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The Effects of Supplemental Anionic Salts and Probiotic in Prepartum Diets on Milk Production and Quality and Incidence of Milk Fever in Dairy Cows*

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Abstract: The nutritional effects of supplementing the diets of dairy cows during the dry period with anionic salts and probiotic, on some blood parameters, milk production and composition and the development of milk fever, were evaluated in 20 Holstein cattle (>3rd lactation). Diets were composed of a mixture of hay (70%) and commercial concentrate (30%) with and without supplemental anionic salts. These diets were given to cows during the 3 weeks prior to calving. Cows in the probiotic group also received 5g/day probiotic orally during this period. The cation/ anion balances of the diets were determined to be –67.77 and +130.73 meq/kg dry matter in the anionic diets and the control diets, respectively. Blood samples were collected 21, 14 and 7 days prior to calving, at calving and days 1, 2, 7, 14, 21 and 42 postpartum. Milk samples were collected on days 7, 14, 21 and 42 postpartum. Urine samples were collected 3 weeks prior to calving, at calving and at the sixth week postpartum. Dry matter intake amongst the groups was not affected by trials. Serum Ca levels of cows consuming the control diet were found to be significantly lower than those of the other groups on the first day postpartum ($p<0.05$). On the day of calving, serum Mg levels of all animals in all groups were found to be increased and serum Ca (except the anionic group) and P_i levels were decreased. However, serum total protein and albumin levels were not affected during this experiment. The urine pH level of the control group was highest at the parturition ($p<0.05$). Milk production in the anionic salt and probiotic groups was increased during the 2nd week of lactation compared with the other groups ($p<0.05$). Milk composition was not affected significantly during the trial. Milk fever developed in one animal in the anionic salt + probiotic group, and in two animals in the control group.

Key Words: Dairy cow, anionic salt, probiotic, milk fever

Kuru Dönemdeki İneklerde Anyonik Tuz ve Probiyotik Katkılı Rasyonla Beslemenin Süt Verimi ve Kalitesi ile Süt Humması Oluşumu Üzerine Etkisi

Özet: Kuru dönemde anyonik tuz ve probiyotik ilaveli rasyonlarla beslenen 20 baş Siyah alaca inekte (≥3. laktasyon), beslemenin bazı kan parametreleri, süt verimi ve bileşimi ile süt humması oluşumu üzerine etkileri saptanmıştır. İnekler, kuru dönemlerinin son üç haftasında kuru ot (%70) ve anyonik tuz katkılı ya da katkısız ticari konsantre yem (%30) ile beslenmiştir. Bu yemler ineklere gebeliğin son üç haftası süresince verilmiştir. Probiyotik gruplarında her hayvana günlük olarak 5 g probiyotik ağızdan verilmiştir. Rasyon katyon-anyon dengeleri anyonik yem ve kontrol rasyonlarında sırası ile -67,77 ve +130,73 meq/kg KM olarak hesaplanmıştır. Kan örnekleri doğumdan 21,14, 7 gün önce, doğumun olduğu gün ve doğumdan sonra 1., 2., 7., 14., 21. ve 42. günlerde, süt örnekleri doğumdan 7, 14, 21 ve 42 gün sonra ve idrar örnekleri ise doğumdan 3 hafta önce, doğumun olduğu gün ve doğumdan 6 hafta sonra alınmıştır. Anyonik tuz içeren rasyonlar ile diğer gruplar arasında kuru madde tüketimi arasında bir fark bulunmamıştır. Kontrol rasyonu ile beslenen ineklerin serum Ca düzeyleri, doğumdan sonraki ilk günde diğer gruplardan önemli düzeyde düşmüştür ($P<0.05$). Doğumun olduğu gün, tüm deneme gruplarındaki hayvanların serum Mg düzeylerinde bir artış, Ca ve P_i düzeylerinde ise bir azalma saptanmıştır. Bununla birlikte serum total protein ve albumin düzeyleri bu besleme denemeleri ile etkilenmemiştir. Doğumun olduğu gün idrar pH'sı kontrole göre tüm deneme gruplarında istatistiksel olarak önemli oranda düşmüştür ($P<0.05$). Süt verimi anyon ve probiyotik gruplarında laktasyonun 2. haftasında diğer gruplara göre artmıştır ($P<0.05$). Süt bileşenleri ise deneme süresince önemli bir farklılık göstermemiştir. Anyonik tuz + probiyotik verilen grupta bir ve kontrol grubunda iki hayvanda süt humması saptanmıştır.

