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DUPHAFRAL® VIT. D_3 1000 AND KATAN® ANIONIC SALTS FOR THE PREVENTION OF MILK FEVER IN DAIRY COWS

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On three dairy farms with similar dietary treatments the effect of intramuscular (i/m) administration of Duphafral® Vit. D_3 1000 and oral administration of KatAn® anionic salts for the prevention of milk fever (MF) was studied in 30 Black and White cows. The first group of cows (n=10; dietary cation-anion difference (DCAD)=+95.99 mEq/kg DMI) 10 ml of Vitamin D_3 was administered one week before the expected calving. KatAn® group (n=10; DCDA=–99.00 mEq/kg DMI) was orally administered 300 g of anionic salts per day two weeks before parturition. The third group of cows (n=10, DCDA=+95.99 mEq/kg DMI) was the control group. During the experiment we controlled the clinical status of cows and blood samples were taken (2 and 1 week before parturition, and 1, 2 and 7 days postpartum) for the determination of plasma Ca, iP and Mg concentrations. It was established that the best results were achieved by i/m administration of 10 million IU cholecalciferol (vit. D_3) one week before calving. Only one cow in this group was affected by MF the second day after calving. In KatAn® group one cow showed typical MF symptoms the first day after calving and another cow had retained foetal membranes (RFM). In the control group the MF incidence was markedly higher. One cow developed MF, one RFM and one cow showed left-sided displacement of the abomasum (LDA). Because the cows refused to eat silage mixed with KatAn salts, each cow was given the solution (300 ml) daily via a manual drencher. On the basis of the analyses, clinical-laboratory data and time-consuming application of anionic salts, we are of the opinion that the best prophylactic measure for the prevention of MF on small and medium large dairy farms in Slovenia is i/m administration of Duphafral®Vit. D_3 1000.

Key words: transition cows, blood biochemical analysis, milk fever, prevention, anionic salts, vitamin D_3

INTRODUCTION

Many of the parturition complex diseases around and after calving can be traced back to nutrition (Bigras-Paulin and Tremblay, 1998; Ziggers, 2004). Milk fever (MF) is a metabolic disease occurring just before, at, or just after calving in high producing cows, and it may be expressed either as a clinical or subclinical condition. As the milk yield of dairy cows in Slovenia has increased over the past 30 years, so has the overall incidence of metabolic disturbances from around 3% to over 7% (Gašperlin *et al*., 2002). In some of the Holstein Frisian herds up to 30% of the animals have contracted MF (Gregorović *et al*., 1967; Klinkon and Klinkon, 1994). This has resulted in a need to find an effective inexpensive preventive treatment which can be used on farms.

MF aries due to high Ca drain from the blood to milk without a concurrent satisfactory compensatory mechanism of absorption from the intestines and/or bones or reabsorption from the kidneys, to keep the concentration of Ca in the blood within the physiological range. Preventive strategies are aimed at ensuring that the strong homeostatic mechanisms that control blood Ca levels are well prepared. Stimulation of release of parathyroid hormone and the formation of 1,25-$(OH)_2$ D_3 vitamin with the resultant increased absorption of Ca from the bone take at least 24 hours. The timing for preventive procedures is therefore important (Andrews, 2000; Goff, 2004).

About 5-20% of adult cows are unable to maintain plasma Ca and consequently develop severe hypocalcaemia or clinical MF, which requires treatment. MF generally occurs at near calving. Both subclinical and clinical apparent MF may present a major economic problem due to reduction of both milk production and the life span of affected cows (Oetzel, 1988; Ruegg, 1991). MF affects smooth muscle function and has been associated with lowered immune function of the transition cow. Both of these disorders can contribute to dystocia, retained foetal membranes, metritis, displaced abomasum and mastitis. The relationship between the metabolic disorders and other post calving diseases is based on the effects of hypocalcaemia on muscle function of the reproductive tract, digestive tract, and sphincter muscle of the teat end. In many of the farm cases, the cows are not experiencing a single disorder, but the animal's condition is an accumulation of problems that affect animal health, production and reproduction (Gröhn *et al*., 1990; Massey *et al*., 1993; Ziggers, 2004).

