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Blood plasma mineral profile and health status in postpartum cows fed an anionic diet before parturition

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Abstract: The effects of an anionic versus cationic (control) diet on plasma mineral levels and health status were investigated in 24 dry pregnant Holstein-Friesian cows during the last 3 weeks of pregnancy. The cows were in their third or fourth parity, had a moderate level of milk production in their previous lactation, and did not have a history of milk fever. The anionic diet significantly improved plasma calcium status, as compared to the control (2.18 vs. 1.99 mmol L^{-1}) and nonsignificantly reduced the incidence of subclinical hypocalcemia (1/12 vs. 5/12) without affecting other periparturient diseases. Other plasma mineral levels were unaffected, except chloride, which was higher in the anionic group. The results show that an anionic diet could improve calcium status around the time of parturition and reduce the incidence of subclinical hypocalcemia when fed to moderate producing dairy cows not predisposed to milk fever.

Key words: Anionic diet, dry cow, health status, plasma minerals

Introduction

An anionic, or acidic, diet is one supplemented with anionic salts to provide more anions (Cl^- and S^{2-}) relative to the cations Na^+ and K^+. It has a negative dietary cation-anion difference (DCAD), calculated as mEq kg^{-1} of dry matter = (Na + K) – (Cl + S). It is well known that anionic diets efficiently maintain blood Ca at parturition and prevent milk fever when fed to cows during the last several weeks of pregnancy (1-4). Milk fever (paresis puerperalis) may have serious consequences for subsequent milk production (1), but some investigations suggest that attention should also be paid to subclinical hypocalcemia (5,6). Reducing smooth muscle contractions, and rumen and intestinal motility (7), subclinical hypocalcemia may have an even greater impact on herd economy than clinical milk fever because of its broader influence on dry matter intake (DMI), secondary disease conditions, and milk production during early lactation (8).

Feeding an anionic diet before parturition has been advised if the incidence of milk fever in a herd

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exceeds 10%, and also when it is desired to improve the health status and production in herds in which clinical milk fever is not a serious problem (8,9). Milk fever occurs more frequently in high producing cows (10) and in cows with a history of the disease (1). Dairy herds in Bosnia and Herzegovina (B&H) generally do not have problems with milk fever and the incidence of the disease seems to be low. Some field experience suggests an incidence of about 2% in herds, but more than 40% of cows may have subclinical hypocalcemia after parturition (V. Sakic, personal communication). It is also important to know that the level of milk production is moderate in most dairy herds in B&H. It remains unknown if use of anionic salts in moderate producing herds can be useful.

The aim of the present study was to examine the effects of an anionic diet fed before parturition on Ca status and other plasma mineral levels after calving, as well as on the health status in moderate producing cows without a history of milk fever.

Materials and methods

The study included 24 pregnant non-lactating Holstein-Friesian cows without a history of milk fever that were 65.5 ± 5 months old (mean ± SD), weighed 660 ± 32 kg, and in their third or fourth parity. The cows were randomly allocated to 2 groups according to parity, expected calving date, and milk yield during the previous lactation (6574 ± 681 kg) (Table 1), which were offered 1 of the 2 experimental diets (control and anionic) during the last 21 days of pregnancy. The experiment was conducted at Butmir Dairy Farm, Sarajevo city region, B&H. Cows were housed and fed in a tie stall with drinking water available ad libitum. Feeding of anionic salts ended at parturition. Immediately after calving, cows entered the routine nutrition and management program used at Butmir Dairy Farm.

Table 1. Baseline analysis of the cows (mean ± SD) fed anionic or control diets in the prepartum period.

	Control group	Anionic group	P =
Age, months	65.7 ± 5.9	65.4 ± 4.2	0.907
Parity	3.4 ± 0.5	3.6 ± 0.5	0.436
Milk production, kg[a]	6557 ± 719	6592 ± 671	0.901
Body weight, kg	657 ± 33	661 ± 31	0.767
Days on prepartum diet	19.1 ± 5.5	18.3 ± 3.9	0.705

[a] In previous lactation.

The diets consisted of corn silage with grass hay and additional amounts of sunflower meal, wheat bran, and commercial feed mixture, as well as salt and mineral-vitamin supplement (Table 2). All the concentrates and supplements were mixed before feeding and fed as a concentrate mixture. Pre-calving DMI was set at 10.1 kg cow^{-1} day^{-1} in both groups (1.53% of body weight) and contained about 75% dry matter (DM) from roughage. The cows were fed 5%-10% less than the expected voluntary intake to ensure consumption of the desired amount of feed without refusal. The anionic diet contained an anionic salt mixture that included (as fed) 140 g of $MgSO_4 \times 7H_2O$, 80 g of $CaCl_2 \times 2H_2O$, and 20 g of NH_4Cl (total: 2.6 Eq) for each animal each day. The salts were mixed with the concentrate mixture and offered twice daily at 0800 and 1500, following a portion of grass hay and before corn silage.