Anahtar Sözcükler: Süt ineği, anyonik tuz, probiyotik, süt humması

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Introduction

Milk fever is a hypocalcemic disease seen mainly in high production dairy cows soon after parturition and usually associated with the onset of lactation. The incidence of postpartum disorders such as abomasal dystorcia and retention of placenta may increase 3-9 fold as a complication of milk fever in these animals (1). The disease arises as the regulatory mechanisms cannot meet the excess demand of calcium at the onset of lactation. Thus, the NRC (2) recommends Ca and P levels of 0.39% (33-43g /day) and 0.24% (20-26g /day) respectively in the diets of dairy cows in order to stimulate the secretion of parathormone (PTH). Furthermore, there are studies indicating that diets containing calcium at a level of 20 g/day or less during the dry period can provide more protection against milk (3, 4). This approach, however, causes difficulties in practice, since it is considered that during the dry period the diet is mainly composed of hay that contains high levels of Ca. On the other hand, Oetzel et al. (5) have reported that the incidence of milk fever or subclinical hypocalcaemia did not change in Holstein cows receiving daily 53 – 105 g Ca in their diets.

Studies in recent years have shown that diet cation-anion balance in the dry period was more important in preventing milk fever than the diet Ca level (6, 7). There are numerous studies on reducing the incidence of milk fever by altering the diet cation-anion balance in a negative direction (8-12). It is postulated that the optimal diet cation-anion balance should be between –50 and –100 meq/kg to prevent milk fever (13). Beede (14) has reported that providing anionic diets to dairy cows during the dry period results in an increase in the total milk production, better reproductive performance and reduction in the incidence of udder oedema and retention of placenta. However, there are also authors reporting that when compared with cationic diets, anionic diets provide no reduction in the incidence of milk fever (15,16). Tucker et al. (16) have not determined any significant difference in milk production and milk composition between two groups receiving diets with a cation-anion balance of –3 and +9 meq/kg DM during the dry period.

Robinson (17) has reported that dry matter intake was reduced by 15% during the dry period in mature dairy cows during their second and following pregnancies. Accordingly, it was indicated that the incidence of metabolic disorders (ketosis and milk fever) could also increase.

It is reported that under stress conditions the intestinal flora of animals can change (18). In recent years, probiotics have been used in order to regulate the intestinal flora of animals. Probiotics are described as living microbial cultures, which increase animals' performance by regulating the intestinal microbial balance (19). Physiological states such as pregnancy and parturition are causes of stress in animals. The ruminal microflora balance, which is affected by the described stress factors and is due to reduced dry matter consumption, can be corrected by the use of probiotics.

The term probiotic is often used to describe the yeast cultures, enzymes and some fermentation by-products as well as lactic acid bacteria (20). Lactic acid bacteria produce lactic and acetic acids as their metabolisms' end-products.

These organic acids prevent the colonisation of pathogenic bacteria in the intestine by reducing the pH of the intestinal content (20-23). Probiotics prevent the multiplication of bacteria that produce toxic amines and ammonia, thus preventing the accumulation of such substances within the intestines. Therefore, by reducing the inflammation of the intestinal wall that is triggered by these toxins, digestive efficiency is increased. Both the pH reducing effect and the reduction in inflammation of the intestinal wall due to probiotic use can help to increase the Ca absorption from the intestines and thus may help in preventing milk fever.

