Preventive Effect of Mildly Altering Dietary Cation-Anion Difference on Milk Fever in Dairy Cows

Naotoshi KUROSAKI1,2), Osamu YAMATO1)*, Fuminobu MORI2), Seiichi IMOTO3) and Yoshimitsu MAEDE1)

1)Laboratory of Internal Medicine, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Kita-18 Nishi-9, Kita-ku, Sapporo 060–0818, 2)Total Herd Management Service, Ltd., 22-banchi Asahi-machi, Kamishunbetsu, Bekkai-chio, Notsuke-gun 088–2722 and 3)Toxicology Consultant, 2-jo 8-choume, Hiragishi, Toyohira-ku, Sapporo 062–0932, Japan

(Received 5 September 2006/Accepted 1 November 2006)

ABSTRACT. In the present study, we examined whether mildly altering dietary cation-anion difference (DCAD) contributes to the prevention of milk fever in dairy cows. Thirty multiparous cows and ten primiparous cows (heifer group) were used in this study and the multiparous cows were randomly divided into three groups of ten animals each (anion, non-anion and control groups). The cows in the anion group were given supplemental salts that slightly lowered DCAD. These salts consisted of 115 g of CaCO₃, 42 g of CaHPO₄, 65 g of MgSO₄•7H₂O and 80 g of CaCl₂•2H₂O as a daily dose for each cow, using a catheter from 21 days before the expected date of parturition until parturition. The cows in the non-anion group were given only the same Ca, Mg and ip supplement but no sulfate and chloride salts as that in the anion group. The cows in the control and heifer groups were not given any additional supplement. The incidence of hypocalcemia in the anion group decreased to approximately half of those in the non-anion and control groups, while the heifer group did not develop hypocalcemia at all. In addition, the number of days spent for the treatment of hypocalcemia and the number of drug bottles (calcium borogluconate solution) used for the treatment decreased to less than half in the anion group compared with those in the non-anion and control groups. At parturition, the serum Ca concentration in the control (6.2±1.9 mg/dl, mean ± standard deviation) and non-anion groups (6.4±1.7 mg/dl) were significantly lower than that in the heifer group (8.3±0.4 mg/dl), and the level in the anion group was intermediate (7.3±1.3 mg/dl). The change in ionized Ca concentration in the anion group was almost the same as that in serum Ca concentration, but only the concentration in the anion group tended to increase slightly from a week before parturition and was significantly higher than that in all other groups three days before parturition. Urinary pH in the anion group was maintained at a mildly acidic level (6.8–7.0) for the last two weeks before parturition, compared with those in the control (7.3–7.5) and non-anion groups (7.9–8.1), and similar to that in the heifer group (6.3–7.3). The urinary Ca excretion was the highest in the anion group among all groups during the prepartum period. There were no specific changes in the excretion of parathyroid hormone and 1,25-dihydroxyvitamin D in all groups of multiparous cows while the levels of these hormones remained low in the heifer group throughout the experimental period. The data in the present study indicates that the administration of anion salts that slightly lowered DCAD in the prepartum period was effective for preventing milk fever in multiparous cows. Safe and mild metabolic acidosis induced by the anion salts could be evaluated by urinary pH (6.8–7.0), and might increase the responsiveness to Ca requirement at parturition through some complex mechanisms unrelated to the excretion of Ca-related hormones. In addition, it was clarified that primiparous cows have a high potential to respond to sudden Ca demand unrelated to hormone excretion, and their Ca metabolism was in some respects similar to that in multiparous cows fed anion salts. Therefore, manipulating mildly DCAD is expected to be an effective, safe and natural method for preventing milk fever in dairy cows.

KEY WORDS: dairy cow, dietary anion-cation difference, heifer, hypocalcemia, milk fever.

Milk fever, even the sub-clinical type of this disease without parturient paresis, is a critical issue in dairy farming because it decreases the productivity of dairy cows and induces many other diseases [20, 30, 32]. Therefore, investigators have proposed many methods for preventing milk fever, including a prepartum low-calciu diet, prepartum administration of 1,25-dihydroxyvitamin D (1,25-(OH)₂D) and so on [3, 4, 15, 16, 24, 27], but ideal protocols have not yet been established.