The after effects of hypocalcaemia conditions are very costly, illustrating the importance of prevention. Compounding the problem are the ever changing nutritional needs of the cow, her lactation/dry period needs, feed quality changes, and producer personal management practices (Gašperlin *et al*., 2002; Rice, 2005).

When the herd incidence of MF increases to above 5% of high-risk cows (third or later lactations), a specific control program is necessary. Various methods for the control of MF in dairy cows are available. They include dietary management during the pre calving period (Goff and Horst, 1993; Block, 1994), oral administration of calcium gels at the time of parturition (Pherson and Jonsson, 1991) and administration of vitamin D_3 or its metabolites immediately

before parturition to enhance the mobilization of Ca (Seekles *et al*., 1958; Gregorović *et al*., 1967; Yamagishi *et al*., 2005).

The incidence of MF appears to be dependent on the metabolic state of the cow in the dry period. Diets fed to prepartum cows are commonly alkalogenic and have a DCAD of about +50 to +300 ore more mEq/kg on a dry matter basis. Alkalogenic diets tend to cause MF, whereas acidogenic diets tend to prevent it (Oetzel, 1993).

The aim of our research was to verify the prophylactic effects of intramuscular administration Duphafral® Vit. D_3 1000 by injection and oral administration of KatAn® anionic salts on the incidence of manifested MF and with it related diseases.

MATERIAL AND METHODS

Cows and herd management

The research took place on three dairy farms with a total of 124 Black and White (88%) and Simmental (12%) cows. For the experiment 30 high-yielding Black and White dairy cows that had previously been stricken with parturient paresis were selected. Their age ranged from 4 years 3 months to 10 years 3 months, with average 5.10 ± 1.4 years. All cows were clinically healthy and in good condition (mean BCS = 3.33 ± 0.77). For the evaluation of body condition score we used a BCS chart for Holstein dairy cows (Edmonson, *et al*., 1989). For measuring live-weight of cows we used Krauuse VE-BO combi body weight measuring tape. In experimental groups the cows were selected according to the method of random sampling.

The first group of cows (n=10; DCAD=+95.99 mEq/kg DMI) – Duphafral® group – was i/m administered 10 mL (10 million IU D_3) Duphafral® Vit. D_3 1000 (Fort Doge®) one week before expected calving. KatAn® group (n=10; DCAD=–99.00 mEq/kg DMI) started with the oral administration of 300 g KatAn® (Kimtec International) anionic salts per day (6.5% Mg, 7.3% S and 18.2% Cl; DCAD=[(Na+K) – (Cl+S)]=–9800 mEq/kg) two weeks before parturition. Because the cows refused the adding of anion salts to the silage, they were administered daily the solution (300 ml) via a manual drencher. The third group of cows (n=10, DCDA=+95.99 mEq/kg DMI) was used as the control group.

Blood sampling and laboratory analysis

During the experimental period blood samples (v. caudalis mediana) were taken 5 times: 2 and 1 week before parturition, and 1, 2 and 7 days postpartum. A Cobas Mira La Roche automatic biochemical analyzer (Hoffman - La Roche) was used to determinate Ca, iP and Mg in the blood sera.

Experimental protocol

Throughout the periparturient time we checked the clinical status of cows. The observed clinical disturbances were registered in electronic form. A table of clinical-laboratory results was made for this purpose. The Microsoft Excel program was used.

Diet of cows and dietary cation anion difference analysis

During the close-up period right to the first day after parturition cows were fed twice-daily corn and grass silage, hay and concentrates for dry cows with vitamin-mineral supplementation (46.0 g/cow/day). The average daily feed intake was 11.0 kg of grass silage, 14.0 kg of corn silage, 2.0 kg of hay, 1.2 kg of squash corn grains and 0.8 kg mill prepared concentrates for dry cows. Samples of hay, corn silage, and grass silage, squash corn grains, concentrates and vitamin-mineral supplementation were analyzed once during the trial. The chemical composition of the fodder was determined by proximal analysis, calcium, sodium, potassium and magnesium were analyzed by an atomic absorption spectrometer and the phosphorus with molybdovanadate reagent. Analyses on sulfur and chlorine were made by x–ray fluorescence spectrometry. The formula for calculating the dietary cation-anion difference was as follows: DCAD (mEq/kg DMI) = $[(Na^+ + K^+) - (Cl^- + S^{--})]$.

