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Effects of dietary cation-anion difference on milk fever, subclinical hypocalcemia and negative energy balance in transition dairy cows

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Abstract

Objective of this study was to determine the effects of varying dietary cation-anion differences (DCAD) in prepartum period on milk fever, subclinical hypocalcemia and negative energy balance in dairy cows of Urmia, Iran. Ninety nine Holstein cows assigned to three groups (n=33) were fed diets with DCAD of -24 (anionic), +80 (cationic) and +230 (cationic) mEq/Kg dry matter for 3 weeks before expected calving. Total serum calcium concentration was increased with decreasing DCAD. At calving, mean total calcium concentration in serum was 9.42, 8.44 and 7.87 mg/dl in cows fed diets containing -24, +80 and +230 DCAD respectively. Prevalence of clinical hypocalcemia in cows being fed with -24, +80 and +230 DCAD diets were 0%, 3.03% and 12.12% respectively. Also, prevalence of subclinical hypocalcemia was 9.09%, 15.15% and 27.27% in cows fed -24, +80 and +230 DCAD diets respectively. The prevalence of clinical and subclinical hypocalcemia was lower in cows fed anionic diet than cows fed cationic diets. Prevalence of negative energy balance in cows fed with -24, +80 and +230 DCAD diets was 33.33%, 18.18% and 21.21% respectively. Also, the mean β-hydroxybutyrate in cows fed anionic diet was higher than those fed with cationic diets. There was no significant difference in serum glucose concentrations among the three groups. The prevalence of postpartum negative energy balance was higher in cows fed anionic diet. These findings showed that use of anionic diets during three weeks before calving can protect dairy cows from clinical and subclinical hypocalcemia by increasing the calcium level in serum. To reduce the postpartum negative energy balance, replacement of anionic diet by cationic ions soon after calving is suggested.

Keywords: dietary cation-anion difference; milk fever; Non-esterified fatty acid; negative energy balance

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Introduction

The onset of lactation results in a sudden large demand for calcium (Ca) homeostasis. A cow producing 10 kg of colostrums will lose 23g of Ca (2.3g of Ca/kg) in a single milking (Radostits et al., 2007). Lost Ca from the plasma pool must be replaced by increasing intestinal absorption and bone resorption. Most cows adapt within 48 hours after calving by increasing plasma concentrations of parathyroid hormone (PTH) and 1,25-$(OH)_2D$ vitamin at the onset of the hypocalcemia (Radostits et al., 2007). However, 5-20% of adult cows do not adapt and they develop parturient paresis which requires treatment (Radostits et al., 2007). Using anionic diets in prepartum period is one of the effective methods to prevent hypocalcemia (Oetzal et al., 1988; Tucker et al., 1991; Moore et al., 2000).

Dietary cation-anion differences (DCAD) measure the levels of four macro minerals in the diet. Positively charged cations, potassium (K) and sodium (Na), and negatively charged anions, chloride (Cl) and sulphur (S) (Tucker et al., 1991; Moore et al., 2000; Radostits et al., 2007). DCAD affects blood buffering capacity and acidity of the blood in a cow (Muhammad et al., 2010). The cation-anion difference of a diet is commonly described in milli equivalents (mEq) per Kg dry matter

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(DM) (mEq/kg DM). This value is calculated as DCAD = (Na+K) - (Cl+S) as reported elsewhere (Radostits et al., 2007; Muhammad et al., 2010).

A diet high in cations, especially Na and K, tends to induce milk fever compared with those high in anions, primarily Cl and S, which can reduce this incidence (Radostits et al., 2007). Dietary cations are alkalogenic while dietary anions are acidogenic (Radostits et al., 2007). Metabolic alkalosis predisposes cows to milk fever. Under most circumstances, the alkalosis is induced by K in the diet (Goff and Horst, 2003). Addition of anions to the diet of dairy cows prior to parturition effectively reduces the incidence of milk fever by inducing a metabolic acidosis which facilitates bone resorption of Ca (Radostits et al., 2007). Systemic acidosis induced by anionic supplementation affects the function of the parathyroid hormone (PTH) (Block, 1984; Fredeen et al., 1988; Muhammad et al., 2010). The PTH dependent functions including bone resorption and renal production of 1,25-dihydroxy vitamin D, are enhanced in cows fed diets with added anions which increase their resistance to milk fever and hypocalcemia (Block, 1984; Goff and Horst, 2003; Muhammad et al., 2010).

