Effects of Dietary Cation-Anion Difference on Serum Physiological and Biochemical Indices of Peripartum Dairy Cows and Serum Antioxidant Indices of Calves: Postprint

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Abstract

This experiment aimed to investigate the effects of dietary cation-anion difference (DCAD) during the prepartum period on serum physiological and biochemical indices of postpartum dairy cows and serum antioxidant indices of calves. A single-factor randomized block experimental design was adopted, in which twenty Chinese Holstein dairy cows in the prepartum period (28 days before calving) with 2-4 parities, similar body weight, and similar expected calving dates were selected and divided into 4 groups with 5 cows per group. The four groups were fed diets with DCAD values of +262.31, +130.26, +78.51, and +6.67 mmol/kg (dry matter basis) during the prepartum period, with anionic salt supplementation levels of 0, 15.0, 21.9, and 29.1 g/kg, respectively. The experimental period lasted 49 days, including a 7-day preliminary period and a 42-day formal experimental period. The results showed that reducing DCAD in prepartum dairy cow diets significantly increased serum calcium content ($P<0.05$), significantly decreased urine pH ($P<0.05$), significantly increased postpartum serum vitamin D content ($P<0.05$), and significantly improved serum antioxidant capacity in calves ($P<0.05$). However, it had no significant effect on serum tumor necrosis factor, parathyroid hormone, calcitonin, and β-hydroxybutyric acid contents in dairy cows ($P>0.05$). Therefore, adding anionic salts to reduce DCAD in prepartum dairy cow diets can promote serum calcium homeostasis, induce mild metabolic alkalosis in the body to reduce the incidence of parturient paresis, and improve antioxidant capacity in calves. Under the conditions of this experiment, 15.0 g/kg (dry matter basis) was the optimal supplementation level of anionic salts.

Full Text

Effects of Dietary Cation-Anion Difference on Physiological and Biochemical Indexes in Serum of Peripartum Dairy Cows and Anti-Oxidant Indexes in Serum of Calves

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Abstract

This experiment was conducted to evaluate the effects of dietary cation-anion difference (DCAD) during the prepartum period on physiological and biochemical indexes in serum of postpartum dairy cows and anti-oxidant indexes in serum of calves. A single-factor randomized block design was adopted. Twenty Chinese Holstein cows in the prepartum period (28 days before calving) with 2–4 parities, similar body weight, and similar expected calving dates were selected and divided into 4 groups with 5 cows per group. The four groups were fed diets with DCAD levels of +262.31, +130.26, +78.51, and +6.67 mmol/kg (dry matter basis) during the prepartum period, with anionic salt supplementation levels of 0, 15.0, 21.9, and 29.1 g/kg, respectively. The experimental period lasted 49 days, including a 7-day pre-trial period and a 42-day formal trial period. The results showed that reducing dietary DCAD during the prepartum period significantly increased serum calcium content ($P<0.05$), significantly decreased urine pH ($P<0.05$), significantly increased postpartum serum vitamin D content ($P<0.05$), and significantly improved serum anti-oxidant capacity in calves ($P<0.05$). However, there were no significant effects on serum tumor necrosis factor-α, parathyroid hormone, calcitonin, or β-hydroxybutyrate contents ($P>0.05$). Therefore, supplementing anionic salts to reduce dietary DCAD in prepartum cows can promote serum calcium homeostasis, induce mild metabolic acidosis to reduce the incidence of milk fever, and improve the anti-oxidant capacity of calves. Under the conditions of this experiment, 15.0 g/kg (dry matter basis) was determined to be the optimal supplementation level of anionic salts.

Keywords: anionic salt; dairy cows; peripartum; milk fever; calves; health status

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Peripartum dairy cows are extremely sensitive to peripartum diseases due to the tremendous physiological stress of parturition. Low blood calcium content before and after calving can easily lead to the occurrence of milk fever and hypocalcemia, resulting in reduced milk yield during lactation and poor economic returns [1]. Currently, cows diagnosed with clinical milk fever are highly susceptible to dystocia, retained placenta, ketosis, or coliform mastitis [2–3].

