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EFFECTS OF DIETARY CATION-ANION DIFFERENCES ON SERUM CALCIUM, PHOSPHORUS AND MAGNESIUM CONCENTRATIONS IN PERIPARTURIENT DAIRY COWS

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Key words: veterinary medicine; cows; parturient paresis; dietary cation-anion differences; anionic salts; macro elements in blood

The aim of this study was to investigate the influence of two dietary cation-anion differences (DCAD) on the serum calcium, phosphorus and magnesium of forty dairy cows directly endangered by parturient paresis. The cows were chosen from three farms where, altogether, 736 Friesian dairy cows were bred. They were divided randomly into two groups. The control - cation group (n = 15) received a ration with an average DCAD of 243 ± 132 mEq/kg and the experimental - anion group - (n = 25) received a ration with an average DCAD of -28 ± 136 mEq/kg of DM. We found that when the calcium levels in the sera of the experimental group were compared to those of the control group they were statistically significantly higher (P = 0.0095) two days prior to parturition. They were also higher the day before (P = 0.0007) and two days after (P = 0.0296) parturition, which confirmed the positive effects of an anionic diet on the mechanisms regulating calcium's absorption from the intestine and its mobilization from the bones during the most critical days immediately before and after parturition. The anionic salts supplement had no significant statistical affect on the level of serum phosphorus and magnesium. However, the expected decrease of the phosphorus level that occurs around the parturition period was established in both groups of caws, though the mean values of these blood elements were at an adequate level throughout the experiment. Five cows were affected by parturient paresis: two (13.3%) from the controls and three (12.0 %) from the experimental group. The occurrence of the disease was confirmed by serum analyses which showed that the affected animals had statistically significant lower calcium (P = 0.0332) and phosphorus (P = 0.0411) concentrations in comparison with clinically normal animals. Considering the analyses of the ration and the fact that all the cows developed the disease after parturition, it is our opinion that the major cause for the

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occurrence of the disease in the group fed anionic salts was an insufficient calcium intake after parturition.

VPLIV KATIONSKO-ANIONSKE RAZLIKE OBROKA NA DINAMIKO KALCIJA, ANORGANSKEGA FOSFORJA IN MAGNEZIJA V KRVI MOLZNIC V OBPORODNEM OBDOBJU

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Ključne besede: veterinarska medicina; krave, puerperalna pareza; kationsko-anionska razlika obroka; anionske soli; makro elementi v krvi

V raziskavi smo ugotavljali vplive kationsko-anionskih razlik obroka (DCAD) na vsebnost serumskega kalcija, fosforja in magnezija pri 40 molznicah neposredno ogroženih od poporodne pareze. Krave smo izbrali na treh farmah, kjer redijo 736 molznic črno-bele pasme in jih po naključju razdelili v dve skupini. Kontrolna - kationska skupina 15 krav je dobivala obrok s povprečno DCAD 243 ± 132 mEq/kg, eksperimentalna - anionska skupina 25 krav pa obrok s povprečno DCAD -28 ± 136 mEq/kg suhe snovi obroka. Ugotovili smo, da je bila raven kalcija v krvi molznic eksperimentalne skupine v primerjavi s kontrolno skupino statistično značilno povečana dva (P = 0,0095) in en dan (P = 0,0007) pred porodom ter drugi dan po porodu (P = 0,0296), kar potrjuje pozitivne učinke anionoske diete na mehanizme, ki uravnavajo absorpcijo kalcija iz črevesja in mobilizacijo iz kosti v najbolj kritičnih dnevih pred telitvijo in neposredno po njej. Dodatek anionskih soli pa ni pomembno vplival na raven serumskega anorganskega fosforja in magnezija. Ugotovili smo le poznano obporodno znižanje ravni serumskega fosforja pri kravah v obeh skupinah, sicer pa so bile povprečne vrednosti teh prvin v krvi ves čas poskusa na ustrezni ravni. Za poporodno mrzlico je klinično zbolelo pet krav; in sicer dve (13,3 %) iz kontrolne in tri (12,0 %) iz eksperimentalne skupine. Da so krave obolele za tipično poporodno mrzlico, potrjujejo tudi analize krvnega seruma, kjer smo v primerjavi s klinično zdravimi živalmi ugotovili statistično značilno nižje vrednosti kalcija (P = 0,0332) in anorganskega fosforja (P = 0,0411). Glede na analizo krmnega obroka in na dejstvo, da so vse krave zbolele v poporodnem obdobju, utemeljeno domnevamo, da je bil najpomembnejši vzrok za presenetljiv pojav bolezni tudi v skupini krav, ki je dobila anionske soli, premajhna količina kalcija v obroku po porodu.