Statistical analysis

The One-Way program analyses of variance from Statistical Package for the Social Science made the statistical evaluations and ANOVA was used to calculate the significance of differences of mean Ca, iP and Mg values within and among groups of cows.

RESULTS

Calculation results of DCAD in dry cows during close-up period

The concentration of DMI macro-elements and the DCAD estimate in feed intake of dry pregnant cows (close-up period) are presented in Table 1. Supplementation with minerals in all three groups was practically the same with the exception of Cl and S, which significantly increased by the addition of anionic salts in KatAn® group of cows. A comparison between the actual supplementation of cows in the close-up period with up to – date recommendations in the literature (Van Saun, 1991; Ziggers, 2004) which are for DMI 10.0 to 13.0 kg, for Ca 7.0 g, for P 3.0 g, for Mg 2.0 g, for Na 1.2 – 1.5 g, for K 10.0 g, for Cl 1.5 g and for S 2.0 g calculated for kg of dry matter basis reveals that the intake of dry cows was in our circumstances rather imbalanced and variable. Quite higher than the requirements were Na, K, and Cl levels. Because of the predominant part of maize silage the Ca level was low and the P concentration at the lowest level of the recommended range. Mg concentration was adequate and in fact it exceeded the recommended value.

According to some researchers the close-up diet (DCAD +95.99 mEq/kg DMI), which was established in the control and Duphafral group was already effective enough for the prevention of MF (Oetzel, 1993).

Adding of anion salts decreased the value of DCAD in KatAn® group of cows to mean –99.00 mEq/kg DMI, S concentration, however, did not exceed the critical value which is 4.0 g/kg DMI (Van Saun, 1991; Ziggers, 2004).

Table 1. Mean DMI/kg, macro-elements' concentrations (g/kg DMI) and calculated DCAD (mEq/kg DMI) in feed of the three trial groups of dry cows (n = 10) in close-up period

Group of cows	DMI	Ca	P	Mg	Na	K	Cl	S	DCAD
Control	14.3	5.25	2.91	2.25	1.87	12.75	6.42	2.11	+95.99
Duphafral® Vit. D_3 1000	14.3	5.25	2.91	2.25	1.87	12.75	6.42	2.11	+95.99
KatAn®	14.6	5.14	2.84	2.20	1.84	12.49	10.02	3.56	–99.00

Clinical observations and laboratory results

All cows in the experiment were clinically observed. We focused on the periparturient period especially on the day of calving and one week following it. Within this critical period for MF development and blood samples were taken. Close attention was paid to disease symptoms associated with dystocia, prolonged calving time, depression, gait stumbling, recumbency and coma. Cows were clinically observed until one month after calving. The signs of retarded foetal membranes, metritis, dislocation of the abomasum and mastitis were recorded. The clinical results and laboratory analyses of affected cows are shown in Table 3 and Table 4.

The analysis of variance of mean values did not show any significant (P0.05) differences among cows with regard to age, milk production, body score condition (BSC), and body weight (Table 2). As statistically significant ($P<0.001$) difference was established only for body weight. The results show that the groups of cows were homogenous and that the hypothetical choice was a good done.

Table 2. Mean values and standard deviations (± SD) for age (year), milk production (kg), body score condition (BCS), body weight (kg) and P values calculated with the analyses of variance among groups

Group of cows	n	Age	Milk production	BCS	Bodyweight
Control	10	4.70 ± 1.476	8.870 ± 891.6	3.10 ± 0.56	578.20 ± 27.30
Duphafral® Vit. D_3	10	5.20 ± 1.647	8.430 ± 835.4	3.40 ± 0.69	623.25 ± 57.44
KatAn®	10	5.40 ± 1.160	8.882 ± 813.5	3.50 ± 0.85	631.82 ± 32.50
Total	30	5.10 ± 1.423	8.677 ± 836.9	3.33 ± 0.77	611.09 ± 41.74
P values		0.562	0.595	0.467	0.034

In our experiment three cows, one from each group, were affected by MF. All cows with MF were recumbent and showed characteristic clinical signs of the disease within 48 hours after calving. The results of the blood analyses taken immediately before therapy also confirmed that cows were affected by typical MF (Table 4).