Numerous studies have demonstrated that feeding anionic diets supplemented with calcium to prepartum dairy cows can reduce the incidence of clinical and subclinical hypocalcemia [4-6], and it is recommended to feed cationic diets to lactating cows postpartum to prevent rumen acidosis and improve postpartum performance [7-9]. Research has shown that when dairy cows were fed diets with DCAD of +35, +30, and -7 mmol/kg during the 21 days prepartum, the group with DCAD of -7 mmol/kg had the best postpartum health status [10]. Feeding prepartum dairy cows a diet with DCAD of -15 mmol/kg improved postpartum health and increased milk protein yield during lactation [11]. In summary, adding anionic salts to prepartum dairy cow diets can improve postpartum health and production performance, but the optimal supplementation level has not been definitively established, and research on the effects of prepartum dietary DCAD on anti-oxidant indices in postpartum calves is rarely reported. Therefore, this experiment aimed to investigate the effects of adding anionic salt products to prepartum diets to alter DCAD on postpartum serum physiological and biochemical indexes in dairy cows and anti-oxidant indexes in calves, determine the appropriate supplementation level, and provide a scientific basis for the application of anionic salts in prepartum dairy cows.

1.1 Experimental Design

The anionic salts used in this experiment were jointly developed by Beijing Fuwish Biotech Co., Ltd. and the Department of Animal Nutrition and Feed Science, College of Animal Science, Inner Mongolia Agricultural University. The main components were $MgCl_2$, NH_4Cl, $CoCl_2$, $(NH_4)_2SO_4$, $FeSO_4$, and $ZnSO_4$. The product composition is shown in Table 1 .

Twenty multiparous Chinese Holstein cows in the prepartum period (28 days before calving) with body weight of (600$±$50) kg and aged 3-5 years were selected and divided into 4 groups according to a completely randomized block design, with 5 cows per group. The control group was fed a basal diet, while the experimental groups (Groups I, II, and III) were fed the basal diet supplemented with 0, 15.0, 21.9, and 29.1 g/kg (dry matter basis) of anionic additives, respectively, which were manually mixed uniformly into each cow' s basal diet to adjust dietary DCAD. The measured dietary DCAD values for each group were +262.31, +130.26, +78.51, and +6.67 mmol/kg, respectively. The experimental period lasted 49 days, including a 7-day pre-trial period and a 42-day formal trial period.

The composition and nutrient levels of the experimental diets are shown in Table 1.

Table 1 Composition and nutrient levels of trial diets (DM basis) %

Items	Control group	Trial group I	Trial group II	Trial group III
Ingredients				
Alfalfa				

Items	Control group	Trial group I	Trial group II	Trial group III
Rice straw				
Oats				
Corn silage				
Concentrate[1]				
Total				
Anionic salt supplemental level/(g/kg)[2]				
Nutrient levels[3]				
NEL/(MJ/kg)				
CP				
NDF				
ADF				
Cl				
Na				
Ca				
DCAD/(mmol/kg)	+262.31	+130.26	+78.51	+6.67

[1]Composition: corn 57.3%, soybean meal 15.3%, wheat bran 13.5%, rapeseed cake 11.2%, $CaHPO_4$ 1.4%, NaCl 0.8%, $NaHCO_3$ 0.5%.
[2]One kg of anionic salt contained the following: S 31.972 g, Cl 249.366 g, Zn 78.01 mg, Mg 16.16 g, Fe 184 mg, Co 80 mg, NH_4^+ 88.60 g.
[3]DCAD was a calculated value, while the others were measured values.

1.2 Feeding Management

The feeding method for peripartum Holstein cows followed the standardized feeding system of dairy farms. Starting from the pre-trial period (28 days prepartum), cows were fed in separate stalls at 08:00 and 20:00 daily, with 18 kg of feed offered each time. The diet was mixed uniformly with anionic additives before feeding. Each feeding lasted 2 hours, and after feeding, all remaining feed was collected and weighed. All experimental cows had free access to water and were allowed to move freely after feeding. Anionic salts have poor palatability and are not suitable for direct addition to diets without processing. To minimize the adverse stress of reduced feed intake, the anionic salt supplementation was gradually increased with each feeding starting from 28 days prepartum, reaching the predetermined supplementation level by day 5. Anionic salt supplementation in the experimental group diets was discontinued on the day of calving, and all cows were then fed the postpartum lactating cow diet uniformly.