Decreasing the DCAD during the last 3 to 4 weeks before calving has beneficial effects on systemic acid-base status including Ca metabolism, peripartum health and postpartum productive and reproductive performance (Tucker et al., 1991; Horst et al., 1994; Goff and Horst, 2003). A method to reduce the DCAD of prepartum diets is to feed anionic salts magnesium sulphate ($MgSO_4$), magnesium chloride ($MgCl_2$), ammonium chloride (NH_4Cl) and ammonium sulphate $(NH4)_2SO_4$ as described somewhere else (Moore et al., 2000; Radostits et al., 2007).

Effects of decreasing the DCAD on milk fever and subclinical hypocalcemia in dairy farms of Iran is not well documented. Decreasing the DCAD to prevent milk fever in transition dairy cows (3-4 weeks before to 3-4 weeks after calving), will lead to reduce dry matter intake (DMI) (Moore et al., 2000; Radostits et al., 2007). Therefore, we hypothesized that the use of negative DCAD diets during transitional period may worsen the negative energy balance (NEB) in dairy cows. On the other hand, effects of DCAD on energy balance in transition dairy cows is unknown, so we decided to investigate its effects on body fat stores and plasma concentration of Non-esterified fatty acid (NEFA) and β-hydroxybutyrate (BHBA), markers of negative energy balance.

Material and Methods

Animal and diets

Ninety nine multiparous pregnant Holstein cows from dairy farm around Urmia city, Iran, were selected for the experiment. Animals were randomly assigned to three groups (n=33) and were fed diets with varying DCAD 24 (anionic), +80 (cationic) and +230 (cationic) mEq/Kg DM for three weeks before expected calving.

The DCAD was calculated using the equation DCAD= (Na+K) - (Cl+S) (Block, 1984; Goff and Horst, 2003). Diets were fed two times a day and in the form of Total Mixed Ration (TMR). The compositions of diets are shown in Table 1. Feed samples were collected and analyzed via wet chemistry (Animal Science Research Institute, Karaj, Iran) for measurement of Na and K. The concentration of Cl and S were measured using titration and spectrophotometric methods, respectively.

Sample collection

A total number of 198 blood samples from 99 dairy cows were aseptically obtained via jugular vein at 2 and 12 days postpartum for measurement of blood Ca and NEFA respectively. Samples were numbered and characteristics of each cow including number of parturition, body temperature, heart rate, history of dystocia, retained fetal membranes and mastitis were recorded. Blood samples were transported to laboratory in a cooler with ice packs, and then centrifuged at 2000×g for 15 min. Serum was separated and stored at -20°C until analysis.

Urine samples were collected from seven cows in each group by perineal stimulation and midstream urine was collected in plastic containers. The pH was immediately measured using urine stripe test.

Biochemical analysis

Serum concentrations of BHBA and NEFA were measured by commercial kits kit (Randox, United Kingdom). Ca and glucose were measured by kit method (Bionik, Farasamed, Iran). These assays were analyzed using a Biotecnica 1500 Auto Analyzer, Rome, Italy).

Cows were considered to be affected by clinical and subclinical hypocalcemia when their serum Ca concentrations were <5 mg/dl and <7.5 mg/dl respectively (Radostits et al., 2007). Also, cows were considered to be affected by postpartum NEB when their serum NEFA concentrations were >0.7 mmol/L (Drackley, 2001; Garcia et al., 2011).

Statistical Analysis

Data were analyzed using the statistical software, SPSS 15. Independent samples T-test to investigate the correlation of variables, one way ANOVA to evaluate the analysis of variance and Chi-square test for evaluating the differences in prevalence of diseases between three groups were used. The spearman correlation test was used for assessment of the correlation between variables and linear regression. Significance was declared at P<0.05.

Results

Mean serum Ca concentrations on day 2 after calving is shown in Table 2. Cows fed anionic diet (-24 mEq/Kg DM) had higher serum Ca than those fed cationic diets (+80 and +230 mEq/Kg DM) (P<0.05). No cow on the

Table 1: Ingredients and chemical composition of diets

Group	Alfalfa hay (g/kg)	Concentrate mixture* (g/kg)	Corn silage (g/kg)	Barley (g/kg)	Straw (g/kg)	Na (%DM)	K (%DM)	Cl (%DM)	S (%DM)
DCAD = -24	400	300	230	70	---	0.154	0.283	0.167	0.187
DCAD = +80	400	300	200	---	100	0.158	0.420	0.116	0.103
DCAD=+230	500	200	100	---	200	0.328	0.751	0.148	0.102

* Concentrated mixture is composed of barley, wheat barn, soybean meal and corn

anionic diet was affected by clinical hypocalcemia (milk fever). Clinical hypocalcemia was observed in cows fed +80 and +230 DCAD diets respectively. The prevalence of clinical and subclinical hypocalcemia in cows fed anionic diet was significantly lower than those fed cationic diets (P<0.05). The correlation coefficient between DCAD and serum Ca concentration was -0.388 which is a reverse correlation, and shows that as DCAD decreases, the Ca concentration of serum increases significantly (P<0.05) (Fig. 1).