Introduction

Both subclinical and clinically apparent parturient pareses may present major economic problems due to the reduction of both the milk production and the life spans of affected high-producing dairy (1, 2, 3). There is also a highly significant relationship between milk fever and dystocia, retained foetal membranes, ketosis, mastitis, displacement of the abomasum and uterine prolapse (4, 5, 6, 7, 8). Dairy cows are relatively sensitive and more susceptible to parturient paresis around the time of parturition. At the beginning of lactation, and in the dry period 30 to 60 days before parturition, oscillations occur in the level of minerals in the blood serum (9, 10, 11, 12). If the homeostatic mechanisms in this stressful period are not effective enough then parturient paresis may develop. Various prophylactic measures may be used in order to prevent the development of hypocalcaemia in the period around parturition. Among them are the feeding of diets with low rates of calcium during the dry period, applying high doses of D_3 vitamin or analogues before parturition and, calcium-gel dosing. However, the most reliable method when the calcium intake is high is the manipulation of the cation-anion difference in the daily ration (13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 32). This method has gained in prominence because of the difficulty in formulating diets low in calcium during the dry period and manipulating the necessary abrupt change from a low to a high calcium diet 1 to 2 days before calving. The recommended values of dietary cation-anion difference (DCAD) are from -100 to -150 mEq/kg of the dry matter (18, 22, 23, 24, 29, 33). Low DCAD values influence the acid-basic balance in a cow's organisms, - the kidneys and bones become more responsive to the parathyroid hormone and consequently the concentrations of calcium, phosphorus and magnesium in the blood change. An excess of acid-forming elements in the daily ration can be achieved, usually through the feeding of chloride and sulphate salts. Unfortunately to avoid the risk of ammonia toxicosis and because of the bitter taste of the salts their intake is limited (25, 26, 27, 28, 29, 31).

The intention of this research was to establish what influence a daily ration with a moderately low DCAD values would have on the concentrations of calcium, inorganic phosphorus and magnesium in the blood of the experimental animals (anion group) compared to the controls, with the aim of preventing the parturient paresis of high-producing dairy cows.

Materials and methods

The research took place in three major farms with a combined total of 736 Holstein cows. For the experiments 40 high-yielding dairy cows, that had previously been stricken with parturient paresis were selected. Their ages ranged from 5 years 3 months to 14 years 4 months, with an average age of 8 years. All the cows were clinically healthy and in good condition. Their average milk production during their last standard lactation was 7,772.7 kg of milk, which contained 4.00 % fat and 3.14% protein. During the dry period right through to the third days after parturition the animals were feed with corn and grass silage, hay and domestically prepared concentrates of ground corn or barley without mineral supplementation. The average daily feed intake was 11.5 kg of grass silage, 15 kg of corn silage, 1.5 kg of hay and 0.8 kg of the concentrates. The experimental cows were divided randomly in two groups. The experimental - anion group consisted of 25 cows and there were 15 cows in the control - cation group. In order to achieve the low dietary cation-anion difference of the experimental cows, a daily oral administration of 100 g of ammonium chloride and 100 g of ammonium sulphate in one liter of water beginning six weeks prior to the expected parturition. The fifteen controls only received rations for dry cows.

During the experimental period venal blood samples were taken once a week for six weeks, than daily for 7 days prior the expect parturition, as well as twelve hours immediately after, and on the second and third days after the parturition. A Cobas Mira automatic biochemical analyzer (Hoffman-La Roche) was used to determine calcium (Ca), inorganic phosphorous (iP) and magnesium (Mg) in the blood sera. Samples of hay, corn silage, grass silage and a corn-grass silage mixture were analyzed twice during the trial. The chemical compositions of the fodder were determined by proximal analyse, the level of calcium, sodium, potassium and magnesium were analyzed by an atomic absorption spectrometer and the phosphorus levels with a molybdovanadate reagent. Analyses on sulphur and chlorine were made using x-ray fluorescence spectrometry. The formula for calculating the dietary cation-anion difference of a diet was follows: DCAD (mEq/kg DM) = [(sodium + potassium + calcium + magnesium) - (chlorine + sulphur + phosphorus)]. This seemed the most appropriate method because al the major acid- and alkali-forming elements were considered. The DCAD program, written in Excel, version 7.0, Windows 95.