Table 3. Milk fever (MF) incidence and related diseases retained foetal membranes (RFM) and left-side displaced of the abomasum (LDA) in three groups of cows

Group of cows	Healthy	MF	RFM	LDA	Total
Control	7 (70%)	1 (10%)	1 (10%)	1 (10%)	10 (100%)
Duphafral® Vit. D_3 1000	9 (90%)	1 (10%)	–	–	10 (100%)
KatAn®	8 (90%)	1 (10%)	1 (10%)	–	10 (100%)

From Table 3 it is evident that the incidence of postpartum diseases related to MF, i.e. postpartal hypocalcaemia, was highest in the control group of cows. In this group three cows were affected: one by MF, the second had retained foetal membranes and the third left-side displacement of the abomasum. In KatAn® group one cow had MF and another cow retained foetal membranes. The analyses of blood serum confirmed that cows were affected by typical signs of MF and related diseases, as shown by low Ca and iP values compared to clinically normal cows. The unexpected occurrence of the disease is to our belief associated with deficient Ca concentration in the feed intake after calving when the normal requirements for this essential element are suddenly elevated. During the close-up period the feed intake is often reduced. The most severe depression in feed intake takes place just before or at calving when the cow needs energy and especially Ca to expel the foetus and increases colostrum production (Bertics *et al*., 1992; Grummer, 1995).

These results obtained in an objective study of homogeneous groups of cows clearly demonstrate that the i/m administrated Duphafral® Vit. D_3 1000 one week before calving is a very effective and economical preventive method for MF and accompanying diseases.

Dynamics of mean Ca, iP and Mg concentrations in the blood of cows and P values obtained via analysis of variance

Tables 5, 6, and 7 represent the results of analyses of variance of mean Ca, iP and Mg plasma concentrations during the periparturient period of three trial groups with regard to timing of blood sampling. Because only one cow from each group was stricken with MF, the results of biochemical analyses of blood from these animals are not taken into account in the tables. The expected day of calving was calculated according to the tables; however, it did not work out with all cows. Thus, six cows calved a bit too early and therefore certain samples are missing in Table 5, 6, and 7. Table 5 presents the dynamics of mean serum Ca values for all five blood samplings in all three trial groups. It is evident that in all three groups Ca level significantly ($P<0.05$) decreased. The decrease was highest in KatAn® group. According to the standard for the period immediately before calving and after it, which in the literature is between 1.75 and 2.25 mmol/L, all cows in our experiment showed Ca level to be within these limits (Jazbec *et al*., 1970; Pherson and Jonsson, 1991; Goff and Horst, 1993). Low hypocalcaemia (Table 5) in cows did not cause the development of clinical signs of MF.

Table 4. Hemato-biochemical* analysis of six cows affected by milk fever (MF), retained foetal membranes (RFM) and left-side displacement of the abomasum (LDA)

Group of cows	Name of the cow	Diagnosis	Ca mmol/L	iP mmol/L	Mg mmol/L	Na mmol/L	K mmol/L	AST U/L	ALT U/L	GGT U/L	CK U/L	E $x10^{12}$/L	L $x10^9$/L
Control	Meli	MF	1.34	0.83	1.48	149	5.94	59	10	11	198	6.69	3.4
	Živa	RFM	2.15	1.79	0.99	143	6.34	63	12	16	92	6.14	8.0
	Una	LDA	2.34	1.49	0.90	144	5.10	56	9	31	56	5.56	8.1
Duphafral® Vit.$D_3$1000	Muta	MF	1.47	0.71	1.37	147	5.97	55	13	19	332	6.66	7.0
KatAn®	Švica	MF	1.65	1.40	1.25	154	6.46	86	16	17	1538	7.19	10.6
	Rika	RFM	2.13	1.84	1.00	145	6.01	55	14	13	87	6.23	9.2

*Blood samples were drawn just about making diagnosis; MF = 1 or 2 days after calving, RFM = 24 hours after calving, LDA = 1 week after calving

