47 in the 450-mL treatment group). Biochemical recovery was defined as plasma ICa concentration ≥ 0.95 mmol/L at 48 hours after the first or subsequent treatment. After 1 or more administrations, 31 of 83 (37%) cows were not biochemically recovered. Following treatment with 1 or more administrations of either volume of calcium solution, 52 of the 83 cows were biochemically recovered. The 450-mL treatment group included 22 biochemically recovered cows, of which 16 recovered biochemically after only 1 administration; 14 cows did not achieve biochemical recovery. In comparison, the 750-mL treatment group included 30 cows, of which 19 recovered biochemically after only 1 administration; 17 cows did not achieve biochemical recovery. The number of cows that required a single administration of calcium solution to achieve biochemical recovery did not differ significantly (P = 0.476) between treatment groups. The number of cows requiring multiple (2 to 5) treatments in the 450- and 750-mL treatment groups was 6 and 11, respectively. Only cows with plasma ICa concentration < 0.48 mmol/L (1.92 mg/dL) before first treatment had to be treated ≥ 3 times.

Among the cows that recovered biochemically after 1 administration of either volume of calcium solution, plasma ICa concentrations before and at 10 seconds and 48 hours after treatment were compared (Table 1).
Peak values in the 10-second samples collected from cows in the 2 treatment groups differed by 0.70 mmol/L (2.81 mg/dL); the value in the cows receiving 750 mL of calcium solution was significantly (P < 0.001) greater than the value in cows receiving 450 mL of calcium solution. The mean maximum increase in plasma I Ca concentration following administration of 450 mL was 1.29 mmol/L (95% CI, 1.05 to 1.52 mmol/L [5.17 mg/dL]; 95% CI, 4.21 to 6.09 mg/dL). The mean maximum increase in plasma I Ca concentration following administration of 750 mL was 2.01 mmol/L (95% CI, 1.71 to 2.31 mmol/L [8.06 mg/dL]; 95% CI, 6.85 to 9.26 mg/dL).

Effects of storage on plasma biochemical variables—The effects of storage for 20 days at −20°C on heparinized plasma samples obtained from the control cows were assessed. Following storage, the mean concentration of I Ca was 1.13 ± 0.09 mmol/L (4.53 ± 0.36 mg/dL), compared with 1.16 ± 0.07 mmol/L (4.65 ± 0.28 mg/dL) before storage (P = 0.267); T Ca concentration was 2.42 ± 0.14 mmol/L (9.70 ± 0.56 mg/dL), compared with 2.59 ± 0.09 mmol/L (10.38 ± 0.36 mg/dL) before storage (P = 0.000). pH was 7.457 ± 0.040, compared with 7.452 ± 0.020 before storage (P = 0.639); and total protein concentration was 89 ± 8 g/L, compared with 89 ± 7 g/L (P = 0.965). In addition, linear regression revealed the estimated decrease in plasma I Ca concentration was < 5% over a period of 25 days storage at −20°C.

On the basis of the data collected following storage of heparinized plasma at −20°C, reference ranges (95% CIs) were calculated as follows: pH, 7.377 to 7.337; T Ca concentration, 2.14 to 2.70 mmol/L (8.58 to 10.82 mg/dL); I Ca concentration, 0.95 to 1.31 mmol/L (3.81 to 5.25 mg/dL); and total protein concentration, 73 to 105 g/L. The lower limit for plasma I Ca concentration (0.95 mmol/L or 3.81 mg/dL) was used as the marker for biochemical recovery from naturally occurring hypocalcemia in the study.

**Discussion**

In the present study, values of heparinized blood pH as well as the plasma T Ca and serum protein concentrations determined in samples obtained from the control cows were within the reference range of our laboratory, which supported the fact that clinically healthy cows were used. In addition, analyses revealed no hypoproteinemia in the cows with hypocalcemia. On the basis of our findings, blood samples collected in tubes containing lithium heparin under field conditions provided plasma samples that were valid for later assessment of I Ca concentrations. Unfortunately, it was not possible to analyze all samples within 20 days. Nevertheless, linear regression revealed that the estimated decrease in I Ca concentration was acceptable (< 5%) over a 25-day storage period at −20°C.

Twelve of the 135 (9%) cows initially suspected of having hypocalcemia were not included for further analysis because their plasma I Ca concentration was not < 0.95 mmol/L (3.81 mg/dL) at the first examination. To our knowledge, comparable data with regard to false-positive clinical diagnosis of hypocalcemia are scarce. By use of the ARD (appetite, rumen, and defecation) test, researchers identified that 3.2%15 and 4.6%16 of clinical diagnoses of hypocalcemia among 31 and 108 lactating cows, respectively, 1 week prior to or after parturition were false-positive diagnoses (ie, cows were considered clinically affected, but plasma I Ca concentration was ≥ 0.95 mmol/L). However, misclassification of cows that did not have milk fever by use of the ARD test is much more difficult (misclassification of 12.5%15 and 4.2%16 of 8 and 19 unaffected cows, respectively).