The One-Way program from the Statistical Package for the Social Science made the statistical evaluations and ANOVA was used to calculate the significance of differences between the test group of cows and the controls.

Results

The concentrations of the macro elements and the DCAD in the diet of the dry cows are shown in Table 1. The dietary supply, was the same for both groups, was increased significantly in the experimental group with the application of the anionic salts. Comparing the mineral intake of the cows to the requirements reported in literature (39) (calcium 4.4 g, phosphorus 2.2 g, magnesium 1.1 g, potassium 5.1 g, sodium 1.0 g chlorine 1.3 g and sulphur 2.0 g/kg DM), established that the ratio for the dry cows was rather imbalanced and variable. There was a deficiency of sodium and chlorine and the level of calcium because of high contents of corn silage. The phosphorus and sulphur concentrations were adequate, while the levels of potassium and magnesium were higher than required. Because the ration contained an excess of alkaline the DCAD value was high. With the anionic salts supplement the DCAD values in the experimental group decreased to an average of -28 ± 136 mEq/kg of DM, however, the sulphur and non-protein nitrogen levels in the ration did not exceed the critical values of 4 g/kg and 5 g/kg DM.

Table 1: Dry matter intake of cows during the dry period, the concentrations of the macro elements and the DCAD of the diet (mean ± SD, n = 6)

Group	DM kg	Na g	K g	Ca g	Mg g	Cl g	S g	P g	DCAD mEq/kg DM
Control cows	12.0 ±1.2	0.42 ±1.58	11.11 ±3.0	3.51 ±0.66	2.36 ±0.22	0.71 ±0.14	2.01 ±0.28	2.76 ±0.37	243 ±132
Test cows	12.0 ± 1.2	0.42 ± 1.58	11.11 ± 3.70	3.51 ± 0.66	2.36 ± 0.22	6.52 ± 0.36	4.03 ± 0.15	2.76 ± 0.37	-28 ± 136

Table 2 represents the dynamics of the serum calcium in both groups of cows. It shows that decrease in the serum calcium levels occurred in both groups, which was, however, more significant in the controls. Compared to the controls a statistically significant increase in the calcium levels of the experimental group was established two days (P = 0.0095) prior parturition. This was also the case one day (P = 0.0007) before and two days (P = 00.0296) after parturition, which is within the period when parturient paresis most commonly occurs in the majority of cows. According to data available from literature, the reference values for the pre- and postpartum period from 1.75 to 2.25 mmol/l (34, 35). Therefore one day before and after parturition more than 50.0 % of the cows in the control group were hypocalcaemic and during the same period only 8.0 % (before) and 28.0 % hypocalcaemic. Our results showed that the calcium levels of the test cows were fairly stable, and higher than those of the controls during the parturition period.

Table 2: The mean values (x) of the serum calcium (Ca) levels in the control - cation and in the experimental - anion groups, standard deviations (± SD), standard errors (± SE), minimum (Min.) and maximum (Max.) values