Another benefit of the use of probiotics is the return of normal appetite in sick animals. This brings up a discussion as to whether the use of probiotics with anionic diets, which adversely affect animals' appetite, may help them to recover their normal appetite.

In this study, the effects of feeding dairy cows with diets supplemented with anionic salts and/or probiotic that contains lactic acid bacteria during the last 3 weeks of the dry period, on some blood parameters, milk production and the composition and the incidence of milk fever were investigated.

Materials and Methods

Animals and experimental design

The trial was carried out in Sarmısaklı State Farm in Lüleburgaz. Holstein cows each weighing approximately 600 kg in at least their third lactation period were divided into four groups (5 cows per group) according to the expected date of calving.

Diets

Cows were either fed a diet consisting of 70% hay and 30% anionic salt supplemented commercial concentrate (Anyon-tek®, Purina) or the same composition without the addition of anionic salts (Table 1). Each cow in the probiotic groups additionally received 5g/day probiotic (Probios®, Pioneer Hi-Bred International) orally. Probios® (Dispersible powder – 180D) consisted of lyophilised *Enterococcus faecium, Lactobacillus acidophilus, L. casei* and *L. plantarum* and their fermentation derivatives. Groups were formed in the following order: 1) Control , 2) Anionic diet, 3) Probiotic and 4) Anionic diet + Probiotic.

Feed analysis

Nutritional content and mineral levels of the hay and the commercial concentrate which were used during the trial were determined according to AOAC's (24) methods in the laboratory of the Animal Nutrition and Nutritional Diseases Department of the University of Istanbul, Faculty of Veterinary Medicine. The titrimetric method was used for Ca, the spectrophotometric method for P_i, the flame photometric method (JENWAY PFP 7) for Na and K, and the ion selective electrode method (WIW, ION-SET) for Cl.

Feeding trials

Animals were allowed outdoors for an hour per day and kept indoors for the rest of the day during the trial period and each animal was fed individually. A mixture of hay and concentrate was given to each animal once a day at 14:00. The dry matter intake of animals was estimated to be 1.8% of their average live weights. The remaining feed mixture was collected at 13:00 on the following day from each animal's tray and daily feed consumption per animal was estimated. Diets were balanced so as to meet the requirements of the NRC (2) for energy and protein for dry cows. Dietary cation-anion balance was estimated using the formula (DCAD) = (Na+K) – (Cl+S) (25). In this way, the values –67.77 meq/kg DM and +130.73 meq/kg DM were estimated for the anionic and control diets respectively. Following parturition, all animals were returned to their regular dairy cow diets. Clean and fresh water was always made available for the animals during the trial.

Blood sample collection and analysis

Blood samples were collected at 8:00 from the jugular vein via evacuated tubes 21, 14 and 7 days prior to calving, at calving and on days 1, 2, 7, 14, 21 and 42 following parturition. The blood was allowed to clot at room temperature and was centrifuged at 3000 RPM for 10 min and the serum was aspirated. Serum samples were kept in plastic tubes at –20ºC until analysis. Blood samples of animals suffering from milk fever were taken immediately before the application of 23% Ca borogluconate preparation intravenously. Ca, P, Mg, total protein and albumin levels in these samples were determined by autoanalyser (CIBA-Corning Express Plus).

