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Full Length Research Paper

Effect of dietary cation-anion balance on milk production and blood mineral of Holstein cows during the last two months of pregnancy

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This study was conducted to evaluate the effects of three diets with different cation-anion differences ((DCAD: mEq[(Na + K) – (Cl + S)]/100 g of dry matter)) in far-off and close-up period, on milk production and blood mineral of Holstein cows. Eighteen pregnant cows (220 - 225 d) were fed a base diet with three DCAD (+13 (control), 0, –13/100 g dry matter) for 60 ± 5 days. Control diet consisted of 170 g/kg corn silage, 396 g/kg alfalfa hay and 424 g/kg concentrate mix (dry matter basis). For decrease DCAD, two anionic salts such as ammonium chloride and ammonium sulphate were used. Production of milk and 3.5% fat corrected milk (FCM) were increased with decreasing DCAD. However, milk composition and yield of milk fat, protein and lactose were not affected by diets containing +13, 0 and –13 DCAD. In addition, prepartum dry matter intake, BCS change and body weight gains were similar for cows fed the three diets with different cation-anion differences. At calving, plasma calcium concentration was 6.55, 6.46 and 6.78 mg/dl for cows fed diets containing +13, 0 and –13 DCAD, respectively. Blood pH and concentration of K, Mg and Na were not affected by dietary cation-anion balance. Urinary pH of cows was affected by diets and was decreased linearly with decreasing DCAD. The mean urinary pH was 7.99, 6.81 and 6.11 for cows fed diets containing +13, 0 and –13 DCAD, respectively.

Key words: Metabolic disorders, pregnant cow, anionic salts.

INTRODUCTION

The main metabolic disorders of cows at the calving and fresh time are milk fever, udder edema, retained placenta, displaced abomasums, rumen acidosis, mastitis and laminitis. Sudden onset of lactation increases calcium (Ca) demand and could induce hypocalcemia (Block, 1984; Horst et al., 1997; Jackson et al., 2001). Hypocalcemia or low blood Ca (not just milk fever) impairs abomasums contraction leading to more metabolic disorders. Hypocalcemia causes secretion of cortisol, which impairs the immune system of the fresh cow (Wang et al., 1991). Plasma Ca concentration is under the control of parathyroid hormone, calcitonin and the metabolites of vitamin D (Goff et al., 1995). The traditional method of preventing milk fever in fresh dairy cows is by restricting dietary intake of Ca during the prepartum period (Roche et al., 2002). Recently, supplementation of cows precalving with chlorine (Cl) and sulphur (S) (while maintaining or reducing the dietary concentration of sodium (Na) and potassium (K) to reduce blood pH) has been effectively used to prevent hypocalcemia. It has been shown that feeding diets supplemented with Cl and S induced acidosis in cows and increased the size of the exchangeable Ca pool because of alterations in bone metabolism (Tucker et al., 1988).

In Iranian small farms (<100), cows in far-off and close-up period and all cows (1 - 60 days to parturition) were fed similar diet. Decreasing the dietary cation-anion difference (DCAD; milliequivalents [(Na + K)-(Cl + S)]/100 g of DM) during the last 3 to 4 weeks before calving can have beneficial effects on systemic acid-base status, Ca metabolism, peripartum health and postpartum productive and reproductive performance (Horst et al., 1994; Oetzel et

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Abbreviations: DCAD, dietary cation-anion differences; **BCS,** body condition score; **FCM,** fat corrected milk.

Table 1. Ingredient and chemical composition of prepartum diets.

Item	Control 0	DCAD‡	13 DCAD
Dietary ingredients(g/kg DM)			
Corn silage	170	168	166
Alfalfa	396	394	390
Barley	155	154	151
Soybean meal	20	20	20
Cottonseed meal	25	25	25
Corn, ground	55	55	55
Wheat brain	173	171	170
Salt	1.8	1.8	1.8
Limestone	2.5	2.5	2.5
Calcium chloride	0	2.7	4.3
ammonium sulfate	0	4.3	12.7
Trace mineral and vitamin premix□	1.7	1.7	1.7
Chemical composition (g/kg DM)			
NEL† (Mcal/kg)	1.48	1.47	1.45
CP	148	147	146
NDF	408	405	401
K	12	12	12
Na	1.7	1.7	1.7
Ca	5.03	5.03	5.03
P	3.25	3.25	3.25
Mg	3.22	3.22	3.22
Cl	3.2	4.65	6.8
S	2.5	4.0	5.2
Dietary cation-anion balance	+13	0	-13