	Control group				Experimental group				
Blood samples collection	Ca mmol/l x	SD	SE	Min. - max.	Ca mmol/l x	SD	SE	Min. - max.	P
Before parturition									
6 weeks	2.40	0,.8	0.05	2.12 - 2.75	2.51	0.12	0.02	2.25 - 2.80	
5 weeks	2.41	0.21	0.05	2.00 - 2.87	2.48	0.14	0.03	2.14 - 2.83	
4 weeks	2.38	0.10	0.03	2.18 - 2.56	2.45	0.19	0.04	2.20 - 2.88	
3 weeks	2.33	0.18	0.05	1.89 - 2.67	2.51	0.14	0.03	2.24 - 2.76	
2 weeks	2.38	0.20	0.06	2.14 - 2.77	2.48	0.18	0.04	2.03 - 2.72	
7 days	2.30	0.22	0.08	1.89 - 2.63	2.44	0.22	0.06	2.10 - 2.87	
6 days	2.40	0.13	0.05	2.25 - 2.54	2.33	0.31	0.08	1.43 - 2.65	
5 days	2.28	0.10	0.04	2.15 - 2.42	2.35	0.24	0.06	1.90 - 2.75	
4 days	2.35	0.13	0.04	2.10 - 2.52	2.43	0.22	0.05	1.96 - 2.74	
3 days	2.31	0.21	0.07	1.97 - 2.60	2.42	0.18	0.04	1.99 - 2.70	
2 days	2.17	0.21	0.06	1.84 - 2.59	2.40	0.23	0.05	1.84 - 2.71	0.0095
1day	1.76	0.53	0.14	0.43 - 2.58	2.26	0.30	0.06	1.38 - 2.63	0.0007
After parturition									
12 hours	1.77	0.30	0.08	1.23 - 2.27	1.97	0.36	0.07	1.28 - 2.72	0.0958
2 days	1.81	0.44	0.11	0.86 - 2.33	2.09	0.34	0.07	0.95 - 2.58	0.0296
3 days	1.98	0.36	0.09	0.91 - 2.42	2.15	0.38	0.08	1.10 - 2.91	

Table 3 represents the dynamics of the serum phosphorus levels in the control and experimental group of cows. It shows that in both groups the characteristic in the serum phosphorus levels occurred around the parturition period and that no statistically significant differences between the two groups were noted. Considering that the normal values of serum phosphorus range from 1.61 to 2.25 mmol/l, and that directly before and after parturition they decreased by about 30.0 % (9, 36, 37), we may conclude that the concentrations of inorganic phosphorus in the blood were at adequate levels during the experiment. It also indicates that the test cows' dietary supply of phosphorus was adequate and that the effects of anionic salts on the absorption and metabolism of this element.

Table 3: Serum inorganic phosphorus (iP) level in control - cation and in experimental - anion group - mean values (x), standard deviations (±SD), standard errors (±SE) minimal (Min.) and maximal (Max.) values

Control group					Experimental group				
Blood samples collection	iP mmol/l x	SD	SE	Min. - max.	iP mmol/ x	SD	SE	Min. - max.	P
Before parturition									
6 weeks	2.12	0.47	0.12	1.35 - 3.00	2.00	0.38	0.08	1.42 - 2.75	
5 weeks	2.08	0.53	0.14	1.06 - 2.95	2.01	0.28	0.06	1.59 - 2.59	
4 weeks	1.89	0.38	0.10	1.50 - 2.70	1.91	0.18	0.04	1.65 - 2.26	
3 weeks	2.05	0.58	0.15	1.29 - 3.51	1.93	0.32	0.06	1.36 - 2.54	
2 weeks	1.92	0.33	0.10	1.47 - 2.52	1.91	0.38	0.08	1.03 - 2.64	
7 days	1.93	0.35	0.13	1.62 - 2.62	1.89	0.27	0.07	1.38 - 2.42	
6 days	1.87	0.35	0.14	1.40 - 2.30	2.00	0.71	0.18	1.42 - 4.45	
5 days	1.85	0.53	0.19	0.68 - 2.48	1.79	0.28	0.07	1.43 - 2.32	
4 days	1.95	0.28	0.09	1.65 - 2.39	1.83	0.31	0.07	1.39 - 2.52	
3 days	1.90	0.37	0.12	1.15 - 2.49	1.72	0.20	0.04	1.32 - 2.02	
2 days	1.80	0.38	0.11	1.27 - 2.40	1.78	0.33	0.07	1.23 - 2.29	0.8853
1 day	1.58	0.44	0.11	0.94 - 2.32	1.70	0.38	0.08	0.88 - 2.49	0.3986
After parturition									
12 hours	1.62	0.53	0.14	0.91 - 3.00	1.41	0.34	0.07	0.66 - 2.07	0.1279
2 days	1.75	0.81	0.21	0.59 - 3.72	1.51	0.34	0.07	0.74 - 2.17	0.2052
3 days	1.73	0.61	0.16	0.42 - 2.62	1.53	0.51	0.10	0.51 - 2.64	

Table 4 represents the serum magnesium levels of all the blood samples collected from the control - cation and the experimental - anion groups of cows. Data processing did not reveal any statistically significant differences between, or within, the groups at any stage of blood collection. This shows that the anionic salts used in our experiment did not significantly affect the absorption and metabolism of magnesium. The normal serum magnesium values of mature cows are between 0.69 and 1.23 mmol/l according to the adequate data (9,38). Therefore we may conclude that the animals were given an adequate supply of this element during the experiment and it did not affect the mechanisms which regulate calcium absorption from the intestines and its mobilization from the bones.