As one of the complex causes of milk fever, the defect in calcium homeostasis appears to reside in the sensitivity of bone and kidney tissues to parathyroid hormone (PTH) stimulation. Evidence suggests that the acid-base status of the cow dictates the sensitivity of the tissues to PTH stimulation, and that metabolic alkalosis is responsible for blunting PTH responsiveness in tissues [13]. Excessive dietary potassium is very common and the most important factor causing metabolic alkalosis in dairy cows. Formulation of rations to reduce metabolic alkalosis and/or induce compensated metabolic acidosis in prepartum cows has proved a useful strategy for preventing milk fever.

In several studies, manipulation of dietary cation-anion difference (DCAD) in periparturient diets has been successful in lowering the blood pH and reducing the incidence of hypocalcemia [10, 14, 36]. Most of these studies recommend lowering urinary pH to 6.0–6.5 to achieve sufficient effect [22]. However, reducing urinary pH to less than 6.0 may cause severe metabolic acidosis resulting in a decline in

* PRESENT ADDRESS: YAMATO, O., Laboratory of Clinical Pathology, Department of Veterinary Clinical Sciences, Faculty of Agriculture, Kagoshima University, 1–21–24 Kohrimoto, Kagoshima 890-0065, Japan.

dry matter intake (DMI) [5, 20, 22]. These risks are more critical in practical sites such as dairy farming because mineral balance in forage alters day by day and thus sub-clinical metabolic acid-base disturbances can be caused by an ordinary diet [9]. In addition, a large amount of anion salt supplement can reduce DMI due to the unpleasant taste, which may cause other disorders in dairy cows [11, 14]. Although urinary pH should be checked after every ration change to prevent severe metabolic acidosis, there are practical difficulties in performing urinary pH check so frequently in dairy farming. To avoid both the risk of severe metabolic acidosis and the reduction of DMI, it is desirable to feed animals a smaller amount of anion salts inducing a mild and safe level of acidosis [33]. However, there have been few reports about mildly altered DCAD, which can induce mild and safe acidotic status that definitely prevents postpartum hypocalcemia and milk fever.

Further study about periparturient Ca metabolism in primiparous cows is required because primiparous cows almost never develop milk fever [19, 25] and therefore can be an ideal model for studies of postpartum hypocalcemia and milk fever. The periparturient Ca metabolism and hormonal status in primiparous cows will contribute to preventing milk fever in multiparous cows. However, there have been few reports concerning milk fever in primiparous cows or heifers. Therefore, primiparous cows were also investigated in the present study.

The present study investigated whether mildly altered DCAD contributes to the prevention of milk fever in dairy cows. For the purpose, anion salts that slightly lowered DCAD were given to multiparous cows. The incidence of milk fever, Ca metabolism and hormonal status in multiparous cows fed anion salts were compared with those in multiparous and primiparous cows not fed anion salts.

MATERIALS AND METHODS

Cows: Forty pregnant Holstein cows were used in the present study. Thirty of them were multiparous cows and were divided into three groups of ten animals each, called the anion, non-anion and control groups, respectively. The average numbers of lactation was 3.8, 3.9 and 4.2 in anion, non-anion and control groups, respectively. The average body condition scores were almost the same (3.1–3.4) in the three groups. Another group, called the heifer group, consisted of ten heifers that had not experienced parturition or milk fever. Thereafter, the animals were fed the same diet as the other groups. The diet placed into the feed bank twice a day ad libitum, and the orts were weighed back once a day. Cows in the anion group were given anion salts along with high Ca content (Table 1), which were dissolved with 500 ml of tap water, using a stomach catheter at 6 am every morning, from 21 days before the expected date of parturition until the actual date of parturition. The cows in the non-anion group were given only the high Ca supplement but no sulfate and chloride salts (Table 1), in the same manner as in the anion group. The concentrations of minerals in the diet and supplement and DCAD calculated using these data are shown in Table 2. In the present study, DCAD in the anion group remained higher (+1.2 mEq/100 g of dietary dry matter) compared with those in other researches concerning DCAD in dairy cows.

Treatment of cows with parturient paresis: In the present study, cows were diagnosed as having severe hypocalcemia when their Ca concentration in serum was less than 5 mg/dl, and moderate hypocalcemia when the concentration ranged from 5 to 7 mg/dl. A 500-ml bottle of 20% calcium borogluconate solution was used to treat cows with parturient paresis. Only cows with astasia or dysstasia due to hypocalcemia were treated by a private veterinarian. The treatment was done by intravenous injection after the collection of blood and urine samples for the measurement of variables in the present study when the cow was recumbent. The number of days and bottles required for treatment was counted and recorded.

