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Effects of Anionic Salts on Hypocalcaemia and Ca Homeostasis in Periparturient Dairy Cows in China

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Abstract: This study assessed the effect of anionic salts in the prevention of hypocalcaemia in dairy cows. Fifty multiparous Holstein cows were randomly divided into 5 groups: Control group (C) and 4 treatment (T1-T4) groups. The T groups were fed different doses of anionic salts added to their diets from day 21 pre-calving and The Dietary Cation-Anion Difference (DCAD) was 85 mEq kg^{-1} DM (C), -30 mEq kg^{-1} DM (T1), -80 mEq kg^{-1} DM (T2), -130 mEq kg^{-1} DM (T3) and -180 mEq kg^{-1} DM (T4). Plasma Ca^{2+}, Hydroxyproline (HYP), 1,25-Dihydroxyvitamin D_3 (DHVD), Parathyroid Hormone (PTH) and pH were determined during the transition period. The results revealed that there was a relatively high incidence of hypocalcaemia (80%) in the C group compared with the T groups (35-65%). Furthermore, plasma concentrations of Ca^{2+} and HYP in the T groups were higher than those of the C group at calving ($p<0.05$) and plasma pH value ($p<0.05$) and PTH concentration ($p<0.05$ or $p>0.05$) in the T groups were lower than those of the C group at calving. These results suggest that anionic salts may induce metabolic acidosis and reduce the incidence of hypocalcaemia. Compared to all groups, cows in the T3 group had the lowest incidence of hypocalcaemia and plasma PTH concentrations ($p<0.05$) and the highest plasma Ca ($p<0.05$) and HYP ($p<0.05$) concentrations at calving indicating that cows fed a DCAD diet of -130 meq kg^{-1} DM prior to calving may efficiently activate Ca homeostatic mechanisms. In conclusion, anionic salts prior to calving may enhance Ca homeostasis and reduce the incidence of hypocalcaemia in dairy cows.

Key words: Dairy cows, hypocalcaemia, DCAD, Ca homeostasis, transition period

INTRODUCTION

Milk fever, also known as clinical hypocalcaemia, usually occurs within 24-48 h after calving. At the beginning of lactation, homeostatic mechanisms are activated to meet the increased calcium demands. Increased calcium mobilization from bones and intestinal calcium absorption are required to maintain calcium homeostasis (Goff, 2006, 2008). Calcium homeostasis is regulated by systemic hormones including Parathyroid Hormone (PTH), Calcitonin (CT) and 1,25-Dihydroxy-Vitamin D_3 (DHVD) (Goff *et al*., 1991; Horst *et al*., 1994). Thus, cows may affect clinical hypocalcaemia after calving once the calcium homeostatic mechanisms is unable to meet the increased calcium demands for lactation.

The kidney and bone response to PTH can be impaired in cows affected milk fever as a result of a high positive Dietary Cation-Anion Difference (DCAD) which may be modified by adequate prepartum diets (Block, 1984; Goff *et al*., 1991). The high dietary anions in prepartum diets can successfully reduce the incidence of milk fever during transition period in dairy cows but the poor palatability of the anionic salts reduces both appetite and intake in cows (Goff, 2006; Heron *et al*., 2009; Horst *et al*., 1997). A rumen by-pass technique has been widely used to prevent the degradation of proteins, amino acids, choline and vitamins by ruminal bacteria (Mikolayunas *et al*., 2011). Even though studies have shown positive effects of anionic salts on milk fever no study has evaluated the effects of anionic salts on hypocalcemia in dairy cows during the transition period.

The previous investigations showed that the incidence of hypocalcaemia was >75% in certain dairy farms from the province of Heilongjiang in China which was attributed to high DCAD as a result of high dietary

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intakes of K and low dietary intakes of Cl and S. Therefore, the objectives of this study were to investigate the effect of anionic salts on hypocalcaemia and calcium homeostasis and select the optimum DCAD to prevent hypocalcaemia in periparturient dairy cows.

MATERIALS AND METHODS

Experimental animals: The animals in this study were treated according to the International Guiding Principles for Biomedical Research Involving Animals. Fifty Holstein dairy cows were used in this experiment, the cows were in their 3th-5th lactation. The cows were housed in individual pens bedded with sawdust at a dairy farm in Heilongjiang, China and fed the same diet. The cows were randomly divided into 5 groups with 10 cows per group: Control group (C) which was fed a diet without anionic salts and Treatment groups (T) which were fed the same diet supplemented with different doses of anionic salts (i.e., 12, 16, 22 or 26 g kg^{-1} dry matter) (Beijing Feedstuff Science and Technology Ltd. Company, Beijing, China), corresponding to groups T1-T4, respectively from 21 days prepartum to calving. All animals had *ad libitum* access to water, diets were fed to the groups 3 times daily.