Table 4: The mean values (x) of the serum magnesium levels (Mg) in the separate stages of collection in the control - cation and in experimental - anion groups of cows, standard deviations (± SD), standard errors (± SE), minimum (Min.) and maximum (Max.) values

Control group	Experimental group								
Blood samples collection	Mg mmol/l x	SD	SE	Min. - max.	Mg mmol/l X	SD	SE	Min. - max.	P
Before parturition									
6 weeks	0.87	0.10	0.02	0.66 - 1.01	0.96	0.12	0.02	0.62 - 1.12	
5 weeks	0.86	0.13	0.03	0.64 - 1.06	0.96	0.12	0.02	0.66 - 1.15	
4 weeks	0.89	0.08	0.02	0.70 - 0.98	0.94	0.14	0.03	0.70 - 1.20	
3 weeks	0.83	0.11	0.03	0.59 -1.01	0.94	0.19	0.02	0.67 - 1.14	
2 weeks	0.86	0.07	0.02	0.73 - 0.96	0.92	0.13	0.03	0.69 - 1.08	
7 days	0.91	0.09	0.03	0.80 - 1.07	0.93	0.10	0.03	0.68 - 1.09	
6 days	0.92	0.11	0.05	0.78 - 1.07	0.92	0.11	0.03	0.66 - 1.05	
sb1205 days	0.89	0.10	0.04	0.78 - 1.10	0.95	0.17	0.04	0.75 - 1.44	
4 days	0.86	0.12	0.04	0.72 - 1.11	0.92	0.15	0.03	0.69 - 1.19	
intbl3 days	0.86	0.14	0.04	0.73 - 1.16	0.90	0.12	0.03	0.60 - 1.08	
2 days	0.91	0.17	0.05	0.66 - 1.12	0.90	0.16	0.03	0.69 - 1.27	0.8852
palpha1 day	0.91	0.31	0.08	0.12 - 1.56	0.89	0.22	0.04	0.51 - 1.48	0.8174
After parturition									
12 hours	0.95	0.25	0.06	0.48 - 1.43	0.97	0.19	0.04	0.63 - 1.41	0.7670
2 days	0.86	0.21	0.05	0.45 - 1.24	0.87	0.16	0.03	0.61 - 1.27	0.8728
3 days	0.79	0.20	0.05	0.44 - 1.22	0.86	0.15	0.03	0.62 - 1.21	

Five cows developed clinically apparent parturient paresis; two (13.3 %) from the controls and three (12.0 %) from test group. All were recumbent, showing characteristic clinical signs of the disease on the first day after calving. The diagnosis was confirmed by analyses of the blood samples collected twelve hours after parturition (Table 5). It is, however, difficult to explain, why an almost identical percentage of cows from two groups developed the disease, as the experimental group received anionic salts and showed a significantly higher serum calcium level. We have reason to believe that the major cause of the similar incidence rate in both groups was an insufficient level of calcium in the diet immediately following parturition, when the needs for this element dramatically increase.

Table 5: Results of the serum analyses, of blood collected 12 hours after parturition in clinically normal cows and in cows with puerperal paresis (mean values ± SD)

Group of cows	N	Ca/ mmol/l	iP/ mmol/l	Mg/ mmol/l
Healthy	35	1.94 ± 0.32	1.55 ± 0.41	0.98 ± 0.21
Clinically ill	5	1.58 ± 0.42	1.13 ± 0.41	0.86 ± 0.22
		P = 0.0332	P = 0.0411*	P = 0.2545

*<0,05

Discussion

Several formulas for calculating the DCAD of a diet are cited in the available literature (24, 25, 29, 31). In our experiment we used the difference between the sums of sodium, potassium, calcium and magnesium and that of chlorine, sulphur and phosphorus. We are of the opinion that calculations that take into account a greater number of elements more precisely reflect their actual influence on the acid-base balance than calculations that only take into account the difference between the sums of sodium and potassium and chlorine and sulphur. However, it is the second method that has been more frequently used by researchers. According to that calculation the control animals received a ration with a mean DCAD value of 158 mEq/kg and the DCAD value of the experimental group was -126 mEq/kg DM. A comparison between both ways of calculating the DCAD revealed a statistically significant correlation ($r = 0.94236$, $P < 0.0001$).