In the present study, the diagnosis of hypocalcemia on the basis of the defined clinical signs was a false-negative finding in 8.9% of 135 cows. In the present study, plasma I Ca concentrations at 48 hours following treatment indicated that 31 of 83 (37%) treated cows were not biochemically recovered (ie, plasma I Ca concentration was not ≥ 0.95 mmol/L). Hypocalcemia was more accurately identified by the variables evaluated in the ARD test, compared with measures evaluated clinically by veterinarians in our study. The ARD test is fast and practical, but its validity was proven in only 2 studies,15,16 to our knowledge. In addition, it has been determined that severe hypocalcemia (plasma I Ca concentration < 0.48 mmol/L [1.92 mg/dL]) in cows can be predicted on the basis of certain clinical signs: somnolence or coma, low pulse frequency, low rectal temperature, cold ears, dry nose, and low frequency of rumen contractions.17

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**Table 1**—Mean ± SD plasma I Ca concentration (mmol/L [mg/dL]) assessed prior to (pretreatment) and 10 seconds and 48 hours after IV administration of 450 or 750 mL of calcium solution that resulted in biochemical recovery in 35 lactating dairy cows with hypocalcemia. Cows initially had plasma I Ca concentration < 0.95 mmol/L (3.81 mg/dL), and biochemical recovery was defined as plasma I Ca concentration ≥ 0.95 mmol/L 48 hours following the single treatment.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Volume administered (mL)</th>
<th>Pretreatment*</th>
<th>10 seconds after treatment</th>
<th>48 hours after treatment</th>
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<tbody>
<tr>
<td></td>
<td>450 (n = 16)</td>
<td>750 (n = 19)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.58 ± 0.15 (2.32 ± 0.60)</td>
<td>0.56 ± 0.18 (2.24 ± 0.72)</td>
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<td></td>
<td>1.87 ± 0.28 (7.49 ± 1.12)*</td>
<td>2.57 ± 1.08 (10.30 ± 4.33)*</td>
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<td></td>
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<td></td>
<td>1.12</td>
<td>1.03 ± 0.05 (4.13 ± 0.26)</td>
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</table>

*For the 450- and 750-mL treatment groups, the ranges of the pretreatment sample values were 0.30 to 0.81 mmol/L (1.20 to 3.25 mg/dL) and 0.35 to 0.91 mmol/L (1.40 to 3.65 mg/dL), respectively.

**In a row, values with different superscript letters are significantly (P < 0.05) different.
Among the cows with hypocalcemia that achieved biochemical recovery after the initial treatment in the present study, mean plasma ICa concentrations at 10 seconds after administration of a 450- or 750-mL volume of calcium solution were increased (compared with pretreatment values), and the difference in values at this time point between the 2 groups was significant. However, this difference did not affect clinical or biochemical recovery. In another study,13 the severity of hypercalcemia in cows with naturally occurring milk fever was positively related to the amount of calcium solution administered.

Findings of the present study are in agreement with results of other investigations,15-20 indicating that increases in infusion volume do not positively influence recovery of cows with hypocalcemia. However, in those other reports, no clear end point was given in terms of plasma ICa concentration. Despite the fact that administration of a 450-mL volume of calcium solution induced a lesser increase in plasma ICa concentration, compared with the effect of a 750-mL volume of calcium solution, it induced similar clinical and biochemical recovery among affected cows in our study. Taking into account the mean plasma ionized calcium concentration prior to administration of either volume, the 100% clinical recovery achieved among the study cows was not surprising.

Most severely affected cows in the present study (those with plasma ionized calcium concentrations < 0.48 mmol/L [1.92 mg/dL]) had to be treated ≥ 3 times, which is in accordance with a report indicating that the required number of treatments for resolution of hypocalcemia is greatest in cows with the lowest plasma ionized calcium concentrations.21 Severely affected cows can be identified on the basis of the aforementioned specific clinical signs.17

On the basis of the findings of our study, the increase in plasma ICa concentration achieved via IV administration of 1 or more 450-mL doses of calcium solution was sufficient both in terms of clinical and biochemical recovery. The data collected did not support the need to increase the administered volume of calcium solution from 450 to 750 mL for treatment of naturally occurring hypocalcemia in lactating cows. Interestingly, numerous cows were judged as clinically recovered following treatment, yet plasma ICa concentrations were still low.

### References