Table 1. Nutrient composition, energy level and cation-anion difference of the treatment diets.[a]

	Hay	Concentrate for dry cows	Anionic concentrate	Control diet[b]	Anionic diet[b]
Crude protein	10.62	19.18	18.91	13.19	13.11
Crude fibre	27.00	11.80	11.46	22.44	22.34
Ca	0.70	0.95	1.05	0.78	0.81
P	0.32	0.64	0.67	0.42	0.43
Na	0.04	0.34	0.36	0.13	0.14
K	1.70	1.05	1.00	1.51	1.49
Cl	0.70	0.59	1.80	0.67	1.03
S[c]	0.20	0.20	0.70	0.20	0.35
DCAD[d], meq/kg	132.96	125.52	-536.15	130.73	-67.77
NEL[c], Mcal/kg	1.15	1.86	1.86	1.44	1.43

[a] Percentage of total dietary DM unless otherwise noted
[b] Calculation based on 70:30 (hay: concentrate)
[c] Value calculated on book value
[d] DCAD = (Na+K) – (Cl+S)

Urine sample collection and analysis

Urine samples were collected 3 weeks prior to calving, on the day of calving and one week after parturition at the same time as the blood samples were collected, and pH levels were determined within an hour of collection by using a digital pH meter (WIW, pH 340/ion-set).

Milk sample collection and analysis

Animals were milked twice daily, at 6:00 and at 16:00. Milk samples were collected on days 7, 14, 21 and 42 after parturition. Morning and evening samples were mixed and homogenised, and were stored at –20ºC in containers containing $K_2Cr_2O_7$ as a preservative. Milk yield per animal was also registered during the same period. Dry matter, fat and protein content of the samples were determined by autoanalyser (Milko Scan S 50 Type 7500 Foss Electric- Denmark). The 4% fat corrected milk yield figures were determined by Alpan's formula (26).

Statistical analysis

Changes with time in the parameters Ca, Pi, Mg, total protein and albumin were examined by Repeated Measurement Analysis, using the General Linear Models procedures of the MINITAB Statistical Package (27). Statistical differences amongst the 4 groups of cows were evaluated by ANOVA, followed by the Tukey (HSD) procedure. The level of significance was fixed at 0.05 (28).

Results

No statistical difference was found amongst the groups regarding dry matter intake during the last three weeks of the dry period (Table 2).

Serum Ca, Pi, Mg, total protein and albumin levels are shown in Table 3. Serum Ca levels in the control group were numerically higher than in the other groups at the beginning of the trial, and dropped considerably at parturition. Serum P_i levels were significantly lower in the control group than in the other groups one week prior to calving (P<0.05). Serum Ca concentrations of the anionic diet + probiotic group were found to be significantly lower than in the other groups at parturition (P<0.05). The lowest serum Ca levels were encountered in the control group one day after parturition (P<0.05). However, the serum Ca levels of the anionic diet group were highest on the day of calving.

In all groups, serum P_i concentrations decreased along with the onset of parturition. Furthermore, Mg concentrations were elevated considerably in the anionic diet and slightly in the control and probiotic groups. Statistically, there was no difference in the total protein and albumin values amongst the groups (Table 3).

Urine pH levels were found to be similar in all groups at the beginning of the trial; however, they were significantly lower in all groups except the control group on the date of parturition (P<0.05). Statistically, there was no difference amongst the groups a week after parturition, despite the fact that urine pH levels of animals in the anionic diet group were found to be lower than in the other groups (Table 4).

Milk yield and the milk composition values of the 1st , 2nd, 3rd and 6th week of lactation are given in Table 5. Milk yield and the 4% fat corrected milk yield figures of the anionic diet and probiotic groups were higher than those of the other groups at the 2nd week of lactation (P<0.05). Milk yield figures as of the 6th week of lactation - the end of the trial - were not statistically significant amongst the concerned groups. There was no statistical difference amongst the groups regarding skimmed milk dry matter content (SNF), milk fat and milk protein levels.

Two cows out of five in the control group and one cow in the anionic diet+probiotic group developed milk fever during the trial period. No such cases were

Table 2. Dry matter, Ca and P intake and DCAD means of dairy cows in the control and treatment groups.