Sample collection: Venous blood was collected by jugular puncture at 11 pm (5 hr after feeding) on 40, 14, 7 and 3 days before the expected date of parturition (days −40, −14, −7 and −3), and on 3 and 7 days after parturition (days +3 and +7). The sample at parturition (day 0) was collected immediately after parturition. The serum and heparinized plasma were stored at −20°C until use. Urine was also collected in the same way as blood sampling. Urine was divided into two parts. One part was acidified with concentrated HCl for analysis of Ca and stored at −20°C until use. The residual part of intact urine was also stored for the measurement of creatinine concentration after pH was measured.

Table 1. Concentrations of salts and minerals in the dietary supplement

<table>
<thead>
<tr>
<th>Salts (g/cow/day)</th>
<th>Anion group</th>
<th>Non-anion group</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaCO$_3$</td>
<td>115</td>
<td>185</td>
</tr>
<tr>
<td>CaHPO$_4$</td>
<td>42</td>
<td>50</td>
</tr>
<tr>
<td>MgSO$_4$•7H$_2$O</td>
<td>65</td>
<td>0</td>
</tr>
<tr>
<td>CaCl$_2$•2H$_2$O</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>MgO</td>
<td>0</td>
<td>11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Minerals (g/cow/day)</th>
<th>Anion group</th>
<th>Non-anion group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>80.0</td>
<td>88.7</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>9.6</td>
<td>11.4</td>
</tr>
<tr>
<td>Magnesium</td>
<td>6.4</td>
<td>6.4</td>
</tr>
</tbody>
</table>
**Analysis of variables:** The total Ca concentration in serum and urine was determined by atomic absorption spectrophotometry. The concentration of 1,25-(OH)₂D was determined by radioimmunoassay using a commercial kit (Immunodiagnostic Systems, Boldon, UK) [28]. The concentration of PTH was determined by chemiluminescent immunoassay using a commercial kit (Lumico PTH, Nichols Institute Diagnostics, San Clemente, CA, U.S.A.) [17]. Ionized Ca concentration was measured by I-Stat (Fuso Pharmaceutical Industries, Ltd., Osaka, Japan). Urinary pH was measured by a pH meter (HORIBA Twin pH B-212, HORIBA Ltd., Kyoto, Japan). Creatinine concentration in urine was measured using an automated biochemical analyzer (COBAS MIRA plus, Hoffmann-La Roche, Basel, Switzerland). Urinary Ca excretion was expressed as mg of Ca per mg of creatinine.

**Statistics:** Statistical analysis was performed using 1-way factorial analysis of variance with post hoc tests (Tukey method). These analyses were carried out on a computer using a statistical software package (SYSTAT, Evanston, IL, U.S.A.). Values of P<0.05 were considered significant. Marks showing statistical significance were used as followed: * P<0.05, ** P<0.01, *** P<0.005, **** P<0.001 vs heifer group. * P<0.05, ** P<0.01, *** P<0.005, **** P<0.001 vs control group. * P<0.05, ** P<0.01, *** P<0.005, **** P<0.001 vs non-anion group.

**RESULTS**

**Hypocalcemia incidence and treatment:** Incidence of severe hypocalcemia with less than 5 mg/dl of serum Ca in anion, non-anion, control and heifer groups were 11%, 20%, 40% and 0%, respectively. Incidence of moderate hypocalcemia with 5 to 7 mg/dl of serum Ca in anion, non-anion, control and heifer groups were 22%, 40%, 20% and 0%, respectively. The average days spent on treatment in anion, non-anion, control and heifer groups were 1.0, 2.6, 2.3 and 0, respectively (Fig. 1A). The value was significantly higher in the non-anion and control groups than in the heifer group. The average number of drug bottles used for the treatment in anion, non-anion, control and heifer groups was 1.3, 3.4, 2.9 and 0, respectively (Fig. 1B). This value was also significantly higher in the non-anion and control groups than in the heifer group. The days and numbers of drug bottles for the treatment of parturient paresis in the anion group were markedly lower among the three groups of multiparous cows, although there was no significant difference.