‡ = DCAD as [(Na + K) - (Cl + S)] in milliequivalents per 100 g of DM; § = contained 2.0% Mg, 5% K, 3.0% Zn, 2.0% Mn, 3.0% Fe, 0.3% Cu, 0.001% Se, 0.01% Co, distillate, 0.01% I, 500 IU/g of vitamin A, 100 IU/g of vitamin D and 1 IU/g of vitamin E. † = Calculated according to NRC (2001).

al.,1988; Tucker et al., 1991). However, decreasing the dietary cation-anion difference during the last 7 to 8 weeks before calving has not being well established. For this purpose, the present study was carried out to compare the effect of diet formulated with +13, 0, -13 mEq/ 100 g DM DCAD on plasma concentrations of calcium and other macro mineral elements, urine and fecal pH, in the peripartum period and milk production in postpartum.

MATERIALS AND METHODS

Cows, diet and treatments

Eighteen multiparous pregnant Holstein cows, 60 ± 5 days before calving with an average body weight of 620 ± 20 kg were randomly assigned to three dietary treatments in a completely randomized design (CRD) and were fed during far-off and close-up periods. The experiment was carried out at the dairy barn of the Department of Animal Science of Tehran University in Karaj, Iran. Cows were placed in individual pens with concrete floors that were cleaned regularly and fed a total mixed ration *ad libitum*. Control diet consisted of 170 g/kg corn silage, 396 g/kg alfalfa hay and 424 g/kg concentrate mix (dry matter [DM] basis) (Table 1). Treatment were: control (no anionic salt supplementation, +13 mEq[(Na + K) – (Cl + S)]/100 g of dry matter) and supplemented with 7 g/kg anionic salt (0 mEq[(Na + K)-(Cl + S)]/100 g of dry matter) or 17 g/kg anionic salt (-13 mEq[(Na + K)-(Cl + S)]/100 g of dry matter). The DCAD was calculated by measured quantities of Na, K, Cl and S in the diets. To formulate diet with different DCAD, the anionic salts (NH_4Cl, $(NH_4)_2SO_4$) was added to reduce DCAD. Cows were fed twice daily at 0800 and 1600 h allowing for 5 - 10% orts, which were weighed daily. Water was available for cows throughout the entire experiment.

Feed samples were collected once per week and analyzed via wet chemistry (animal nutrition laboratory, karaj) for K, Na, Ca, Mg and Cl (determined by plasma emission spectroscopy; AA 670; Shimatzu, Japan) and DM content (ID 934.01; AOAC, 2000). Twice a month, feed samples were also analyzed via wet chemistry for N and neutral detergent fibre (NDF). Content of N in the samples were determined by Kjeldahl method in an automated Kjelfoss apparatus (Foss Electric, Copenhagen, Denmark). The NDF was sequentially determined using a fiber analyzer (Fiber tic system, Tecator, 1010, Denmark) according to the methodology supplied by the company, which was based on the methods described by Van Soest et al. (1991).

After calving, all animals were placed on the same lactation diet

Table 2. Feed intake and BW change in cows with +13(control), 0, or -13 dietary cation-anion balance diets.

Item	Control	0 DCAD‡	-13 DCAD	SEM	P value
Feed intake (kg/d)	11.36	11.44	11.56	.47	NS
Feed intake (kg/d), -2 d parturition	10.2	10.1	10.02	.57	NS
BW[2] change(kg/d)	1.21	1.19	1.25	.21	NS
BCS[3] change	.25	.22	.25	.07	NS
Calve weight[†] (kg)	39.12	42.66	41.00	3.22	NS

‡ = Dietary cation-anion balance; *P < 0.05; **P < 0.01; NS = not significant (P > 0.05); [† =] measurement in calving time; BW = body weight; BCS = body condition score.

Table 3. Milk yield and composition in cows with +13(control), 0, or -13 dietary cation-anion balance diets.

Item	Control	0 DCAD[1]	- 13DCAD	SEM	P value
Actual milk yield (kg/d)	34.34[b]	34.57[b]	35.51 [a]	.62	*
3.5% FCM (kg/d)	32.08[b]	31.83[b]	33.18 [a]	.68	*
Milk Fat (g/kg)	31.3	30.9	31.5	.51	NS
Milk Protein (g/kg)	27	26.5	26.5	.45	NS
Milk Lactose (g/kg)	4.8	47	47. 6	.80	NS

*P < 0.05; **P < 0.01; NS = not significant (P > 0.05); [a.b.c] means within a row with unlike subscript differ (p < 0.05).

that contained 1.70 Mcal/kg of NEL, 17.5% CP, 32.0% NDF, 1.1% K, 0.28% Na, 0.98% Ca, 0.50% P, 0.31% Mg and a DCAD of +32 mEq/100 g of dietary DM.