	Control Diet	Anionic mixed diet	Probiotic	Anionic mixed diet + probiotic
Dry matter intake, kg/day	10.10	9.90	10.20	10.00
Ca intake, g/day	79.60	80.20	78.80	81.00
P intake, g/day	42.80	42.60	42.20	43.00
DCAD, meq/day	1333.40^a	-670.90^b	1320.40^a	-677.70^b

$^{a, b}$ Means with different superscripts in the same row are significantly different (P<0.05).

Table 3. Mean ± SEM of the serum concentrations of Ca, Pi, Mg, Total Protein and Albumin in serum from cows collected around parturition, n = 5.

	Day relative to parturition																			
	-21		-14		-7		0		1		2		7		14		21		42	
	x	SEM	x	SEM	x	SEM	x	SEM	x	SEM	x	SEM	x	SEM	x	SEM	x	SEM	x	SEM
CONTROL																				
Ca, mg/dl	12,40^{a}	0,28	12,98^{a}	0,58	12,66^{a}	0,36	8,38^{bc}*	0,54	7,96	0,77	11,18	0,95	9,96^{b}	0,38	10,70	0,90	10,54	1,27	10,44	0,48
Pi, mg/dl	6,08	0,52	6,38	0,42	5,90^{b}	0,18	3,94*	0,43	5,32	0,73	6,36	0,70	4,88	0,79	4,46	0,38	4,98	0,56	4,36	0,37
Mg, meq/l	2,22	0,12	2,26	0,19	2,28^{ab}	0,12	2,42	0,04	1,94*	0,09	2,08	0,16	1,56^{b}*	0,10	1,94	0,11	1,86	0,16	2,30	0,22
Total pr,,g/dl	6,40	0,25	6,16	0,20	6,32	0,35	6,32	0,14	6,08	0,24	6,28	0,34	5,94	0,39	6,80	0,14	6,70	0,47	6,78	0,40
Albumin, g/dl	3,78	0,24	3,86	0,17	3,84	0,32	3,78	0,14	3,48	0,27	3,84	0,24	3,76	0,32	3,48	0,24	3,56	0,39	3,54	0,21
ANIONIC DIET																				
Ca, mg/dl	11,24^{ab}	0,29	10,42^{ab}	0,20	10,56^{bc}	0,29	11,50^{a}*	0,26	11,14	0,58	12,54	0,44	12,10a	0,51	12,40	0,65	12,86	0,13	12,20	0,31
Pi, mg/dl	8,14	0,47	7,50	0,46	7,38a	0,40	5,54	0,77	6,44	0,95	6,08	0,66	5,16	0,22	4,82	0,50	5,46	0,27	5,44	0,14
Mg, meq/l	1,88	0,15	1,60*	0,17	1,64b	0,25	2,46	0,09	1,92	0,20	1,78	0,31	2,12^{a}	0,13	2,08	0,18	1,96	0,09	1,84	0,05
Total pr,, g/dl	5,58	0,24	5,18	0,18	5,80	0,24	5,70	0,33	0,11	0,31	5,32	0,31	6,26*	0,58	6,08	0,20	6,66	0,10	6,42	0,25
Albumin, g/dl	4,20	0,14	3,78	0,11	3,86	0,17	3,96	0,45	3,68	0,47	3,92	0,11	3,44	0,19	3,30	0,28	3,54	0,14	3,50	0,16
PROBIOITIC																				
Ca, mg/dl	11,08^{ab}	0,83	11,46^{ab}	1,20	12,08^{ab}	0,42	9,56^{b}*	0,56	10,52	0,94	12,22	0,81	12,24a	0,19	11,98	0,25	11,28	0,67	10,68	1,01
Pi, mg/dl	7,50	0,76	7,58	0,89	7,14^{a}	0,32	3,98*	0,40	4,20	0,48	6,56*	0,60	5,52	0,19	4,90	0,49	5,42	0,26	5,02	0,18
Mg, meq/l	1,88	0,12	1,92	0,17	2,26^{ab}	0,14	2,96	0,30	2,22	0,14	2,04	0,12	1,74^{ab}	0,10	2,08	0,16	1,94	0,12	2,16	0,10
Total pr,, g/dl	5,82	0,66	5,28	0,45	6,46*	0,21	5,32	0,46	6,40	0,39	6,16	0,42	6,46	0,25	6,40	0,30	5,96	0,25	6,26	0,25
Albumin, g/dl	4,02	0,18	3,94	0,17	3,74	0,17	3,74	0,13	3,94	0,19	4,46	0,49	3,56	0,34	3,28	0,09	3,58	0,06	3,58	0,14
ANIONIC+PROB																				
Ca, mg/dl	9,72^{b}	0,30	8,86^{b}	0,54	9,90^{c}	0,56	7,62^{c}*	0,40	9,72	0,88	10,18	0,45	11,18^{ab}	0,71	10,52	0,51	12,88	0,81	12,46	0,19
Pi, mg/dl	7,08	0,50	6,58	0,61	6,90^{ab}	0,26	6,16	0,92	5,88	0,82	6,00	0,59	5,78	0,53	5,86	0,25	6,44	0,30	4,78	0,72
Mg, meq/l	1,72	0,12	2,24	0,18	2,38^{a}	0,15	2,30	0,26	1,84	0,17	1,86	0,13	1,54^{b}	0,15	1,84	0,18	2,32	0,16	2,10	0,13
Total pr,, g/dl	5,40	0,16	5,44	0,34	5,74	0,45	5,68	0,30	5,56	0,15	5,54	0,16	5,74	0,32	5,64	0,53	6,94*	0,16	6,78	0,25
Albumin, g/dl	3,58	0,26	3,68	0,22	3,90	0,25	4,28	0,29	3,88	0,31	3,82	0,21	3,30*	0,24	3,26	0,14	3,32	0,25	3,36	0,16

