Mineral Metabolism in Plasma, Urine and Bone of Periparturient Cows Fed Anionic Diets with Different Calcium and Phosphorus Contents

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(Received 23 April 1996/Accepted 10 July 1996)

ABSTRACT. The objective of this experiment was to evaluate the influences of Ca and P contents in an anionic diet on the mineral metabolism in plasma, urine and bone in periparturient dairy cows. Fifteen multiparous Holstein-Friesian cows were divided into 3 dietary groups (5 cows/group) by dietary Ca and P contents and dietary cation-anion balance $[(Na + K) - (Cl + S) \text{ mEq/kg DM}]$: diet 1 [low Ca (0.46%), low P (0.24%), cationic (+195.8 mEq/kg DM)]; diet 2 [low Ca (0.46%), low P (0.24%), anionic (-32.4 mEq/kg DM)]; and diet 3 [high Ca (0.93%), high P (0.60%), anionic (-41.0 mEq/kg DM)]. Cows were fed one of these 3 diets from approximately 4 weeks before the expected calving date to 5 days after calving. There was no outbreak of milk fever in any cows fed these 3 diets; however, plasma Ca levels at 1 and 2 days after calving tended to be higher in the cows fed diet 3 than those in the cows fed diets 1 or 2. Fractional urinary excretion of Ca in the cows fed diet 2 or 3 was higher than that in the cows fed diet 1. Fractional urinary excretion and plasma level of Pi were higher during the periparturient period in the cows fed diet 3 than those in the cows fed diets 1 or 2. There were no significant differences in plasma parathyroid hormone levels among the 3 groups. In the spongy substance of ilium at 5 days after calving, the Ca and Mg contents, bone volume and trabecular thickness were the lowest, but not significant, in the cows fed diet 2. These data suggest that sufficient Ca and P contents in an anionic diet may be effective in maintaining plasma Ca and Pi levels of periparturient cows and further in preventing of potential bone damage brought about by increased urinary mineral excretion following the feeding of an anionic diet. — **KEY WORDS:** bone change, dairy cattle, dietary calcium content, dietary cation-anion balance, dietary phosphorus content.

J. Vet. Med. Sci. 58(12): 1187–1192, 1996

Milk fever (parturient paresis, parturient hypocalcemia) is mainly caused by an imbalance between the calcium (Ca) uptake by the mammary gland and the influx of Ca from bone and intestine [1, 17]. The dietary cation-anion balance (DCAB) and dietary Ca and phosphorus (P) contents during the dry period may play an important role in the incidence of milk fever. The DCAB is defined by most nutritionists as milliequivalent [sodium (Na) + potassium (K)] – [chloride (Cl) + sulfur (S)]/kg of dry matter (DM) [6, 13, 18, 23]. Feeding low Ca-low P or anionic (low DCAB) diets during the dry period tend to reduce milk fever, while high Ca-high P or cationic (high DCAB) diets tend to induce it [5–7, 10, 19]. However, the effects of Ca and P contents in the anionic diet on mineral metabolism have not been fully understood.

Skeletal minerals are decreased during lactation, and these are replenished during late lactation and dry period in ruminants [2]. An anionic diet is generally fed for 3 to 6 weeks prior to parturition [5, 6, 13, 18]. An anionic diet is thought to induce a metabolic acidosis which increases the bone Ca resorption and urinary Ca excretion [5, 6, 22, 23], whereas, intestinal Ca absorption usually does not increase during metabolic acidosis [4, 13, 22]. Nevertheless, the influence of feeding of anionic diet for a long period on the bone change has not been studied.

The objective of the present study, therefore, was to evaluate the influences of Ca and P contents in the anionic diet on the mineral metabolism in plasma, urine and bone in

periparturient cows.

MATERIALS AND METHODS

Fifteen multiparous Holstein-Friesian cows were divided into 3 dietary groups (5 cows/group) based on DCAB, dietary Ca and P contents. The ingredients and nutrient composition of the 3 experimental diets are given in Table 1. Diet 1 (low Ca, low P, cationic) was based on corn silage and orchardgrass haylage. The basal ration was consisted of 1.68 kg corn silage, 1.0 kg orchardgrass, 0.87 kg beet pulp, and 1.31 kg concentrate mixture on a DM basis. Diet 2 (low Ca, low P, anionic) was formulated by the addition of NH_4Cl (120 g/cow/day) to the basal ration. Diet 3 (high Ca, high P, anionic) was formulated by the addition of NH_4Cl (120 g/cow/day) and $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ (200 g/cow/day) to the basal ration. The DCAB (mEq/kg DM), calculated as $(Na + K) - (Cl + S)$, was +195.8, -32.4, and -41.0 in diets 1, 2 and 3, respectively. Cows fed the diet 1 or 2 received approximately 41 g of Ca and 21 g of P/day. Cows fed the diet 3 received approximately 85 g of Ca and 55 g of P/day. The average age (years) at parturition was 4.9, 4.6, and 4.0 in the groups receiving diets 1, 2 and 3, respectively. The diets met NRC [16] protein and energy requirements for dry pregnant and lactation cows.