Serum NEFA, BHBA and glucose concentrations in day 12 after calving are shown in Table 2. Serum NEFA concentrations were higher for cows fed the anionic diet than for those fed cationic diets. Prevalence of postpartum NEB was significantly higher in cows fed anionic diet than those fed cationic diets (P<0.05).

There was significant difference in serum BHBA concentrations between cows fed -24 DCAD diet and cows fed +230 DCAD diet (P<0.05), but there was no significant difference between cows received +80 DCAD diet with those fed -24 and +230 DCAD diets (P<0.05). There was no significant difference in serum glucose concentrations between the three groups (P<0.05).

Discussion

Various methods have been suggested to prevent hypocalcemia in dairy cows. One of the most effective of them is the application of diets with negative DCAD during 3 or 4 weeks before calving (Oetzel et al., 1988; Tucker et al., 1991; Moore et al., 2000). Such a diet enhances cow's resistance to hypocalcemia by producing a mild metabolic acidosis which leads to an increase in the activity of PTH (Block, 1984; Moore et al., 2000; Muhammad et al., 2010).

According to the results of this study, the mean total blood Ca concentration in cows received -24, +80 and +230 DCAD diets were 9.42, 8.44 and 7.87 mg/dl respectively. Cows fed anionic diet had higher serum Ca than those fed cationic diets. Also, there was a reverse linear relation between DCAD and serum Ca. As DCAD decreases Ca concentration is significantly increased. These results agree with those of numerous previous works that reported a positive effect of anionic diets on Ca status in pregnant cows (Wang and Beede, 1992; Moore et al., 2000; Hu and Murphy, 2004). Block (1984) reported that cows fed anionic diets had higher Ca concentration in their blood probably caused by enhanced bone resorption of Ca. Oetzel et al. (1988) reported that use of an anionic diet prior to parturition increases the plasma Ca concentration at calving.

Cows were considered to be affected by clinical hypocalcemia (milk fever) when their serum Ca concentration was <5 mg/dl along with recumbency and considered to be affected by subclinical hypocalcemia when their serum Ca concentration was <7.5 mg/dl (without any clinical findings). Prevalence of clinical hypocalcemia in cows being fed with -24, +80 and +230 DCAD diets were 0%, 3.03% and 12.12% respectively. No cow on the anionic diet was affected by clinical hypocalcemia. Also, prevalence of subclinical hypocalcemia was 9.09%, 15.15% and 27.27% for cows that received -24, +80 and +230 DCAD diets respectively. Results shows that cows fed anionic diet had significantly lower incidence of milk fever and subclinical hypocalcemia which agrees with previous reports (Dishington, 1975; Muhammad et al., 2010). Therefore, use of anionic diets 3 weeks before calving will reduce cow's susceptibility to clinical and subclinical hypocalcemia by increase serum Ca concentration at calving. Dishington (1975) succeeded to prevent milk fever by using anionic diets. Block (1984) reported that prevalence of milk fever was 47% in the case of using a diet with DCAD of 330 mEq/Kg DM and 0.00% in the case of using a diet with DCAD of -128 mEq/Kg DM. Crnkic et al. (2010) reported that the prevalence of subclinical hypocalcemia was lower in cows fed with DCAD of -107 mEq/Kg DM than those fed DCAD of +127 mEq/Kg DM.

To investigate the effect of DCAD on the acid-base balance of body, measurement of urinary pH has been suggested (Horst et al., 1997; Hu and Murphy, 2004). For this reason urine samples were collected from 7 cows in each group by perineal stimulation and midstream urine was collected in plastic containers during last week before parturition. Urine pH was immediately measured using urine stripe test. The ideal pH range to prevent dairy cows from hypocalcemia has been reported to be 5.5-6.5 (Horst et al., 1997; Radostits et al., 2007).

The mean urinary pH in cows fed -24, +80 and +230 DCAD diets were 6.35, 7.14 and 7.35 respectively. This result shows that urinary pH for cows fed anionic diet was in suggested spectrum for prevention of hypocalcemia. Hu and Murphy (2004) suggested that increasing of DCAD leads to increase blood bicarbonate and pH and suggested that increasing blood bicarbonate and decreasing urinary secretion of H^+ are the reasons for increasing urinary pH.