* A mean with asterisk is significantly different from the former means without asterisk in the same row (p<0,05),

$^{a\text{-}c}$, Means with different superscript for the same data in columns are significantly different (p<0,05),

observed, however, in the anionic diet or probiotic group. All the milk fever cases were diagnosed within the first 24 hours following parturition and a single dose of Ca borogluconate injection was sufficient for the treatment.

Discussion

Statistically, there was no difference amongst the groups regarding the dry matter intake during the last three weeks of the dry period (Table 2). This result supports the reports that the addition of anionic salts (29,30) and yeast cultures (31) to the diet does not significantly change the dry matter intake of animals. However, Vagnoni and Oetzel (32) have reported that feed intake was significantly reduced in animals which received four different diets with DCAD levels of +203, -51, -40 and –63 meq/kg during the last two weeks of the dry period due to the development metabolic acidosis.

Serum Ca (except in the anionic group) and Pi levels were decreased and Mg levels were increased on the day of parturition (Table 3). These results are in line with many other reports (10,33). Milk fever usually develops during the first 48 hours following parturition (34). In this study, serum Ca levels in all the treatment groups were higher (P<0.05) than in the control group on the first day following parturition and continued to be so until the end of the trial, with the exception of the anionic diet + probiotic group on day 14 postpartum. This result supports the findings of similar trials done by Oetzel et al. (5), Tucker et al. (16) and Won et al. (35), using diets that were negatively balanced for their cation-anion levels during the dry period in order to prevent the development of milk fever. Goff et al. (36) have reported that cationic diets reduce the level of PTH, which stimulates the mobilisation of Ca from bone tissues in animals. In contrast, anionic diets increase this level. In

Table 4. Concentration of pH value in urinary of dairy cows in control and experimental groups, n=5.

	Days relative to parturition					
	-21		0		7	
	x	SEM	x	SEM	x	SEM
Control	8.37	0.06	8.16[a]	0.06	8.17	0.08
Anionic diet	8.35	0.06	7.25[c]	0.12	7.89	0.21
Probiotic	8.22	0.02	7.56[bc]	0.03	8.04	0.04
Anionic diet + probiotic	8.27	0.10	7.74[b]	0.15	8.19	0.03

[a-c] Means with different superscripts in the same column are significantly different ($p<0.05$).