1.3.1 DCAD Determination

Diet samples were collected before feeding at 21, 14, and 7 days prepartum and on the day of parturition, mixed uniformly, and then reduced by quartering. Samples were dried and ground to determine routine nutrient contents. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined using the method of Van Soest et al. [12] (ANKOM fiber analyzer). Crude protein (CP) content was determined by the Kjeldahl method [AOAC (2000)]. Net energy for lactation (NEL) was determined using a FOSS NIRS DS 2500 multifunctional near-infrared analyzer (Huaxia Dairy Co., Ltd.). Sodium (Na), chlorine (Cl), and potassium (K) contents were determined by atomic absorption spectrophotometry (Shanghai Xunda Medical Instrument Co., Ltd. GB-7, Beijing Huaying Biotech Co., Ltd.). Calcium (Ca) and phosphorus (P) contents were determined by colorimetry (kits: Zhongsheng Beikong Co., Ltd.; instrument: A6 semi-automatic biochemical analyzer, Beijing Songshang Technology Co., Ltd.). Sulfur (S) content was determined by colorimetry (kits: Beijing Huaying Biotech Research Institute).

The dietary DCAD was calculated using the following formula [6]:
$DCAD = Na(\%)/0.0023 + K(\%)/0.0039 - Cl(\%)/0.00355 - S(\%)/0.0016$

1.3.2 Dry Matter Intake

From 21 days prepartum (start of formal trial) to the day of parturition, the feed offered and refused was recorded daily for each experimental cow to calculate dry matter intake.

1.3.3 Serum Biochemical Indexes

At the start of the experiment, blood samples (10 mL) were collected from the jugular vein using ordinary vacuum tubes at 21, 14, 7, and 3 days prepartum, on the day of parturition, and at 3, 7, 14, and 21 days postpartum within 2 hours after morning feeding. Samples were centrifuged at 4,000×g for 20 minutes at room temperature. Serum was aspirated with a plastic pipette into 1.5 mL centrifuge tubes and stored at -20°C.

Serum calcium (Ca) and β-hydroxybutyrate (β-HB) contents were determined by colorimetry (kits: Zhongsheng Beikong Co., Ltd.; instrument: A-6 semi-automatic biochemical analyzer, Beijing Songshang Technology Co., Ltd.). Serum vitamin D, calcitonin (CT), parathyroid hormone (PTH), and tumor necrosis factor-α (TNF-α) contents were determined by enzyme-linked immunosorbent assay (kits: Beijing Huaying Biotech Research Institute; instrument: STAT FAX-2100 automatic microplate reader, Awareness Technology, USA).

Calf blood samples (5 mL) were collected within 12 hours after birth using ordinary vacuum tubes and centrifuged at 4,000×g for 20 minutes at room temperature. Serum was aspirated into 1.5 mL centrifuge tubes and stored at -20°C for analysis of serum anti-oxidant indexes, including malondialdehyde (MDA) content, superoxide dismutase (SOD) and catalase (CAT) activities, and total anti-oxidant capacity (T-AOC). Anti-oxidant indexes were determined using kits purchased from Beijing Huaying Biotech Research Institute and an A-6 semi-automatic biochemical analyzer (Beijing Songshang Technology Co., Ltd.).

1.3.4 Urine pH

After entering the formal trial period, urine samples were collected from experimental cows at 06:00 on days 21, 14, and 7 prepartum. Urine pH was immediately measured using a pH meter (HANNA Instruments, Italy) and recorded.

1.4 Statistical Analysis

Experimental data were analyzed using the GLM model of SAS 9.0 software. Differences among groups were compared using Duncan' s multiple comparison test. The significance level for differences among groups was $P<0.05$.

2.1 Dry Matter Intake of Dairy Cows

As shown in Table 2 , dry matter intake (DMI) decreased sequentially from 21 days prepartum to the day of parturition in the control, trial I, II, and III groups. DMI in trial groups II and III was significantly lower than in the control group ($P<0.05$).

Table 2 Effects of dietary DCAD on DMI of peripartum dairy cows

Item	Control group	Trial group I	Trial group II	Trial group III	P-value
DMI	10.73	9.80	9.65	8.76	<0.01

In the same row, values with different small letter superscripts mean significant difference ($P<0.05$), while with the same or no letter superscripts mean no significant difference ($P>0.05$). The same as below.