**Change in blood Ca:** Between day −40 and day −3 in the prepartum period, the serum Ca concentration in all groups did not change, but it decreased rapidly on day 0 (Fig. 2A). However, the extent of decrease in serum Ca concentration in the heifer group on day 0 was the least among all groups, and the level in the heifer group (8.3 ± 0.4 mg/dl, mean ±
standard deviation) was significantly higher than that in the control (6.2 ± 1.9 mg/dl) and non-anion groups (6.5 ± 1.7 mg/dl). The serum Ca concentration in the anion group on day 0 was intermediate (7.3 ± 1.3 mg/dl) between the heifer group and control or non-anion group. The serum Ca concentration in all groups returned to the prepartum level on day +7.

The change in the ionized Ca concentration was similar to that in serum Ca concentration, but they were different in the prepartum period (Fig. 2B). The ionized Ca concentration in the anion group slightly increased on days –7 and –3 while that in other groups already tended to decrease on day –3 in the prepartum period. Consequently, the ionized Ca concentration was significantly higher in the anion group than in the heifer group on day –7 and than in all other groups on day –3.

Change in urinary pH and Ca: In the control group without any dietary mineral supplement, urinary pH was maintained at a slightly lower level (7.3–7.5) during the prepartum period (days –14 to –3) compared with that (8.0–8.2) during the postpartum period, i.e., on days +3 and +7 (Fig. 3A). In the anion group, the change in urinary pH was marked compared with that in the control group, although changes in both groups were basically similar. The level of urinary pH during the prepartum period was lower in the anion group (6.8–7.0) than that in the control group. There was a significant difference on days –14 and –7 between the two groups. The change in urinary pH in the heifer group (6.3–7.3) during the prepartum period was similar to that in the anion group. In contrast, the urinary pH in the non-anion group (7.9–8.1) was higher than that in all other groups during the prepartum period. As a result, there was a significant difference on days –14, –7, –3 and 0 between the non-anion and anion groups. After parturition, there were no significant differences among the groups.

In the control group, the urinary Ca excretion increased during the prepartum period, then decreased at parturition and remained low after parturition (Fig. 3B). In the anion group, the urinary Ca excretion was higher during the prepartum period compared with that in the control group, and there was a significant difference on day –3 between the two groups. However, the urinary Ca excretion in the non-anion group was lower compared with that in the control and anion groups, and consequently there was a significant difference on days –14, –7, –3 and 0 between the non-anion and anion groups. The change in the heifer group was slight and similar to that in the non-anion group.
Change in endocrine status: In serum PTH concentration, there were no significant differences among the groups during the prepartum period (Fig. 4A). However, the level in the control and anion groups rapidly increased on day 0, then decreased gradually, but remained high on day +7. In contrast, the serum PTH concentration in the heifer group hardly changed even after parturition, and consequently was significantly lower than that in the control group on days 0 and in the anion group on days 0 and +3. The extent of increase in serum PTH concentration in the non-anion group was intermediate between the anion and heifer groups.

During the prepartum period, serum 1,25-(OH)₂D concentration hardly changed in all groups although there was occasionally a significant difference between each pair of groups (Fig. 4B). However, there was a marked change after parturition. In the control group, the serum 1,25-(OH)₂D concentration rapidly increased on day 0, then decreased after parturition and returned to the initial level on day +7. In the anion and non-anion groups, serum 1,25-(OH)₂D concentration began to increase on day 0, and reached a peak on day +3. As a result, there was a significant difference between the control group and the anion or non-anion group on day 0. In contrast, the serum 1,25-(OH)₂D concentration in the heifer group hardly changed during the experimental period, and consequently was significantly lower than that in the control group on day 0 and that in the non-anion group on day +3.

DISCUSSION

In multiparous cows, the total incidence of severe and moderate hypocalcemia decreased to approximately half in the anion group compared with that in the non-anion and control groups. In addition, the number of days spent for the treatment of hypocalcemia and the number of drug bottles used for treatment was decreased to less than half compared with those in the non-anion and control groups (Fig. 1). These data suggest that the administration of anion salts containing Ca, which was expected to slightly lower DCAD, has the potential to prevent milk fever in multiparous cows. However, the administration of Ca supplement alone without sulfate and chloride salts did not contribute to the prevention of milk fever as shown in the results obtained from the non-anion group. Therefore, it was demonstrated that mildly altering DCAD contributes to the prevention of milk fever in practice.

The direct reason cows in the anion group were prevented from developing milk fever seems to be the higher concentrations of serum and ionized Ca at parturition, compared with those in the control and non-anion groups (Fig. 2). This may be because the mobilization of Ca into blood was smoother in the anion group than in the other groups of multiparous cows. The relatively higher Ca level at parturition in the anion group might be explained by the increase in Ca absorption from the intestine, mobilization of bone Ca, or dissociation of ionized Ca from the protein-bound form.