Table 5. Milk production and composition of dairy cows in control and experimental groups, n=5.

	Days of lactation							
	7		14		21		42	
	`x	SEM	`x	SEM	`x	SEM	`x	SEM
CONTROL								
Milk, kg/day	19.72	0.95	24.60[b]	0.93	32.20[ab]	1.88	36.60	2.25
4% FCM*, kg/day	20.57	0.90	23.76[bc]	0.82	25.83[b]	1.18	34.44	2.07
SNF[1],%	11.16	0.07	10.63	0.08	10.40	0.12	10.33	0.13
Fat, %	4.29	0.06	3.77	0.05	3.51	0.03	3.61	0.05
Protein, %	3.31	0.02	3.63	0.01	3.34	0.01	3.32	0.01
ANIONIC DIET								
Milk, kg/day	20.60	0.93	27.40[a]	1.50	36.40[a]	4.16	39.20	4.60
4% FCM, kg/day	21.54	0.76	26.48[a]	1.43	29.21[a]	1.28	36.34	2.00
SNF,%	11.32	0.10	10.54	0.10	10.44	0.08	10.43	0.14
Fat, %	4.32	0.07	3.78	0.04	3.57	0.02	3.51	0.03
Protein, %	3.24	0.03	3.54	0.01	3.36	0.01	3.34	0.01
PROBIOTIC								
Milk, kg/day	19.80	0.37	28.00[a]	0.71	34.00[a]	1.52	38.00	3.38
4% FCM, kg/day	20.95	0.41	26.94[a]	0.62	28.73[a]	0.64	35.41	3.00
SNF,%	11.29	0.11	10.44	0.08	10.44	0.11	10.28	0.04
Fat, %	4.39	0.08	3.75	0.02	3.60	0.03	3.55	0.03
Protein, %	3.27	0.02	3.56	0.01	3.35	0.01	3.36	0.01
ANIONIC DIET + PROBIOTIC								
Milk, kg/day	19.10	0.87	22.60[c]	1.29	28.80[b]	1.46	34.00	2.93
4% FCM, kg/day	20.21	0.71	21.63[c]	1.26	23.44[b]	1.03	31.70	2.59
SNF,%	11.49	0.10	10.39	0.15	10.40	0.22	10.30	0.17
Fat, %	4.40	0.07	3.71	0.03	3.52	0.02	3.56	0.03
Protein, %	3.30	0.02	3.59	0.01	3.32	0.01	3.32	0.01

[a-c] Means with different superscripts for the same data in columns are significantly different, $p<0.05$.
* 4% fat corrected milk, Alpan 1994.
[1]SNF: Skimmed milk dry matter content.

the trial it was observed that the reduction in serum Ca levels of animals postpartum in all the treatment groups which received anionic diets was less than in the control group, leading to the opinion that anionic diets provide better absorption of Ca from the intestine (37,38) as well as increasing the mobilisation of Ca from bones. In contrast to this, Gaynor et al. (39) have reported that diets with low-level cation-anion balance applied in the dry period do not increase plasma Ca levels prior to calving. However, the lowest diet DCAD level was +26.5 meq/100g DM in Gaynor's study (39), compared to the anionic diet DCAD level of –67.77 meq/kg DM in this study.

The effect of the Ca levels of diets on the development of milk fever is not linear. When this level is lower than 0.5% or higher than 2.0% in the diet, the risk of milk fever increases (40). Ca levels in all treatment group diets were 0.78-0.81% in the study and therefore the risk of milk fever was low. However, while the incidence of milk fever in the control group was 2/5, there was no such case in the anionic diet group, thus supporting the previous reports that the anionic diets do reduce the incidence of milk fever (7-9,12).