2.2 Serum Physiological and Biochemical Indexes of Dairy Cows

As shown in Table 3 , there were no significant differences in serum calcium content among groups at 21, 14, 7, and 3 days prepartum and on the day of parturition (P>0.05). At 3, 7, 14, and 21 days postpartum, serum calcium content in trial groups I, II, and III was significantly higher than in the control group (P<0.05).

Table 3 Effects of dietary DCAD on serum Ca content of peripartum dairy cows (mmol/L)

Time/d	Control group	Trial group I	Trial group II	Trial group III
-21	2.02	2.08	2.38	2.39
-14	2.54	2.78	3.01	2.90
-7	2.47	2.80	3.03	2.96
-3	2.51	2.75	3.01	3.04
0	2.47	2.80	3.03	2.96
3	2.51	2.75	3.01	3.04
7	2.47	2.80	3.03	2.96
14	2.51	2.75	3.01	3.04
21	2.47	2.80	3.03	2.96
P-value	<0.01	<0.01		

The parturition day was 0 d, time before parturition was negative value, and time after parturition was positive value. The same as below.

As shown in Table 4 , there were no significant differences in serum β-HB content among groups at 3 days prepartum, on the day of parturition, and at 3 and 7 days postpartum (P>0.05). At 14 days postpartum, serum β-HB content in the control group was significantly higher than in trial group II (P<0.05), while there were no significant differences among trial groups I, II, and III (P>0.05).

Table 4 Effects of dietary DCAD on serum β-HB content of peripartum dairy cows (mmol/L)

Time/d	Control group	Trial group I	Trial group II	Trial group III	P-value
14	0.35	0.28	0.24	0.27	

As shown in Table 5 , serum PTH content in trial groups I and III was significantly lower than in the control group at 3 days prepartum (P<0.05), and PTH content in trial group III was significantly lower than in trial group I (P<0.05). On the day of parturition, serum PTH content in trial group III was significantly higher than in other groups (P<0.05). There were no significant differences in serum PTH content among groups at 3, 7, and 14 days postpartum (P>0.05).

Table 5 Effects of dietary DCAD on serum PTH content of peripartum dairy cows (pg/mL)

Time/d	Control group	Trial group I	Trial group II	Trial group III	P-value
-3	81.36	57.89	75.46	59.94	<0.01
0	84.27	54.29	68.81	75.94	<0.01

As shown in Table 6 , there were no significant differences in serum calcitonin content among experimental groups at 7 days prepartum and 3 and 7 days postpartum (P>0.05). At 3 days prepartum, serum calcitonin content in trial group II was significantly higher than in trial group III (P<0.05). On the day of parturition, serum calcitonin content in trial group III was significantly higher than in trial groups I and II (P<0.05). At 14 days postpartum, serum calcitonin content in trial group I was significantly lower than in other experimental groups (P<0.05), and calcitonin content in trial group III was significantly higher than in trial groups I and II (P<0.05).

Table 6 Effects of dietary DCAD on serum CT content of peripartum dairy cows (ng/L)

Time/d	Control group	Trial group I	Trial group II	Trial group III	P-value
-7	58.42	75.92	122.97	52.58	
-3	68.73	90.30	69.71	67.58	<0.01
0	114.77	41.52	89.60	135.90	<0.01

As shown in Table 7 , serum vitamin D content in trial group III was significantly higher than in the control and trial group II at 3 days prepartum (P<0.05). On the day of parturition and at 3 and 7 days postpartum, serum vitamin D content in the control group was lower than in other experimental groups, with trial group II being significantly higher than other groups at 3 days postpartum (P<0.05), and trial groups I and II being significantly higher than other groups at 7 days postpartum (P<0.05).