Cows before calving were housed in a concrete open pole barn with free access to a dirt exercise lot and drinking water. Cows after calving were housed in individual tie

Table 1. Ingredients, nutrient composition, and cation-anion balance of the 3 experimental diets

	Diet 1 (Low Ca, low P, cationic)	Diet 2 (Low Ca, low P, anionic)	Diet 3 (High Ca, high P, anionic)
Ingredient, % of DM			
Corn silage	18.98	18.71	18.30
Orchardgrass haylage	56.49	55.68	54.47
Beet pulp	9.78	9.69	9.48
Concentrate mixture ^{a)}	14.74	14.59	14.27
NH ₄ Cl	—	1.34	1.31
CaHOP ₄ ·2H ₂ O	—	—	2.18
Nutrient composition			
DM, %	47.90	48.20	48.70
DM intake, kg/day	8.86	8.98	9.18
Crude protein, % of DM	13.50	13.31	13.02
Net energy lactation, Mcal/kg	1.69	1.67	1.64
Minerals, % of DM			
Ca	0.46	0.46	0.93
P	0.24	0.24	0.60
Mg	0.18	0.17	0.17
Na	0.14	0.16	0.15
K	1.43	1.41	1.38
Cl	0.51	1.31	1.32
S	0.14	0.15	0.14
(Na + K) — (Cl + S), mEq/kg DM	+195.8	— 32.4	— 41.0

a) Concentrate mixture contains 46% shelled corn, 16% corn gluten meal, 12% corn gluten feed and 26% others (mineral salts, alfalfa haylage, beet pulp, molasses and cotton seed) (Nippon Formula Feed Manufacturing Co., Japan).

stalls with water bowl. Cows were fed one of the 3 diets once a day in the stanchion at 14:00, beginning from approximately 4 weeks before the expected calving date to 5 days after calving. Orchardgrass haylage was given *ad libitum* in the open pole barn and tie stalls, and intake was measured 3 times throughout the experiment.

One heparinized blood sample was taken anaerobically from the jugular vein of each cow at 16:00 during the periparturient period for analysis of blood pH. Another heparinized blood sample was taken, and the plasma was stored at -20°C until analyzed. Urine sample was obtained by vulva stimulation at the time of the blood sampling, and stored at -20°C until analyzed. Iliac bone specimen was obtained from all cows at 5 days after calving by biopsy at a site 5 cm vertical from the tuber coxae, with a trephine (0.8 cm internal diameter) under sedation with xylazine and local anesthesia with procaine.

Blood and urine pH were analyzed by a blood gas analyzer (CIBA Corning Co., U.S.A.), and pH meter (Iuchi Co., Japan) within 1 hr of sampling. Blood pH was corrected by body temperature. Iliac bone specimen with only spongy substance and diet were dried at 100°C for 24 hr and then ashed at 600°C for 24 hr in an electric furnace. Resulting ash was dissolved in HNO_3 for analysis of Ca, P and magnesium (Mg) in bone and Cl in diet. Another ash from diet was dissolved in HNO_3 and HClO_4 for analysis of Na, K, S, Ca, P and Mg. Levels of Ca and Mg in plasma, urine, bone and diet, and K in diet were determined by

atomic absorption spectrophotometry. Levels of creatinine and inorganic P (Pi) in plasma and urine, and P in bone and diet were determined by colorimetric procedures. Levels of Na, Cl, and S in diet were determined by flame photometry, a mercurimetric method, and a gravimetric method [12], respectively. Fractional urinary excretion (%) of minerals (Ca, P and Mg) was calculated as [(urine mineral/plasma mineral) $\times$ (plasma creatinine/urine creatinine) $\times$ 100] [11]. Plasma intact parathyroid hormone (PTH) level was determined by an immunoradiometric assay (INCstar, U.S.A.).

Each undecalcified iliac bone specimen with cortex was stained with Villanueva's staining solution and embedded in methylmethacrylate. The specimen was cut longitudinally and ground to 10–15 μm in thickness with grinding glasses (Asahi Glass Co., Japan). Bone volume (trabecular bone including osteoid/total bone) and trabecular thickness in the spongy substance were performed with a semiautomatic image analyzer (Carl Zeiss Co., Germany) [14].