The absorption of Ca from the intestine involves two pathways, which are transcellular and paracellular [6]. The transcellular pathway is an active transport which is subject to physiological and nutritional regulation via 1,25-(OH)₂D and takes place almost only in the duodenum, while the paracellular pathway is a passive transport which is independent of regulation via 1,25-(OH)₂D, concentration-dependent and takes place throughout the small intestine. Karabach [23] shows that 60–70% of the mucosa-to-serosa Ca flux is paracellular and the residual 30–40% was transcellular. In the present study, the higher concentrations of serum and ionized Ca at parturition in the anion group can not be explained by only increased absorption from the intestine, because the concentrations of serum and ionized Ca in the non-anion group given the same Ca supplement as the anion group were lower than those in the anion group (Fig. 2), and the change in 1,25-(OH)₂D was almost the same between the two groups (Fig. 4B).

Beck and Webster [1] investigated the effect of acidosis in rats in which the thyroid and parathyroid glands, intestines and kidneys had been removed by surgical treatment. In this investigation, the concentrations of serum and ionized Ca were greater in acidic rats than in non-acidotic rats. This report shows that metabolic acidosis can cause
high concentrations of serum and ionized Ca due to direct mobilization from bone Ca. In addition, Ender and Dishington [8] reported that diets with high amounts of chloride and sulfur reduce intestinal pH and promote Ca absorption from the intestine. Brainthwaite [5] reported that Ca absorption in sheep increases 1 week after feeding a diet containing NH₄Cl. Freeden et al. [10] reported that true Ca absorption from the intestine calculated using a ⁴⁵Ca marker was increased in goats fed negative DCAD compared with that in those fed positive DCAD. In the present study, the higher concentrations of serum and ionized Ca at parturition in the anion group compared with those in the other groups of multiparous cows may be responsible for increased mobilization of bone Ca and absorption from the intestine, which may result from metabolic acidosis induced by the administration of anion salts.

In the present study, the ionized Ca concentration in the anion group slightly increased before parturition (days –7 and –3) while that in the other groups already started to decrease in this period (Fig. 2B). In contrast, the total serum Ca concentration did not differ among the groups during that period (Fig. 2A). This seems to be mainly due to the increase in ionized Ca dissociation from the protein-bound form [7]. It is well known that the dissociation of ionized Ca from the protein-bound form is associated with low blood pH [7, 29]. The increase of ionized Ca dissociated from the protein-bound form may also contribute to the relatively higher Ca level at parturition and consequently the prevention of milk fever in the anion group.

In the present study, blood pH was preliminarily measured to determine the change in true acid-base status in cows, but the results were ambiguous (data not shown) because it was very difficult to maintain the accuracy of measurement of many samples in a farm rather than laboratory environment. Instead of blood pH, urinary pH was measured and utilized to evaluate the acid-base status in cows. Generally, urinary pH reflects the acid-base status of an animal and monitoring urinary pH can be a sensitive method of evaluating the effect of diet on blood pH [12, 22]. In addition, it has been reported that both blood and urinary pH increase linearly or quadratically with increasing DCAD [34]. In the present study, urinary pH in the anion group was maintained at a mildly acidic level, i.e., pH 6.8–7.0, for the last two weeks before parturition (Fig. 3A). This level of urinary pH was milder in metabolic acidosis compared with the level (6.0–6.8) recommended for preventing milk fever by many researchers [2, 12, 22, 26, 30, 36], but it was the expected urinary pH level to make cows in the anion group mildly acidic in this experiment.

It is well documented that feeding anion salts to lower DCAD reduces urinary pH, while simultaneously increasing urinary Ca excretion, i.e., hypercalciuria [5, 20, 34, 35, 40]. In the present study, marked hypercalciuria was observed in the anion group with mild acidosis and moderate hypercalciuria even in the control group without acidosis (Fig. 3B). However, the mechanism of hypercalciuria has not been understood clearly. It is reported that sulfate salts can form a complex with Ca in the renal tubules and prevent the reabsorption of Ca [31, 35]. Other reports have indicated that either sulfate or chloride interferes with Ca resulting in hypercalciuria [37, 38]. Several researchers have suggested that prepartum hypercalciuria in cows fed anion salts is the result of an increase in Ca eflux from an exchangeable Ca pool such as bone [2, 18, 36]. Hypercalciuria caused by metabolic acidosis in the anion group, but not by Ca intake alone in the non-anion group, seems to indicate activation or flexibility of the Ca pool in the body.