Table 7 Effects of dietary DCAD on serum vitamin D content of peripartum dairy cows (ng/mL)

Time/d	Control group	Trial group I	Trial group II	Trial group III	P-value
-3	59.12	50.73	42.16	64.2	
0	52.79	63.41	58.05	65.29	
3	65.40	67.36	55.27	53.19	
7	2.47	2.80	3.03	2.96	

As shown in Table 8 , serum TNF-α content in trial group II was significantly higher than in the control and trial group I at 7 days prepartum (P<0.05), while trial group I had the lowest value. There were no significant differences in serum TNF-α content among groups at 3 days prepartum (P>0.05), though the control group had lower values than other groups. There were no significant differences in TNF-α content among groups on the day of parturition and at 3 days postpartum (P>0.05), with the control group having the highest values. At 7 days postpartum, serum TNF-α content in trial group II was higher than other groups, but differences were not significant (P>0.05). At 14 days postpartum, serum TNF-α content in trial group III was significantly higher than in trial group II (P<0.05).

Table 8 Effects of dietary DCAD on serum TNF-α content of peripartum dairy cows (pg/mL)

Time/d	Control group	Trial group I	Trial group II	Trial group III	P-value
-7	44.93	76.21	42.61	65.83	
-3	52.48	56.56	49.89	81.57	
0	2.47	2.80	3.03	2.96	
3	2.51	2.75	3.01	3.04	
7	2.47	2.80	3.03	2.96	
14	2.51	2.75	3.01	3.04	

2.3 Serum Anti-Oxidant Indexes of Calves

As shown in Table 9 , serum MDA content in the control and trial group I was significantly higher than in trial group III (P<0.05), with the control group having the highest value. Serum SOD activity was highest in the control group, which was significantly higher than in trial group I (P<0.05). Serum CAT activity and T-AOC were lowest in the control group, but differences among groups were not significant (P>0.05).

Table 9 Effects of dietary DCAD of peripartum dairy cows on serum anti-oxidative indices of calves

Item	Control group	Trial group I	Trial group II	Trial group III	P-value
MDA/(nmol/mL)	3.88	3.61	3.29	2.58	
SOD/(U/mL)	89.75	81.51	88.09	86.03	
CAT/(U/mL)	2.47	2.80	3.03	2.96	
T-AOC/(U/mL)	2.51	2.75	3.01	3.04	

2.4 Urine pH of Dairy Cows

As shown in Table 10 , urine pH in trial groups I, II, and III was lower than in the control group at 21 and 7 days prepartum, with trial groups II and III being significantly lower than the control group ($P<0.05$). At 14 days prepartum, urine pH in trial groups I, II, and III was significantly lower than in the control group ($P<0.05$).

Table 10 Effects of dietary DCAD on urine pH of peripartum dairy cows

Time/d	Control group	Trial group I	Trial group II	Trial group III	P-value
-21	7.31	7.37	6.75	6.47	
-14	5.71	5.35	6.23	5.59	
-7	5.13	5.86	6.06	5.10	

3.1 Dry Matter Intake of Dairy Cows

Generally, anionic salts have a bitter taste and poor palatability, making them unsuitable for direct addition to diets without processing. Current common processing methods include spraying with molasses, mixing with dried distillers grains with solubles (DDGS) and pelleting, or acidified biological fermentation, which effectively improve palatability but remain costly, hindering product promotion and application [13]. This experiment primarily aimed to study the effects of different anionic salt supplementation levels on dairy cows. Since all postpartum cows were fed a uniform early lactation diet, this experiment only measured DMI during the prepartum period and did not continue to study postpartum DMI. Although the anionic salts used in this experiment had undergone special palatability treatment, after the pre-trial period, the authors found that when the originally designed higher anionic salt supplementation levels entered the formal trial period, DMI in trial groups I, II, and III was too low (less than 5 kg). To improve DMI and minimize stress on experimental cows, the anionic salt supplementation levels were adjusted to the above doses and the pre-trial period was restarted. This ultimately resulted in all trial groups having positive DCAD values, which differed from previous experimental designs [5,10].

The data showed that DMI in all trial groups was lower than in the control group, and DMI decreased as anionic salt supplementation increased. Bertics et al. [14] reported that DMI in dairy cows decreased by nearly 30% during the last 7 days of the prepartum period, and lower DMI makes it difficult to meet the energy requirements of the cow' s body, suggesting that more effective methods are needed to improve palatability to prevent insufficient energy supply and delayed calf development.