The effects of DCAD on the serum calcium levels during the parturition period were comparable to those cited in literature. Gaynor et al (17) and Goff et al (23) found that with low DCAD feed the mean serum calcium values ranged from 1.90 to 2.08 mmol/l, while the mean calcium levels produced by high DCAD feed were slightly lower at between 1.64 to 2.07 mmol/l. Seven days before parturition the serum calcium values were 2.27 mmol/l (anion diet) and 2.30 mmol/l (cation diet). One day before parturition the levels of the anionic diet dropped to 2.23 mmol/l and the cation diet dropped to 2.16 mmol/l. One day after parturition the calcium value of the low DCAD diet decreased markedly to 1.74 mmol/l and the high DCAD diet to an even lower of 1.49 mmol/l. However, by the third day after parturition the values had climbed back to 2.21 mmol/l and 1.97 mmol/l respectively. Tucker et al (31) reported that an anionic diet had produced a mean serum calcium value of 4.49 mEq/l and 4.32 mEq/l by using cation diet.

The low DCAD of the diet supplemented with anionic salts did not reduce the rate of clinically apparent parturient paresis as was expected, which was contrary to the results that the majority of other researchers had established. Sanchez and Blauwickel (29) reported that DCAD from - 100 to - 150 mEq/kg of DM in the diet helped prevent parturient paresis because the serum calcium, phosphorus and magnesium that are critical during parturition period are kept stabile and within normal values. Goff et al (25, 31) also came to the conclusion that a DCAD between -100 and -200 mEq/kg in the diet prevented the development of parturient paresis. When Jersey cows fed predominantly cationic diets (+978 mEq/kg) were compared to Jersey cows that received diets high in anions (-228 mEq/kg) the incidence rate of parturient paresis was reduced from 26.0 % to 4.0 %. Other researchers have published similar results (22, 24, 28, 32). The optimal cation-anion ratio for reducing the incidence of parturient paresis was between -100 and -200 mEq/kg of DM. We are of the opinion that the differences between our results and the findings of other researchers are mainly due to the insufficient level of calcium in the diets of our postparturient experimental cows.

Conclusions

The formula [(Na + K + Mg +Ca) - (Cl + S + P)] was used to calculate the DCAD of the rations fed to both the control, whose feed had a DCAD of 243 ± 132 mEq/kg of DM, and the experimental animals, whose rations were supplemented with anionic salts (100 g of ammonium chloride and 100 g of ammonium sulphate) and had a DCAD of -28 ± 136 mEq/kg.

The serum calcium concentrations in the experimental group were, compared to the controls, statistically significantly higher two days (P = 0.0095) prior to parturition. They were also higher the day before (P = 0.0007) and two days after parturition (P = 0.0296), which confirms the positive effects of an anionic diet on the mechanisms calcium's absorption from the intestine and its mobilization from the bones during the most critical days immediately before and after parturition.

The addition of anionic salts did not have a statistically significantl affect on the serum inorganic phosphorus and magnesium concentrations. We established, in both groups of cows, the expected decrease in the inorganic phosphorus concentrations that occurs around the parturition period, though the mean values of these blood elements were at adequate levels throughout the experiment.

Five cows suffered from disease; two (13.3 %) from the controls and three (12.0 %) from test group. The diagnosis of the disease was confirmed by serum analyses which showed that the affected animals had statistically significant lower calcium (P = 0.0332) and phosphorus (P = 0.0411) concentrations compared to clinically normal animals. Considering the ration analysis and the fact that all the cows developed the disease after parturition, we have reason to believe that the major cause for the occurrence of the disease in the group fed anionic salts was an insufficient level of calcium in the postpartum diet.

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