Recently, many studies have supported the hypothesis that PTH secretion in response to hypocalcemia at parturition is not directly associated with the incidence of milk fever [14, 21]. Actually in the present study, the PTH excretion pattern after parturition differed in each group of multiparous cows (Fig. 4A), and the pattern was not associated with the incidence of hypocalcemia in each group. Furthermore, in groups of multiparous cows, there was no relationship between the change in serum 1,25-(OH)₂D concentration (Fig. 4B) and the incidence of milk fever. Although it is reported that cows fed anion salts have a higher concentration of 1,25-(OH)₂D in the peripartum period [30], it is likely that the 1,25-(OH)₂D level is affected by dietary Ca concentration, but not by metabolic acidosis [39]. From the data of the present study, the excretion pattern of PTH and 1,25-(OH)₂D in cows do not seem to be the direct cause of milk fever.

Dairy cows almost never develop milk fever during the first lactation [19, 25]. In the present study, there was no incidence of hypocalcemia in primiparous cows fed a standard diet without any salt supplement, and these animals did not need to be treated for hypocalcemia (Fig. 1). Therefore, primiparous cows may be good models for the study of milk fever and it would also be useful to evaluate the Ca metabolism in heifer cows, which may contribute to the elucidation and prevention of milk fever in multiparous cows.

In the present study, changes in serum and ionized Ca concentrations in the heifer group were slight throughout the experimental period (Fig. 2). Even at parturition (day 0), the serum and ionized Ca concentrations in the heifer group decreased very slightly and maintained at a much higher level compared with those in the various multiparous groups. However, the high levels of serum and ionized Ca did not seem to be controlled by the strong secretion of PTH and/or 1,25-(OH)₂D because the serum concentrations of PTH and 1,25-(OH)₂D hardly changed and increased slightly at parturition and in the postpartum period (Fig. 4). These results show that primiparous cows have the high potential for response to Ca requirement and does not rely on the abilities of PTH and/or 1,25-(OH)₂D excretion. The decline in Ca absorption with advancing age is documented in animals and humans [18]. Horst et al. [18] showed that the concentration of vitamin D receptor is significantly lower in intestine and bone in old rats and cows, which is associated with an attenuated response to 1,25-(OH)₂D. Thus, older cows is less able to respond to 1,25-(OH)₂D than younger cows and take longer to adapt intestinal Ca absorp-
tion mechanisms against lactational Ca demands. Therefore, the high responsiveness to the Ca requirement in the heifer group might be partly due to the large number of vitamin D receptors in intestine and bone.

In the present study, the urinary pH in heifer cows decreased during the prepartum period like that in the anion group (Fig. 3A), in which metabolic acidosis was induced by feeding anion salts. As described above, the administration of anion salts induced metabolic acidosis in multiparous cows resulting in the increased response to Ca requirement and consequently the prevention of milk fever. Primiparous cows in the heifer group may have the potential to down-regulate blood pH to increase the response to Ca demands in the anion group (Fig. 3A), in which metabolic acidosis was induced during the prepartum period like that in the anion group. The reason urinary Ca excretion did not increase in the heifer group as it did in the anion group can not be explained (Fig. 3B), but heifer cows without Ca supplement might inhibit the outflow of Ca from kidneys to allow Ca utilization in the body.

In conclusion, the administration of anion salts slightly lowered DCAD during the prepartum period and effectively prevented milk fever in multiparous cows. The safe and mild metabolic acidosis induced by anion salts could be evaluated by urinary pH (6.8–7.0), and might increase the responsiveness to Ca requirement at parturition through some complex mechanisms unrelated to the excretion of PTH and 1,25-(OH)2D. In addition, it was demonstrated that primiparous cows have the potential to respond to sudden Ca demand unrelated to the excretion of hormones, and their Ca metabolism was similar to that in multiparous cows fed anion salts in some respects. Therefore, manipulating mildly DCAD is expected to be an effective, safe and natural method for preventing milk fever in multiparous cows, although further studies are needed to establish the protocol for practical use.

ACKNOWLEDGEMENT. The authors wish to thank all staff of Yamagishi dairy farm, Hokkaido, Japan, for cooperation.

REFERENCES