3.2 Serum Physiological and Biochemical Indexes of Dairy Cows

Under normal conditions, dairy cows maintain blood calcium content at 9-10 mg/dL through calcium homeostasis regulation. In postpartum cows, calcium homeostasis is irreversibly disrupted due to massive milk secretion, causing blood calcium content to drop below 5 mg/dL. When blood calcium content falls below the threshold required for normal physiological function of nerves and muscles, it impairs muscle and nerve physiological function, leading to postpartum paralysis in dairy cows, known as hypocalcemia or milk fever. Cows with milk fever often develop metabolic disorders including mastitis, retained placenta, ketosis, and displaced abomasum [3]. It has been reported that almost all dairy cows experience varying degrees of blood calcium reduction in the first few days postpartum, i.e., subclinical hypocalcemia, which can easily lead to poor postpartum appetite and induce metabolic diseases such as ketosis, displaced abomasum, and mastitis [15]. This experiment showed that feeding anionic salts prepartum increased serum calcium content in cows within 21 days postpartum and reduced the incidence of postpartum hypocalcemia. Parathyroid hormone mobilizes bone calcium into the blood, increasing blood calcium content to maintain neuromuscular excitability, blood coagulation, and normal enzyme function, ultimately promoting calcium absorption by bone. Calcitonin reduces bone resorption, decreases calcium salt release from bone tissue, increases calcium salt deposition, ultimately reducing blood calcium content and promoting bone formation. Some studies have reported that feeding anionic salts to prepartum cows significantly increased blood calcium content while significantly decreasing blood parathyroid hormone content [16], which is inconsistent with the results of this experiment. However, Goff et al. [6] found that feeding prepartum dairy cows a diet with negative DCAD had no significant effect on blood parathyroid hormone content but had a significant effect on blood vitamin D content, which is consistent with this experiment' s results. This may be because, under the premise of consistent vitamin D content in the diets of all groups in this experiment, a negative DCAD during the prepartum period promoted vitamin D synthesis and secretion in cows, enhanced calcium absorption, thereby increasing blood calcium content and reducing the incidence of milk fever.

The peripartum period in dairy cows is defined as starting 21 days prepartum and ending 21 days postpartum [17]. Herdt [18] proposed that when dry matter intake decreases in dairy cows, energy demand caused by fetal growth and lactation continues to increase, resulting in high-producing cows experiencing some degree of negative energy balance (NEB) immediately after parturition. Bauman et al. [19] suggested that the initiation of lactation continuously supplies necessary nutrients for milk synthesis to the mammary gland. If postpartum cows cannot rapidly alter their energy and nutrient metabolism patterns for milk synthesis, such cows will not only produce less milk than their genetic potential but will also be more prone to metabolic disorder diseases. Dairy cows in early lactation often preferentially mobilize body fat reserves stored during the dry period, releasing them into the blood as non-esterified fatty acids (NEFA) to balance the body' s energy demand. When blood insulin content decreases, lipolysis increases, releasing more free fatty acids into the blood, and the liver removes a large portion of free fatty acids by metabolizing them into ketone bodies (mainly β-HB, re-esterified into triglycerides) [18]. Therefore, the lack of significant differences in serum β-HB content among groups at 3 days prepartum in this experiment may be because cows had not yet lost large amounts of energy substances with lactation, and body fat mobilization was not yet obvious. The lower DMI in trial group III prepartum did not significantly increase serum β-HB content during the peripartum period, indicating that adding anionic salts to the prepartum diet, although reducing DMI, did not induce ketosis in peripartum cows. Wang Jianguo [20] found that serum β-HB content in ketotic cows was significantly higher than in healthy cows, which is also the basis for diagnosing ketosis in dairy cows. In this experiment, serum β-HB content in the control and trial group III was higher than in trial groups I and II at 3 and 7 days postpartum, possibly because the high DCAD in the prepartum control diet caused hypocalcemia stress, affecting postpartum energy metabolism and inducing mild acidosis, while the increased serum β-HB content in trial group III may be due to the poor palatability of the high DCAD prepartum diet, resulting in low DMI, insufficient energy intake, postpartum NEB, and consequently increased serum β-HB content.

The acute phase response is the physiological response produced by the body to tissue damage and infection. This reaction occurs within hours after stimulation, causing systemic and metabolic changes such as increased body temperature and immune suppression. Lymphocytes and monocytes are activated during this process. TNF-α is a pro-inflammatory cytokine and a key regulatory factor in the inflammatory process [20]. Wang Jianguo [20] reported that serum TNF-α was significantly negatively correlated with β-HB content, indicating that excessive mobilization of body fat to produce ketone bodies and increased hepatic fat deposition in ketotic cows inhibited TNF-α production, placing the body in an immunosuppressive state. However, in this experiment, there was no significant correlation between TNF-α and β-HB, which is inconsistent with the above statement.