Table 2. Ingredient composition (DM, %) of prepartum diets used in an experiment to determine the effects of a prepartum anionic diet on plasma mineral and health status after calving.

	Control diet	Anionic diet
Grass hay	30.19	30.20
Corn silage	45.53	45.55
Wheat bran	3.46	3.47
Sunflower meal	9.21	9.21
Feed mixture [a]	10.89	9.51
Min-vit. supplement [b]	0.38	0.38
NaCl	0.20	0.20
$CaCl_2 \times 2H_2O$	-	0.60
$MgSO_4 \times 7H_2O$	0.14	0.68
NH_4Cl	-	0.20

[a] Supplemented per 1 kg with: vitamin A 10,000 IU, vitamin D_3 1500 IU, vitamin E 20 mg, J 1 mg, Mn 50 mg, Co 0.12 mg, Se 0.1 mg, Fe 25 mg, and Zn 45 mg.

[b] Contained per 1 kg: Ca 160 g, P 80 g, Na 100 g, Mg 20 g, Cl 154 g, vitamin A 1,000,000 IU, vitamin D_3 120,000 IU, vitamin E 1200 mg, vitamin B_1 60 mg, vitamin B_2 30 mg, vitamin B_6 15 mg, vitamin B_{12} 100 µg, niacin 600 mg, Zn 9000 mg, Fe 800 mg, Mn 5000 mg, Cu 1000 mg, Co 20 mg, J 100 mg, and Se 40 mg.

Feed samples were analyzed for DM, crude protein, fiber, and ether extract, as well as Ca, P, Mg, Na, K, Cl, and S. Absolute DM content of each feed was determined by drying at 103-105 °C for 24 h. Dried samples were ground through a 1-mm screen and ashed at 550 °C overnight. Nitrogen content was determined using a Kjeltec Model 1030 automatic analyzer (Tecator AB, Hoganos, Sweden). Crude fiber analysis and fat extraction were performed according to standard ISO procedures.

Ca, Mg, Na, and K concentrations were determined via atomic absorption spectrophotometry (AAnalyst 300, Perkin Elmer Corp., Norwalk, CT, USA) in ashed feed samples dissolved in a 3*N* HCl solution and diluted (1:50) either with strontium chloride for Ca and Mg or with deionized water for Na and K analysis. Phosphorus was measured colorimetrically using the ammonium molybdate procedure. Sulfur was measured using the gravimetric method, with magnesium nitrate and precipitation with barium chloride. Chlorine was measured using sodium carbonate and precipitation with silver nitrate.

Analysis of Ca and Mg in anionic salts was performed in a water solution using the same procedure as for the feeds, while S and Cl were measured using the gravimetric method, with barium chloride precipitation, and the titrimetric method of Mohr, respectively. Chemical analysis of mineral-vitamin supplements was not performed, except for DM and ash content; therefore the reported composition of the product was used for calculations.

Whole blood samples were collected anaerobically from the jugular vein of each cow into 10-mL heparinized vacutainers (Becton Dickinson VACUTAINER Systems, Rutherford, NJ, USA). All blood samples were obtained within 8 h of parturition, immediately placed on ice, and transported to the lab, where they were centrifuged at 800 ×g for 20 min. After centrifugation plasma was transferred to plastic tubes and frozen at −20 °C until mineral element analysis. The entire procedure (from blood sample collection to plasma separation) was performed within 2 h of sampling. Prior to analysis, plasma samples were thawed at room temperature overnight and the mineral concentrations were determined the next day. Total Ca, Mg, Na, and K concentrations were analyzed via atomic absorption spectrophotometry. Samples were diluted 1:50 with 0.1% (wt.:vol.) lanthanum-chloride for Ca and Mg, and with deionized water for Na and K analysis. Inorganic plasma P was measured colorimetrically with ammonium chloride after samples were deproteinized with 20% trichloroacetic acid. Plasma Cl was measured using the method of Mohr.

All clinical diseases were diagnosed and treated by the farm's veterinary service, and hypocalcemia (plasma Ca <2 mmol L^{-1}) and hypomagnesemia (plasma Mg <0.75 mmol L^{-1}) were diagnosed by laboratory analysis of blood plasma samples. Student's t-test for independent samples was used to determine the significance of the differences between corresponding means in the 2 groups. Differences in disease incidences were tested by the 2-tailed Fisher's exact test. P values less than or equal to 0.05 were considered statistically significant.