Table 2: Comparison between serum concentration (± SD) of Ca, NEFA, BHBA, glucose and negative energy balance with prevalence of clinical and subclinical hypocalcemia between three groups

DCAD (mEq/Kg DM)	Mean Ca (mg/dl)	Mean NEFA (mmol/L)	Mean BHBA (µmol/L)	Mean glucose (mg/dl)	Milk fever (%)	Subclinical hypocalcemia (%)	Negative Energy Balance (%)
DCAD= -24	9.42±1.36	0.55±0.21	648±452	53.09±15.20	0(n=0)	9.09(n=3)	33.33(n=11)
DCAD=+80	8.44±1.21	0.43±0.20	542±261	54.03±18.37	3.03(n=1)	15.15(n=5)	18.18(n=6)
DCAD=+230	7.87±1.89	0.46±0.23	472±209	53.39±14.26	12.12(n=4)	27.27(n=9)	21.21(n=7)

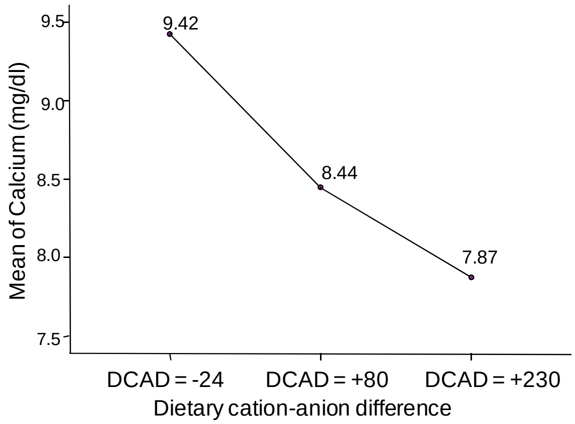

Fig. 1: Correlation between DCAD and serum Ca concentration. (As DCAD decreases, the Ca concentration of serum increases significantly)

In high producing dairy cows, there is often a negative energy balance in the first few weeks of lactation. The DMI does not occur until 8-10 weeks after calving but peak milk production is at 4-6 weeks, and energy intake may not keep up with demand (Radostits et al., 2007). In this situation, using anionic diets in postpartum period (that leads to decrease in DMI) will deteriorate the NEB. Decreasing in DMI due to anionic diets had been shown in different studies (Tucker et al., 1991; Hu and Murphy, 2004). Plasma concentration of NEFA and BHBA (markers of NEB) are the markers of NEB (Drackley, 2001; Garcia et al., 2011). Cows were considered to be affected by postpartum NEB when their serum NEFA concentrations were >0.7 mmol/L (Drackley, 2001; Garcia et al., 2011). Prevalence of NEB in cows fed with -24, +80 and +230 DCAD diets were 33.33%, 18.18% and 21.21% respectively. Also, the mean BHBA in cows fed anionic diet was higher than those fed with cationic diets. There was no significant difference in serum glucose concentrations between three groups.

High prevalence of NEB and also higher concentration of BHBA in cows fed with -24 DCAD diet shows the state of energy balance in this group. This condition can be explained by decrease in DMI due to anionic diets and also metabolic acidosis suggested by many authors (Tucker et al., 1991; Hu and Murphy, 2004). Wang and Beede (1992) reported that increasing DCAD tends to increase DMI by increasing ruminal pH that improves ruminal flora activity. Hu and Murphy (2004) reported that decrease of DMI occurs after using anionic diets because of decreasing diet's palatability. Goff et al. (1991) reported that decrease in diet's palatability is the reason for decrease DMI. More energy imbalance due to anionic diets leads to more mobilization of body fat stores (to produce energy) that increases plasma NEFA concentration and will cause high incidence of related disorders such as ketosis (Drackley, 2001).

Conclusions

According to results of this study, using -24 DCAD diet during 3 weeks before parturition leads to increase in serum Ca concentration, so, these cows seem more resistant to occurrence of milk fever or even subclinical hypocalcemia. On the other hand, the occurrence of NEB in cows fed -24 DCAD diet was higher than cows fed cationic diets. Therefore, feeding the dairy cow with anionic DCAD diets during 3 weeks before calving suggested as an effective method to maintain calcium homeostasis, but the diet should be changed to cationic DCAD immediately after calving because of possible effects on negative energy balance.

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