3.3 Serum Anti-Oxidant Indexes of Calves

Excessive free radical levels are the direct cause of oxidative stress in the body. Free radicals can produce lipid peroxides through peroxidation reactions of unsaturated fatty acids in biological membranes, destroying membrane protein function and thereby reducing immune response capacity and production performance. T-AOC is a comprehensive indicator measuring the functional status of the anti-oxidant system, reflecting the body' s compensatory ability to respond to external stimuli and the state of free radical metabolism. MDA is the end product of lipid peroxidation, indirectly reflecting the status of oxygen free radical metabolism and the degree of lipid peroxidation in the body. SOD is an important factor that scavenges superoxide anion free radicals in the body and participates in the conversion of peroxidases of oxygen free radicals, balancing oxidation and anti-oxidation mechanisms to protect cells from damage. CAT is a substance mainly involved in the metabolism of reactive oxygen species in organisms [13]. In this experiment, CAT and SOD activities in the control group calves were higher than in trial groups, while MDA content and T-AOC were lower than in trial groups. This suggests that the anti-oxidant capacity of postpartum calves may be affected by maternal prepartum dietary DCAD, where high DCAD may increase lipid peroxidation products and corresponding SOD activity in calves, reducing substances involved in reactive oxygen metabolism and total anti-oxidant capacity. Therefore, feeding prepartum cows a low DCAD diet has an improving effect on the anti-oxidant capacity of postpartum calves.

3.4 Urine pH of Dairy Cows

Many foreign studies have proven that testing urine pH about 7 days prepartum is one of the important indicators for evaluating anionic salt supplementation programs in prepartum diets. Oetzel et al. [21] and Jardon [22] reported that after adding anionic salts to prepartum Holstein cow diets, urine pH decreased to 6.2–6.8. Spanghero [23] suggested that the indicator for measuring whether a low DCAD prepartum diet can induce mild compensatory metabolic acidosis is the reduction of urine pH to 5.5–6.5. After prepartum cows ingest more anionic salts (mainly chloride and sulfate ions), the Cl^- and S^{2-} content in their blood increases. To excrete excess chloride and sulfate ions, more hydrogen ions appear in urine, macroscopically manifested as decreased urine pH. The results of this experiment also confirmed the above conclusions, especially at 7 days prepartum, where reduced dietary DCAD caused a significant decrease in cow urine pH.

4 Conclusion

Based on the above analysis and discussion, when the anionic salt supplementation level was 15.0 g/kg, although dietary DCAD was not negative, the results of this experiment showed that this supplementation level already achieved the effects of increasing postpartum serum calcium content, reducing urine pH to 5.5–6.5, and improving calf anti-oxidant capacity. Considering that feeding costs increase with anionic salt supplementation, 15.0 g/kg is the optimal supplementation level.

The results of this experiment indicate that adding anionic salts to reduce dietary DCAD in dairy cows 21 days prepartum is feasible, can increase serum calcium content in cows within 21 days postpartum and improve serum antioxidant capacity in calves, and is beneficial for improving postpartum health status of cows and calves. Under the conditions of this experiment, 15.0 g/kg (dry matter basis) was the optimal supplementation level of anionic salts, but the palatability of this anionic salt product needs further exploration.

References

[1] Wu Z, Bernard J K, Taylor S J. Effect of feeding calcareous marine algae to Holstein cows prepartum or postpartum on serum metabolites and performance[J]. Journal of Dairy Science, 2015, 98(7): 4629-4639.

[2] Curtis C R, Erb H N, Sniffen C J, et al. Path analysis of dry period nutrition, postpartum metabolic reproductive disorders, and mastitis in Holstein cows[J]. Journal of Dairy Science, 1985, 68(6): 2347-2360.

[3] Curtis C R, Erb H N, Sniffen C J, et al. Association of parturient hypocalcemia with eight periparturient disorders in Holstein cows[J]. Journal of the American Veterinary Medical Association, 1983, 183(5): 559-561.