Serum Pi levels were significantly higher ($P<0.05$) in the anionic diet and probiotic groups than in the other groups one week prior to calving (Table 3). This finding can be explained by the mobilisation of P along with Ca from bone tissues since these two minerals are chemically bound in bone tissues.

Oetzel et al. (5) have reported that a diet with low DCAD levels does not significantly affect the plasma Mg level at parturition but reduces it just prior to calving. In this study, the serum Mg levels of the anionic diet group were also numerically lower than those of the other groups one week prior to parturition (Table 3).

It is postulated that the urine pH values 1-2 weeks prior to calving can be used as an indication of a risk of milk fever, especially if the figure is above 8.0 (41). At the beginning of the trial, the urine pH values of all groups were above this figure (Table 4). Approximately 3 weeks later, on the day of calving, the urine pH values of all animals in all treatment groups were significantly lower than those of the control group. However, the correlation between the pH levels and the development of milk fever is an issue that needs further clarification. The ideal pH range proposed for Holstein cattle was 6.0-7.0 by Jardon (41) and 5.5-6.2 by Horst et al. (13). The urine pH of cows which received a diet with an DCAD level of –1185meq/day for 3 weeks was determined to be 6.49 in a trial run by Pherson et al. (42). However, in this trial, the urine pH of animals did not drop below 7.0 despite fact that DCAD levels of the anionic diet and the anionic diet + probiotic groups were –670.9 and –677.7 meq/day respectively (Table 4). In the control and the anionic diet + probiotic groups, in which milk fever cases were reported, the urine pH values were 8.16 and 7.74 respectively on the day of parturition, supporting the view of Jardon (41) that the urine pH values can give an indication of the development of milk fever.

The effect of pushing the diet cation-anion balance in the negative direction by supplementing the diets with anionic salts during the dry period is not only limited to the prevention of milk fever. Animals receiving this type of diet not only produce more milk in the following lactation but also show a better reproductive performance (14,43). In this trial, an increase of around 7.10% was observed in milk production in animals that received the anionic diets, in comparison to the control group, at the 6th week of lactation. However, this increase was not statistically significant ($P>0.05$). The improvement in the milk yield in animals fed on anionic diets may be due to the prevention of secondary diseases as a complication of milk fever. There was no difference in the dry matter content of the skimmed milk, milk protein and fat levels in the anionic diet group, supporting the findings of a similar trial (16).

On the 2nd week of lactation the probiotic or anionic groups showed an increase in milk production compared to the other groups ($P<0.05$). At the end of the trial, the total milk production of the probiotic group was 3.83% higher than the control group but the difference was not found to be statistically significant (Table 5). Studies on the use of probiotics in dairy cattle were mainly conducted by supplementing the diet with yeast cultures (*Saccharomyces cerevisiae*), which resulted in the increase of milk production in the early stages of lactation due to the increase in the total feed intake (44,45). In the present study, it was also the case that the total feed intake of the probiotic group was greater than that of the control group (Table 2). Robinson and Garret (31) have reported in their study that when the animals were fed a probiotic (*Saccharomyces cerevisiae*) supplemented diet, starting 23 days prior to calving and continuing until the 56th day of lactation, dry matter intake, milk production and milk composition values were increased. However, in another study conducted in Turkey on dairy cows, animals were fed a similar yeast supplemented diet, but no difference was found in the milk production and composition values (46). We are unable to explain the fact that during the trial, the anionic diet + probiotic group's milk production was lower than that of all the other groups (Table 5).

No milk fever cases were detected in the group which received an anionic diet, supporting the view that feeding dairy cows anionic diets during the dry period may reduce the incidence of milk fever. On the other hand, the use of probiotic and anionic salts resulted in the reduction of the urine pH values of cows at parturition, and no milk fever cases were reported in these groups. Milk production as of the 2nd week of lactation was increased in the groups which received only anionic salts or probiotic in their diets; however, the milk composition was not affected.

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