Sampling and chemical analyses

Cows were milked three times per day at 0250, 1100 and 1900 h. Daily milk yields were recorded throughout the experiment (for 60 days in milk). Milk samples were collected once per week from each of the three individual milkings for that day and analyzed for fat, protein and lactose by infrared procedures at the animal nutrition laboratory (Milko-scan 133B, Denmark).

Body weight was measured weekly (Monday) at 0750 h a.m. Body condition scores (BCS) were assigned at the start of the experiment and on the -40, -20 and 0, individuals, using a five-point scale(Wildman et al., 1982). Immediately after calving, weights of calves were measured.

Blood samples were obtained from the jugular vein into heparinized blood collection vacutainers each week, at 1000 h a.m. One week before expected date of calving, blood was sampled three times per week (Sunday, Tuesday and Friday). All samples were immediately analyzed for pH and were centrifuged at 800 g for 20 min at 4ºC and plasma stored at -20°C for subsequent mineral analysis. Plasma samples were analyzed for their mineral content. Before calving, the cows were manually stimulated to urinate each week. Urinary samples were collected and urine pH immediately measured.

Fecal pH was measured before calving three times per week to assess acid-base status. Hand-held urine pH meters were calibrated before each sampling of urine and feaces with a pH 7.0 buffer solution as the standard. Water samples were analyzed for Ca, Mg, Na, K, Cl and S and adjusted DCAD diet.

Statistical analysis

Data were analyzed using the PROC MIXED of SAS (2003). Model used is described by the following equation:

$$Y_{ijl} = \mu + T_i + L_j + P_l + E_{ijl}$$

Where μ = overall mean, T_i = effect of treatments (i = 1, 2 and 3), Lj = effect of previous season milk yield, P_l =effect of cow and E_{ijl} = residual.

RESULTS

Intake, body weight change and calf weight at birth

Data on intakes of dry matter, body weight change of cows and calf weight at birth are shown in Table 2. Dry matter intake were not affected by dietary treatment. Dry matter intake ranged from 11.36 to 11.56 kg/d for cows. In the last 5 days of the prepartum period, intake of DM was reduced by 11.71 and 13.32% with reduced DCAD to 0 and -13, respectively, compared with the control diet that were 10.21%. However, feed intake in last 5 days was not affected by DCAD. BW change, BCS change and calve weight at birth were not affected with reduced DCAD.

Milk yield and milk composition

Prepartum diets affected milk yield from week 1 through 10 postpartum (Table 3). Milk contents of fat, protein and lactose were similar among the treatments, but the production of 3.5% fat corrected milk (FCM) was affected by the treatments. Cows fed the –13 DCAD diet had

Table 4. Plasma, urine and fecal pH in cows with +13(control), 0, or -13 dietary cation-anion balance diets (approximately 7 d prepartum).

Item	Control	0 DCAD[1]	- 13DCAD	SEM	P value
Plasma pH before calving	7.41	7.46	7.40	.08	NS
Plasma pH at calving	7.42	7.45	7.42	.10	NS
Plasma pH after calving	7.43	7.41	7.39	.15	NS
Urinary pH	7.99[a]	6.81[b]	6.11[c]	.30	**
Fecal pH	6.90	6.98	6.61	.55	NS

[1] = Dietary cation-anion balance; *P < 0.05; **P < 0.01; NS = not significant (P > 0.05); [a.b.c] means within a row with unlike subscript differ (p < 0.05).

Table 5. Plasma mineral concentration in cows with +13(control), 0, or -13 dietary cation-anion balance diets.

Item	Control	0 DCAD[1]	- 13DCAD	SEM	P value
Ca before calving (mg/ml) †	8.20	8.75	9.31	1.29	NS
Ca calving time (mg/ml) ‡	6.55	6.46	6.78	.64	NS
Ca after calving (mg/ml) §	7.53	7.73	8.58	1.19	NS
Mg before calving (mg/ml)	2.28	2.26	2.29	.54	NS
Mg calving time (mg/ml)	2.18	2.38	2.50	.72	NS
Mg after calving (mg/ml)	2.30	2.40	2.43	.58	NS
K before calving (mg/ml)	4.30	4.41	4.50	.43	NS
K calving time (mg/ml)	4.13	3.95	3.83	.73	NS
K after calving (mg/ml)	4.41	3.89	4.05	.79	NS
Na before calving (mg/ml)	139.16	151.83	152.33	8.76	NS
Na calving time (mg/ml)	119.33	124.50	129.66	5.15	NS
Na after calving (mg/ml)	133.83	142.50	144.83	9.45	NS