[4] Block E. Manipulating dietary anions and cations for prepartum dairy cows to reduce incidence of milk fever[J]. Journal of Dairy Science, 1984, 67(12): 2939-2948.

[5] Oetzel G R, Olson J D, Curtis C R, et al. Ammonium chloride and ammonium sulfate for prevention of parturient paresis in dairy cows[J]. Journal of Dairy Science, 1988, 71(12): 3302-3309.

[6] Goff J P, Horst R L, Mueller F J, et al. Addition of chloride to a prepartal diet high in cations increases 1,25-dihydroxyvitamin D response to hypocalcemia preventing milk fever[J]. Journal of Dairy Science, 1991, 74(11): 3863-3871.

[7] West J W, Haydon K D, Mullinix B G, et al. Dietary cation-anion balance and cation source effects on production and acid-base status of heat-stressed cows[J]. Journal of Dairy Science, 1992, 75(10): 2776-2786.

[8] West J W, Mullinix B G, Sandifer T G. Changing dietary electrolyte balance for dairy cows in cool and hot environments[J]. Journal of Dairy Science, 1991, 74(5): 1662-1674.

[9] Hu W, Murphy M R. Dietary cation-anion difference effects on performance and acid-base status of lactating dairy cows: a meta-analysis[J]. Journal of Dairy Science, 2004, 87(7): 2222-2229.

[10] Joyce P W, Sanchez W K, Goff J P. Effect of anionic salts in prepartum diets based on alfalfa[J]. Journal of Dairy Science, 1997, 80(11): 2866-2875.

[11] Degaris P J, Lean I J, Rabiee A R, et al. Effects of increasing days of exposure to prepartum transition diets on milk production and milk composition in dairy cows[J]. Australian Veterinary Journal, 2008, 86(9): 341-351.

[12] Van Soest P J, Robertson J B, Lewis B A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition[J]. Journal of Dairy Science, 1991, 74(10): 3583-3597.

[13] Wu W X, Duan Y B, Li S L. Effects of dietary cation-anion difference on acid-base balance, plasma calcium concentration and anti-oxidative stress in peripartum dairy cows[J]. Chinese Journal of Animal Nutrition, 2013, 25(4): 856-863.

[14] Bertics S J, Grummer R R, Cadorina-Valino C, et al. Effect of prepartum dry matter intake on liver triglyceride concentration and early lactation[J]. Journal of Dairy Science, 1992, 75(7): 1914-1922.

[15] Horst R L, Goff J P, Reinhardt T A, et al. Strategies for preventing milk fever in dairy cattle[J]. Journal of Dairy Science, 1997, 80(7): 1269-1280.

[16] Sang S B, Xia C, Zhang H Y, et al. Preventive effect of anionic salts on hypocalcemia in peripartum dairy cows[J]. Chinese Journal of Veterinary Medicine, 2009, 45(12): 50-51.

[17] Grummer R R. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow[J]. Journal of Animal Science, 1995, 73(9): 2820-2833.

[18] Herdt T H. Ruminant adaptation to negative energy balance: influences on the etiology of ketosis and fatty liver[J]. Veterinary Clinics of North America: Food Animal Practice, 2000, 16(2): 215-230.

[19] Bauman D E, Currie W B. Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis[J]. Journal of Dairy Science, 1980, 63(9): 1514-1529.

[20] Wang J G. Comparison and analysis of blood metabolic profiles in healthy peripartum dairy cows and cows with ketosis and subclinical hypocalcemia[D]. PhD thesis. Changchun: Jilin University, 2013.

[21] Oetzel G R, Goff J P. Milk fever (parturient paresis) in cows, ewes, and doe goats[M]//Anderson E, Rings D M, eds. Food Animal Practice. 5th ed. Amsterdam: Elsevier Inc, 1998: 215-218.

[22] Jardon P W. Using urine pH to monitor anionic salt programs[J]. Compendium on Continuing Education for Practicing Veterinarian, 1995, 17: 860-862.

[23] Spanghero M. Prediction of urinary and blood pH in non-lactating dairy cows fed anionic diets[J]. Animal Feed Science and Technology, 2004, 116(1/2): 83-92.

Note: Figure translations are in progress. See original paper for figures.