Sample collection and preparation: Dietary samples (200 g) were collected from the prepartum Total Mixed Ration (TMR). The dietary samples were dried, ground, sieved through a 40-60 screen mesh and dried at 65°C until constant weight. The dried samples were allowed to sit at room temperature for 24 h prior to nutrient analyses.

Blood samples were collected on days 21, 14 and 7 precalving, at calving (day 0) and on days 7, 14 and 21 postcalving. Blood samples were collected at 6:00 in the morning from the jugular vein and transferred to tubes containing heparin. Blood was centrifuged at 1,500 g for 10 min within 20 min of collection. Plasma samples were stored at -80°C prior to analysis.

Nutrient analyses: Dietary Energy (NE_l, MJ/kg), Dry Matter (DM%), Crude Fat (CF%), Calcium (Ca%), Phosphorus (P%), sodium (Na, mg kg^{-1}), potassium (K, mg/kg), Chloride (Cl, mg/kg) and sulfur (S, mg/kg) were determined by Chinese GB Methods. DCAD was calculated by the equation:

$$DCAD\ (meq\ kg/DM) = [(Na/23+K/39)-(Cl/35.5+S/16)]$$

The mineral analyses results are shown in Table 1.

Plasma analyses: Plasma Ca^{2+} and HYP (Hydroxyproline) concentrations were determined with commercial kits (Chang Chun Hui Li Bioengineering Ltd. Company, Changchun, China) in a semi-autoanalyzer (ECOM-F 6124, Germany). Plasma PTH concentrations were measured at the Nucleo-Radiology Department of Harbin Medical University in China using a commercial radioimmunoassay (catalog No. 200803-2, Atomic High Technology Limited Company, Beijing, China). Plasma DHVD concentrations were measured using High Performance Liquid Chromatography (HPLC) at the Institute of Product Quality Monitoring in Jilin, China. Plasma pH values were measured on day 7 pre-calving, day 0 and 7 postcalving with pH test paper (Uritest 8A).

Table 1: Mineral composition of the diets

Minerals[1] (DM, %)	C	T1	T2	T3	T4
Na	0.38	0.38	0.38	0.38	0.38
K	0.68	0.68	0.68	0.68	0.68
Cl	0.28	0.39	0.44	0.47	0.51
S	0.28	0.41	0.47	0.50	0.57
DCAD[2] (meq kg^{-1} DM)	85.00	-30.00	-80.00	-130.00	-180.00

[1]Cations and anions DCAD diets of 85 mEq kg^{-1} DM (C); -30 mEq kg^{-1} DM (T1); -80 mEq kg^{-1} DM (T2); -130 mEq kg^{-1} DM (T3) or -180 mEq kg^{-1} DM (T4) prior to calving; [2]DCAD = [(Na/23+K/39)-(Cl/35.5 + S/16)]

Ketosis criteria: Cows were considered to have subclinical hypocalcaemia when plasma Ca^{2+} concentrations were <1.90 mM L^{-1} at any time during the experiment. Cows were considered to be in negative Ca balance when plasma Ca^{2+} concentrations were <2.20 mM L^{-1} at any time during the experiment (Larsen *et al*., 2001).

Statistics analyses: Data were analyzed by ANOVA using SPSS 11 Software. Data in tables are expressed as mean±SD. Values with different lower-case letters are significantly different ($p<0.05$).

RESULTS AND DISCUSSION

The dietary concentrations of Cl and S were lower in the C group than in the T groups. However, DCAD was higher in the C group (85 meq kg^{-1} DM) than in the T groups (-30 to -180 meq kg^{-1} DM).

The incidence of hypocalcaemia in the C group was the highest among all groups during the transition period (Fig. 1). However, the incidence of hypocalcaemia in the T3 group was the lowest at calving. In all groups, the incidence of hypocalcaemia increased prior to calving was the highest at calving and decreased after calving.

Plasma Ca^{2+} concentrations in the C and T groups decreased prior to calving were the lowest at calving and reached normal levels after calving (Table 2). Plasma Ca^{2+} concentrations in the T groups were higher than those in the C group, plasma Ca^{2+} concentrations in the T3 group were the highest at calving among all groups ($p<0.05$). Plasma HYP concentrations showed opposite trends to

plasma Ca^{2+} concentrations. Prior to calving, plasma HYP concentrations in the T3 group were the highest among all groups ($p< 0.05$). In addition, plasma HYP concentrations in the C and T groups increased prior to calving were the highest at calving and decreased after calving. However, plasma PTH concentrations in the T3 group were the lowest among all groups at calving (T3 versus C; $p<0.05$). In addition, plasma DHVD concentrations in the C and T groups decreased prior to calving were the highest at calving and increased after calving. However, plasma DHVD concentrations in the T groups were higher than in the C group, the T3 group had the highest plasma DHVD concentrations ($p>0.05$). As shown in Table 2, plasma pH values in the T groups were lower at calving than at day 7 pre and post-calving ($p<0.05$). Plasma pH values in the C group increased slightly with time and were higher than those in the T groups ($p<0.05$). At calving, plasma pH values of the T4 group were the lowest followed by T3 ($p<0.05$).