[1] = Dietary cation-anion balance; *P < 0.05; **P < 0.01; NS = not significant (P > 0.05); † = Mean of samples taken -7 to -2 d prepartum; ‡ = Means of samples taken at 0 h, 12 h; § = Means of samples taken 1 to 7 d after calving.

higher milk and fat corrected milk than the control and 0 DCAD diet.

pH differences and mineral status

The effects of the range of DCAD on blood pH in calving time, before and after calving time, are shown in Table 4. After calving although blood pH was numerically lower for cows fed diets containing anionic salts than for cows fed the control diet, there was no significant discrepancy. Reduction of DCAD from +13 to -13 mEq/100 g did not affect the concentration of Ca, Mg, Na, or K, or in plasma at any sampling time (Table 5). Also reduction in DCAD from +13 to -13 mEq/100 g in prepartum increased total Ca in plasma on the day of calving and after calving but had no effect on plasma Ca in prepartum. Mg concentration in cows that receive treatment with -13 DCAD was higher than others, however this difference was not significant.

Urinary pH with reduce DCAD, was decreased and urine pH of cows treated with diet content -13 mEq/100 g in week before calving time was 6.11 ± 0.46 which is very close to standard 6.2. The urinary pH for cows treated with 0, +13 was 6.81 ± 0.30 and 7.99 ± 0.14, respectively. The decreased urinary pH indicated that the acid-base status was altered by DCAD. Fecal pH was not affected by dietary treatment.

DISCUSSION

No difference occurred between the three diets for CP, NDF, Na, P, Ca, Mg and Cl content. The result of water analyzed for mineral is not presented because diet mineral content adjusted for this.

DMI (dry matter intake) were unaffected by dietary treatments, although 1-5 day before calving time dry matter intake were decreased in all cows. The present experiment supported previous results in which DCAD from -228 - +978 mEq/kg had no effect on DMI (Oetzel et al. 1993) but this result are inconsistent with the literature in that anionic salts are unpalatable and reduced DMI (Beede, 1995; Horst et al., 1994; Vagnoni and Oetzel,

1998). Vagnoni and Oetzel (1998) reported that the mean DMI of cows fed all diets containing anionic salts was lower than the DMI of cows fed the control diet.

The increased milk yield and 3.5% fat corrected milk with decreasing DCAD and the lack of milk composition response to reducing DCAD during 60 day after calving is in agreement with most findings in the literature (Black, 1984; Moore et al., 2000). Block (1984) reported a 14 and 7% increase in milk production through a low-DCAD induced reduction in the incidence of clinical and sub-clinical hypocalcemia, respectively. However, Goff et al. (1991) have reduced the incidence of hypocalcemia through reducing precalving DCAD and have not found an effect of precalving DCAD on milk production.

After calving, although blood pH was numerically lower for cows fed diets containing anionic salts than for cows fed the control diet, no significant discrepancy was observed. This result was not surprising because maintenance of blood pH is one of the principal objectives of acid-base homeostasis. This result is in agreement with Vagnoni and Oetzel (1998) and was inconsistent with (Wang and Beede, 1992). Wang and Beede (1992) reported that when DCAD was reduced from 69 to -428 mEq/kg, blood pH decreased. Also Tucker et al. (1988) did observe significant decreases in blood pH in response to diets with -168 or -268 mEq/kg.

The effects of anionic salts on serum minerals have been inconsistent (Block, 1984; Oetzel et al., 1988; Tucker et al., 1988). The result of this experiment is consonant with the observation of Tucker et al. (1988) and Delaquis and Block (1995) but contrasted the results of Moore et al. (2000) and Goff et al. (1991). Goff et al. (1991) suggested that dietary anionic salts in prepartum increased total Ca in serum on the day of parturition, but Tucker et al. (1988) concluded that plasma mineral concentrations were much less responsive to DCAD manipulation.

A low urine pH, an indicator of blood pH (Vagnoni and Oetzel, 1998), has been associated with an increased gastrointestinal absorption and urinary output of Ca (Schonewille et al., 1994). However, the significant decrease in urinary pH showed that urine parameters were strong indicators of the systemic acid-base status of the animal. It also suggested that urinary pH measurement might be a useful tool in assessing the degree of metabolic acidosis imposed by dietary anionic salts, a point also considered by Roche et al. (2002).

Conclusion

Supplementing pregnant cow's diets during the last 7 to 8 weeks before calving with anionic salts increased milk production but did not affect DM intake, plasma mineral concentration and milk composition. As a result, urinary pH decreased with anionic salts supplementation. However, the result of the present study indicated that there was no difference for the cows that received negative DCAD content, during the last 7 to 8 weeks before calving.

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