Results

Diet composition and intake

The nutrient composition of the prepartum diets calculated after chemical analysis of the feed ingredients is shown in Table 3. The diets were similar

Table 3. Calculated nutrient composition of prepartum diets (DM basis) used in an experiment to determine the effects of a prepartum anionic diet on plasma mineral and health status after calving.

	Control diet	Anionic diet
Crude protein, %	12.02	12.06
Crude fiber, %	26.39	26.33
Ether extract, %	3.42	3.37
Ash, %	5.69	6.74
NEL, MJ kg^{-1} [a]	5.60	5.49
Calcium, %	0.55	0.76
Phosphorus, %	0.40	0.39
Magnesium, %	0.26	0.36
Sodium, %	0.14	0.14
Potassium, %	1.01	1.01
Chlorine, %	0.25	0.76
Sulfur, %	0.20	0.34
DCAD, mEq kg^{-1} [b]	+ 127.32	− 107.43

[a] Net energy for lactation was based on tabular values for all feeds (18), but calculated for the commercial feed mixture.
[b] Calculated as (Na + K) − (Cl + S).

with respect to all nutrients and energy, except for S and Cl. Levels of Ca and Mg were about 40% higher in the anionic diet because of the high levels of these minerals in anionic salts.

The experimental feeding period was scheduled to last 21 days before parturition, but, because of normal variation in the predicted calving dates, cows in the control and anionic groups spent 19.1 ± 5.5 and 18.3 ± 3.9 days on the treatment (mean ± SD), respectively (Table 1). During the treatment period cows in both groups consumed the diets completely; the only observed difference was that cows in the anionic group took several minutes longer to consume the same amount of concentrate feed than the cows in the control group (data not shown).

Plasma minerals

The mineral concentrations in blood plasma are shown in Table 4. Total plasma Ca was significantly higher at calving in the cows fed the anionic diet than in those fed the control diet. Slightly higher levels of inorganic P and lower levels of plasma Mg were observed in the anionic group, but the differences between the groups were not significant. Concentrations of plasma Na and K did not differ between the groups, but the plasma Cl concentration was significantly higher in the anionic group. The strong ion difference (SID) in blood plasma was lower in the cows fed the anionic diet (as was expected), but this was not significantly different compared to that in the cows fed the control diet.

Table 4. Postparturient plasma mineral concentrations (mean ± SD) in cows fed anionic or control diets in the prepartum period.

Mmol L^{-1}	Control group	Anionic group	P =
Calcium	1.99 ± 0.22	2.18 ± 0.23	0.049
Phosphorus [a]	1.36 ± 0.45	1.48 ± 0.43	0.506
Magnesium	1.06 ± 0.17	0.95 ± 0.16	0.112
Sodium	136.83 ± 6.38	138.28 ± 8.75	0.649
Potassium	4.53 ± 0.40	4.56 ± 0.61	0.891
Chlorine	98.47 ± 4.37	103.83 ± 4.70	0.008
SID, mEq L^{-1} [b]	42.88 ± 6.46	38.98 ± 9.17	0.242

[a] Inorganic.
[b] Strong-ion difference calculated as $(Na^{+} + K^{+}) - Cl^{-}$.

Health status

The health status of the cows after parturition is shown in Table 5. Five cows in the control group had subclinical hypocalcemia after parturition, while only 1 case was observed in the anionic group; the difference was not significant (P = 0.155). Two cows had plasma Mg concentrations below 0.75 mmol L^{-1} (1 in each group). There were no differences in the incidence of retained placenta, metritis, or mastitis between the groups. Three complex cases (cows experiencing 2 or more clinical diseases after parturition) were seen in the control group, while none were observed in the anionic group. Each of the complex cases was hypocalcemic.

Discussion

The use of anionic salts is easier if a total mixed ration (TMR) is used, but it is also possible to use them in component feeding systems if the salts are mixed with at least 2.3 kg of concentrate to mask their bitter taste (11). In B&H roughage and concentrates are always fed separately, and TMRs generally have not been used in the field. Observations made during the present study suggest that the taste of the salts was

Table 5. Incidence of postpartum health disorders in cows fed anionic or control diets during the prepartum period[a].