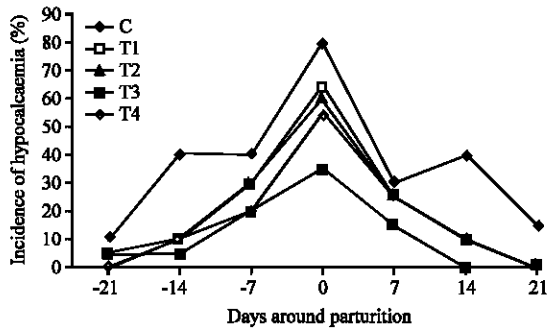

Fig. 1: Incidence of hypocalcaemia in the tested cows during the transition period. Cows fed DCAD diets of 85 mEq kg^{-1} DM (C); -30 mEq kg^{-1} DM (T1); -80 mEq kg^{-1} DM (T2); -130 mEq kg^{-1} DM (T3) or -180 mEq kg^{-1} DM (T4) prior to calving

In this study, there was a significant difference in DCAD among the five groups (85 meq kg^{-1} DM in C, -30 meq kg^{-1} DM in T1, -80 meq kg^{-1} DM in T2, -130 meq kg^{-1} DM in T3 and -180 meq kg^{-1} DM in T4) as a result of the significant difference in dietary Cl and S concentrations (Table 1). High DCAD in prepartum diets is important in the etiology of milk fever and high K or Na concentrations in prepartum diets are more important than high Ca^{2+} concentrations in predisposing cows to milk fever (Heron *et al*., 2009; Horst *et al*., 1997). This study still comfirmed that anionic salts added to prepartum diets with DCAD of -30 to -180 meq kg^{-1} DM may reduce the incidence of hypocalcaemia. Cows fed DCAD of -130 meq kg^{-1} DM had the lowest incidence of hypocalcaemia at calving (Table 2). Thus, the supplementation of anionic salts before calving may become a effective strategy to prevent milk fever during transition period in dairy cows in local dairy farms.

Table 2: Concentration of plasma parameters during the transition period (mean±SD)

		Pre-calving (days)			Calving (days)	Post-calving (days)		
Parameters[1]	Groups[2]	-21	-14	-7	0	7	14	21
Ca^{2+} (mM L^{-1})	C	2.38±0.21	2.19±0.34^{b}	2.21±0.22	1.88±0.34^{c}	2.21±0.23	2.25±0.22^{b}	2.35±0.28
	T1	2.36±0.13	2.33±0.24	2.26±0.23	2.03±0.16^{b}	2.31±0.15	2.33±0.13	2.36±0.21
	T2	2.45±0.13	2.42±0.27	2.17±0.15	2.01±0.17^{b}	2.25±0.23	2.34±0.24	2.38±0.31
	T3	2.33±0.21	2.56±0.17^{a}	2.26±0.15	2.22±0.20^{a}	2.34±0.14	2.59±0.22^{a}	2.42±0.16
	T4	2.59±0.32	2.16±0.16^{b}	2.29±0.22	2.04±0.19^{b}	2.31±0.24	2.41±0.23	2.44±0.22
HYP (ug mL^{-1})	C	2.24±0.16	2.33±0.21	2.41±0.17	2.49±0.27^{b}	2.52±0.13	2.48±0.25	2.43±0.15
	T1	2.23±0.21	2.31±0.15	2.48±0.22	2.61±0.24^{b}	2.55±0.26	2.53±0.18	2.49±0.21
	T2	2.31±0.17	2.43±0.21	2.51±0.18	2.63±0.22^{b}	2.51±0.26	2.49±0.13	2.40±0.12
	T3	2.26±0.18	2.44±0.24	2.65±0.16	2.88±0.24^{a}	2.71±0.21	2.61±0.14	2.55±0.16
	T4	2.20±0.19	2.39±0.16	2.50±0.15	2.66±0.19^{b}	2.50±0.16	2.42±0.15	2.37±0.21
PTH (pmol L^{-1})	C	133±39	164.7±50.3	206±45	268±50^{b}	227±49^{b}	203±52^{b}	188.2±47.5
	T1	130±40	151.6±44.8	181±61	227±49^{b}	175±55	139±48^{a}	142.6±44.8
	T2	142±41	168.2±46.5	195±52	238±52^{b}	188±52	151±55	144.8±47.6
	T3	129±40	161.4±46.7	188±39	216±45^{a}	168±57^{a}	139±46^{a}	136.2±47.3
	T4	129±34	155.6±50.4	211±57	243±51^{b}	190±44	161±39	151.4±37.8
DHVD (ng mL^{-1})	C	19±4	20±6	23±9	23±8	22.8±9	22±9	21±8
	T1	19±5	24±8	26±7	27±9	25.6±8	24±8	22±9
	T2	22±7	25±8	25±8	25±9	24±8	22±9	21±9
	T3	20±8	23±9	24±8	28±8	26±9	24±9	23±8
	T4	18±9	22±8	24±9	25±9	24±9	23±8	22±9
pH	C			7.80±0.25	7.90±0.35^{c}	8.00±0.30		
	T1			7.65±0.35	7.45±0.30^{a}	7.70±0.35		
	T2			7.70±0.30	7.20±0.25^{a}	7.50±0.20		
	T3			7.65±0.25	6.65±0.25^{b}	7.45±0.30		
	T4			7.75±0.30	6.30±0.20^{b}	7.55±0.25		