The data were analyzed using the Statistical Analysis System (SAS Inst., Inc., U.S.A.). Statistical differences among the 3 groups of cows were evaluated by one-way analysis of variance followed by the least significant difference procedure.

RESULTS

No milk fever was developed in any cows fed the 3 diets.

Vein blood pH in the cows fed diets 2 or 3 was significantly lower ($P<0.05$) at 2 and 1 days before calving than that in the cows fed diet 1. Blood pH after calving did not differ significantly among the cows of 3 diets (Fig. 1). Urine pH in the cows fed diets 2 or 3 was significantly lower ($P<0.05$) at 2 days before calving, and 1 and 2 days after calving than that in the cows fed diet 1 (Fig. 1).

Plasma Ca levels in the cows fed diets 1 ($P<0.05$) or 2 were higher at 2 days before calving than those in the cows fed diet 3. However, plasma Ca levels in the cows fed diets 1 and 2 rapidly decreased until day 1 after calving and at calving, respectively. At 1 and 2 days after calving, plasma Ca levels tended to be higher in the cows fed diet 3 than those in the cows fed diets 1 or 2. The cows fed diets 1 or 2 had increased plasma Ca levels at 3 days after calving (Fig. 2). Plasma Pi levels in the cows fed diet 3 were higher ($P<0.05$) at 2 days and 1 day before calving and at 5 days after calving than those in the cows fed diet 1, and higher ($P<0.05$) at 1 day before calving than those in cows fed diet 2 (Fig. 2). Plasma Mg levels did not differ significantly among the cows of all 3 groups, but the values during the periparturient period in the cows fed diet 1 tended to be higher than those in the cows fed diets 2 or 3 (Fig. 2). Plasma PTH levels did not differ significantly among the cows during the periparturient period. In the cows fed diets 1 or 2, plasma PTH levels tended to increase at calving (Fig. 2).

Fractional urinary excretion of Ca in the cows fed diets 2 or 3 was significantly higher ($P<0.05$) at 2 days before calving than that in the cows fed diet 1, and this higher excretion was maintained until 2 days after calving (Fig. 3). Fractional urinary excretion of Pi in the cows fed diet 3 was higher during the periparturient period than that in the cows fed diets 1 or 2, but none of the differences was significant (Fig. 3). Fractional urinary excretion of Mg did not significantly differ among the 3 groups of cows during the periparturient period (Fig. 3).

In the spongy substance of the ilium, the mean contents of Ca, P, and Mg were not significantly different among the 3 groups of cows, but the Ca and Mg contents were the lowest in the cows fed diet 2 (Table 2). Bone volume and trabecular thickness were also not significantly different among the cows, but were the lowest in the cows fed diet 2 (Table 3).

DISCUSSION

Metabolic acid-base status of blood is highly dependent on the dietary balance between cations and anions. Addition of anionic salts such as NH_4Cl , CaCl_2 and MgCl_2 to the diet therefore tends to keep the cow in metabolic acidosis, which may facilitate Ca mobilization favorable for lactation [5, 6, 21–23]. In the present study, cows fed diets 2 or 3 also showed the mild metabolic acidosis until calving. However, blood pH in diets 2 or 3 groups gradually elevated after calving. Such an elevation might be caused by the decreased intake immediately after calving. Urine pH was lower in

Fig. 1. pH values of vein blood and urine in cows fed the 3 experimental diets. Mean $\pm$ SE. a, b; Significant difference between different letters ($P<0.05$).

the cows fed diets 2 or 3 than that in the cows fed diet 1 always, and the urine acidification may be compensatory responses of the kidney function.

Metabolic acidosis increases Ca resorption from bone [6, 13, 22] and decreases Ca accretion into bone and mineralization [22, 24]. Metabolic acidosis usually does not increase intestinal Ca absorption [4, 13, 22], and produces hypercalciuria [15, 22–24]. In the present study, fractional urinary excretion of Ca also was higher in the cows fed diets 2 or 3 than that in cows fed diet 1 during the periparturient period. Bone Ca and Mg contents, bone volume and trabecular thickness were the lowest in the cows fed diet 2, although there was no significant. This result was thought to be caused by the negative mineral balance following the feeding of an anionic diet with low Ca and low P contents, although mineral balance between intake and total excretion was not examined. Won *et al.* [24] indicated that bone mineral loss and bone fragility due to

Fig. 2. Plasma levels of Ca, Pi and Mg in cows fed the 3 experimental diets. Mean $\pm$ SE. a, b; Significant difference between different letters ($P < 0.05$).

metabolic acidosis can be prevented by sufficient Ca intake in rats. Based on our and other studies [2, 24], it is considered that a supplementation of sufficient Ca to the anionic diet may be effective for prevention of potential bone damage.