	Control group	Anionic group	Fisher's, P =
Subclinical hypocalcemia [b]	5/12	1/12	0.155
Hypomagnesemia [c]	1/12	1/12	1.000
Retained placenta [d]	2/12	1/12	1.000
Metritis	3/12	2/12	1.000
Mastitis	2/12	2/12	1.000
Complex cases [e]	3/12	0/12	0.217
Total health disorders [f]	7/36	5/36	0.753

[a] Number of cases per number of cows in each group.
[b] Subclinical hypocalcemia defined as <2 mmol L^{-1} of total Ca in blood plasma.
[c] Hypomagnesemia defined as <0.75 mmol L^{-1} of total Mg in blood plasma.
[d] Fetal membranes retained longer than 12 h after parturition.
[e] Number of cows that experienced 2 or more clinical disorders.
[f] Subclinical hypocalcemia and hypomagnesemia not included in this calculation.

not completely masked in 2.6 kg of concentrate feed, but the amount of concentrate was sufficient to ensure consumption of the desired amount of anionic salts.

The results of this study agree with those of numerous previous works that have reported a positive effect of anionic diets on Ca status in peripartum cows (1,2,4,12-14). The relatively small difference in plasma Ca between the anionic and control groups, although significant, was not sufficient to cause significant changes in plasma P and Mg. Phillippo et al. (15) reported that changes in P and Mg metabolism around the time of parturition are related to hypocalcemia, rather than to the diet. Hypocalcemia releases more parathyroid hormone, which increases reabsorption of Ca and Mg in renal tubules and inhibits reabsorption of P at the same time (16). As a consequence, a reduction in the plasma Ca concentration at parturition is usually accompanied by hypophosphatemia and hypomagnesemia (15). However, this is not always the case, especially if hypocalcemia is not severe and is not clinically manifested. These findings correspond to the results of Oetzel et al. (12) and Joyce et al. (17), who also did not observe any changes in plasma P and Mg concentrations on the day of calving, despite the fact that cows fed anionic diets had significantly higher level of plasma Ca than cows fed cationic diets. The observed changes in plasma Na, K, and Cl in the present study are in accordance with the dietary treatment, as observed by others (15).

Most of the cows in the present study were 5 or more years old, which is considered a critical age for milk fever, but clinical cases of the disease were not apparent. The probability of the incidence of the disease was not high, considering that no cow chosen for this experiment experienced milk fever during previous calving (1) and milk yield during the previous lactation was not high (10). The positive effect of the anionic diet on subclinical hypocalcemia prevention (1/12 vs. 5/12) was in accordance with earlier reports related to high producing cows (2,6,12). The difference in the incidence of hypocalcemia was numerically important, but not significant ($P = 0.155$), most certainly because of the small sample size.

One cow in the control group and another in the anionic group had plasma Mg concentrations below 0.75 mmol L^{-1}. The normal concentration of plasma Mg in cows ranges from 0.75 to 1 mmol L^{-1} and levels below 0.75 mmol L^{-1} suggest insufficient intake or poor bioavailability of Mg in the digestive tract (18). Dietary Mg intake was sufficient in the control group and even increased in the anionic group, but this had no effect on plasma Mg at calving. Increasing Mg intake in cows fed anionic salts from 0.2% to 0.37% also yielded no benefit with regard to Ca or Mg metabolism in a previous experiment (19).

Oetzel et al. (12) reported that cows fed anionic diets are less likely to develop disorders related to hypocalcemia, and recent experiments showed that those diets fed during late gestation significantly reduced the incidence of retained placenta postpartum (14,20). However, anionic diets did not affect the incidence of health disorders in other studies (2,17), which is in accordance with the present study's findings, which might have been related to the relatively good Ca status in both groups of cows. There were 5 hypocalcemic cows in the control group, but plasma Ca values never fell below 1.70 mmol L^{-1}, which is still considered mild hypocalcemia.

All the complex cases (cows that experienced 2 or more clinical diseases) observed in the present study were related to hypocalcemic cows in the control group. The cow with the lowest plasma Ca concentration had retained placenta, followed by coliform mastitis with serious general health disturbances and complete cessation of milk secretion. The other 2 cows had retained placenta, plus metritis and metritis plus mastitis. This finding confirms that periparturient disorders are often seen as a complex and are interrelated (10).

The problem associated with the investigation and interpretation of results on health status often lies in the relatively low number of animals used in such studies. All but 2 (2,20) of the studies cited in this paper were conducted with small samples. The number of cows per group in the present study was also too small to reliably detect differences in disease incidences; therefore caution must be taken when interpreting the results.

In conclusion, the anionic diet fed before parturition in the present study resulted in a tendency towards improved Ca status and a decrease in the incidence of subclinical hypocalcemia in moderate producing dairy cows that were not predisposed to milk fever.

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