[1]Concentrations of plasma Ca^{2+}; Hydroxyproline (HYP), Parathyroid Hormone (PTH) and 1,25-Dihydroxyvitamin D_3 (DHVD) in peri-parturient cows fed DCAD diets of 85 mEq kg^{-1} DM (C); -30 mEq kg^{-1} DM (T1), -80 mEq kg^{-1} DM (T2); -130 mEq kg^{-1} DM (T3) or -180 mEq kg^{-1} DM (T4) prior to calving; [2]Data are expressed as mean±SD. Values with different lower-case letters are significantly different ($p<0.05$)

In addition, cows consuming anionic salts had high plasma H^+ concentrations and low plasma and urine HCO_3^- concentrations and pH values (Penner *et al*., 2008; Rerat *et al*., 2009). Table 2 shows that cows fed negative DCAD diets had lower plasma pH values (pH 6.30-7.45) compared to cows fed positive DCAD diets (pH 7.90) at calving. The lower the value of DCAD, the lower the plasma pH was. At calving, plasma pH was lower in the T groups than in the C group. Therefore, it is more important to maintain mild metabolic acidosis of cows before calving using the negative DCAD diets in order to control successfully milk fever.

Furthermore, PTH controls bone osteoclast activity and renal production of DHVD (Taylor *et al*., 2008). There is increasing evidence that diets with low DCAD result in a state of metabolic acidosis in cows, thereby increasing the responsiveness of bones and kidneys to PTH (Kurosaki *et al*., 2007). Compared to the C group, T groups had lower plasma PTH concentrations ($p<0.05$) and higher plasma HYP ($p<0.05$) concentrations, corresponding to higher plasma Ca^{2+} concentrations at calving. This result suggests that bones of cows consuming negative DCAD diets were responsive to PTH which agrees with the results obtained by other researchers (Heron *et al*., 2009). So, cows consuming negative DCAD diets are is in a state of metabolic acidosis that may stimulates Ca mobilization from bone response to PTH.

Finally, the mammary removal of blood calcium results in a minor decline in blood calcium because cows respond to hypocalcaemia by secreting PTH which stimulates the resportion of calcium from bone, the renal calcium reabsorption and the renal production of DHVD necessary for efficient intestinal calcium absorption (Horst *et al*., 1994). In the study, compared to cows fed positive DCAD diets, cows fed negative DCAD diets had a relatively low PTH secretion. As a result, cows fed negative DCAD diets were in positive Ca balance because low plasma pH may activate calcium homeostatic mechanisms to prevent a severe decline in plasma Ca^{2+} concentrations and reduce the incidence of milk fever or hypocalcaemia. Thereby, it is an important mechanism that the negative DCAD may activate Ca homeostasis to prevent milk fever in periparturient cows by inducing a adequate metabolic acidosis that may stimulate bone and kidney response to PTH.

CONCLUSION

In this study, cows fed negative DCAD diets (especially -130 meq kg^{-1} DM) prior to calving reduced incidence of hypocalcaemia as a result of low plasma PTH, high plasma Ca^{2+}, HYP and low plasma pH. Meanwhile, the negative DCAD diets may induce metabolic acidosis and then activate Ca homeostatic mechanisms to prevent milk fever in periparturient dairy cows. Therefore, the results offer a significant evidence that the use of anionic salts prior to calving may effectively control the incidence of milk fever in local dairy farms in China in the future.

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