Plasma Ca levels in the cows of 3 groups remained at greater than 7.5 mg/dl throughout the experiment, and no milk fever was developed in any cows. Milk fever usually occurs within 48 hr after calving [1, 6]. In the present study, plasma Ca levels before calving were higher in the cows fed diets 1 or 2 of low Ca level than those in the cows fed diet 3 of high Ca level. This result agreed with Green *et al.* [8], and this adaptation in cows fed low Ca diet may play a role in prevention of milk fever. However, plasma Ca levels in the cows fed diet 3 tended to be higher at 1 and 2 days after calving than those in the cows fed diets 1 or 2. Goff *et al.* [6] suggested that a cationic diet decreases the bone response to PTH, and an anionic diet enhances the bone response to PTH. In the cows fed diet 3, the higher plasma Ca levels after calving might have resulted from both the passive transport of Ca and the activation of bone Ca resorption.

Restricting dietary Ca during the dry period is the traditional method of preventing milk fever. Feeding a low Ca diet during the dry period increases the active transport of Ca from intestine and bone Ca resorption prior to the initiation of lactation [7, 8, 10, 20]. However, our present results suggest that sufficient Ca content in an anionic diet (diet 3) is effective for maintaining plasma Ca level and

functional structure of the bone. Oetzel *et al.* [18] also suggest that a high dietary Ca content may be desirable when high anionic diet is given. Plasma PTH levels in the cows fed diets 1 or 2 of low Ca level tended to increase at calving in comparison with those before calving. Such increases may have resulted in the increase in the plasma Ca levels at 3 days after calving in the cows fed diets 1 or 2.

Hypophosphatemia associated with hypocalcemia may also contribute to the pathogenesis of clinical signs in milk fever. Cows with milk fever occasionally remain recumbent as a result of hypophosphatemia alone, even after the hypocalcemia has been corrected [1]. In the present study, diet 3 contained excess P, supplied by the addition of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$. Plasma Pi levels and fractional urinary Pi excretion were higher during the periparturient period in the cows fed diet 3 than those in the cows fed diets 1 or 2. Although feeding a high P diet during the dry period increases the incidence of milk fever [9, 10, 19], our data suggest that the addition of P with an optimal Ca:P ratio to the anionic diet seems to be not harmful for mineral metabolism.

The cause of the increase in the plasma Mg level associated with decreased plasma Ca level is not well understood. In the present study, plasma Mg levels tended to be higher during the periparturient period in the cows fed diet 1 than those in the cows fed diets 2 or 3. This may have been caused by a compensatory mechanism to maintain the systemic anion-cation balance [17].

Fig. 3. Fractional urinary excretions of Ca, Pi and Mg in cows fed the 3 experimental diets. Mean $\pm$ SE. a, b; Significant difference between different letters ($P < 0.05$).

Table 2. Mineral contents^{a)} in the spongy area of ilium in the cows fed the 3 experimental diet at 5 days after calving

	Ca (%)	P (%)	Mg (%)
Diet 1	8.70 $\pm$ 0.74	2.01 $\pm$ 0.35	0.17 $\pm$ 0.02
Diet 2	8.16 $\pm$ 0.37	1.78 $\pm$ 0.37	0.15 $\pm$ 0.02
Diet 3	8.20 $\pm$ 0.84	1.76 $\pm$ 0.33	0.18 $\pm$ 0.02

a) Calculated as percentage of wet weight. Diet 1: low Ca, low P, cationic. Diet 2: low Ca, low P, anionic. Diet 3: high Ca, high P, anionic. Mean $\pm$ SE.

Table 3. Histomorphometric results in the spongy area of ilium in the cows fed the 3 experimental diet at 5 days after calving

	Bone volume (%)	Trabecular thickness (μ m)
Diet 1	23.5 $\pm$ 0.9	70.4 $\pm$ 3.2
Diet 2	21.8 $\pm$ 0.1	64.3 $\pm$ 2.5
Diet 3	24.6 $\pm$ 3.5	75.9 $\pm$ 3.3

Diet 1: low Ca, low P, cationic. Diet 2: low Ca, low P, anionic. Diet 3: high Ca, high P, anionic. Mean $\pm$ SE.

In conclusion, sufficient Ca and P contents in an anionic diet may be effective for the prevention of the potential bone damage that may be due to an increase in urinary mineral excretion by anionic diet, and for maintaining plasma Ca and Pi levels in periparturient cows.

ACKNOWLEDGMENT. The authors thank Messrs. Toshiyuki Okada and Takashi Tanifuji of Iwate Prefectural Animal Husbandry Experimental Station for their technical assistance.

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