Table 5. Dynamics of mean (x) and ±SD serum Ca (mmol/L) levels during puerperal period in three differently treated groups of cows and via analysis of variance obtained P-values within and among groups of cows

Sampling time*	Control		Duphafral® Vit. D_3		KatAn®		P-value among groups
	n	x ± SD	n	x ± SD	n	x ± SD	
– 2	9	2.28 ± 0.25	9	2.32 ± 0.26	9	2.32 ± 0.27	
– 1	6	2.55 ± 0.28	8	2.55 ± 0.27	7	2.46 ± 0.23	
0	9	2.15 ± 0.24	9	2.00 ± 0.34	9	1.95 ± 0.36	
+ 1	9	2.18 ± 0.31	9	2.20 ± 0.39	9	2.06 ± 0.43	
+ 2	9	2.41 ± 0.13	9	2.51 ± 0.26	9	2.47 ± 0.33	
P-value		0.019		0.004		0.006	0.534

*–2=14 day before calving; –1= 1 week before calving; 0 = immediately after calving; +1 = one day after calving; +2 = 1 week after calving

The statistical model for the calculation of multiple analysis of variance of mean Ca levels among groups of cows did not show a significant difference (P=0.543), namely the dynamics of Ca level in the blood during the experiment was not significantly affected by D_3 administration.

Table 6. Dynamics of mean (x) and ± SD serum iP (mmol/L) levels during puerperal period in three differently treated groups of cows and via analysis of variance obtained P-values within and among groups of cows

Sampling time*	Control		Duphafral® Vit. D_3		KatAn®		P-value among groups
	n	x ± SD	n	x ± SD	n	x ± SD	
– 2	9	2.24 ± 0.41	9	2.23 ± 0.17	9	2.21 ± 0.29	
– 1	6	2.15 ± 0.37	8	2.60 ± 1.29	7	2.36 ± 0.17	
0	9	1.66 ± 0.23	9	1.94 ± 0.64	9	1.57 ± 0.40	
+ 1	9	1.64 ± 0.25	9	2.34 ± 0.42	9	1.51 ± 0.46	
+ 2	9	1.77 ± 0.24	9	2.63 ± 0.83	9	2.39 ± 0.45	
P-value		0.000		0.315		0.000	0.000

*-2=14 day before calving; -1= 1 week before calving; 0 = immediately after calving; +1 = one day after calving; +2 = 1 week after calving

The dynamics of mean iP values in all three groups of cows is shown in Table 6. It is evident from the table that in the control group and KatAn® group of cows a statistically significant (P.001) post calving decrease of serum iP occurred. In Duphafral® group there was no such decrease (P>0.05). If we take into account that normal values of iP range from 1.61 to 2.25 mmol/L and that they can decrease directly before and after calving by 30% (Jazbec *et al*., 1970) we can conclude that mean iP values were during the experiment at an adequate level

especially in the group of cows treated with vitamin D_3 injection. Results showed that supplementing the cows with iP was adequate (Table 1). The impact of anion diet on resorption and metabolism of this element was however slight (Table 7).

Table 7. Dynamics of mean (x) and ±SD serum Mg (mmol/L) levels during puerperal period in three differently treated groups of cows and via analysis of variance obtained P-values within and among groups of cows

Sampling time*	Control		Duphafral® Vit. D_3		KatAn®		P-value among groups
	n	x ± SD	n	x ± SD	n	x ± SD	
– 2	9	0.95 ± 0.11	9	1.09 ± 0.06	9	1.09 ± 0.13	
– 1	6	1.01 ± 0.08	8	1.03 ± 0.06	7	1.05 ± 0.07	
0	9	1.08 ± 0.26	9	1.15 ± 0.21	9	1.23 ± 0.11	
+ 1	9	1.02 ± 0.27	9	1.03 ± 0.26	9	1.19 ± 0.13	
+ 2	9	0.92 ± 0.15	9	1.03 ± 0.13	9	1.06 ± 0.07	
P-value		0.473		0.490		0.002	0.001

*–2=14 day before calving; –1= 1 week before calving; 0 = immediately after calving; +1 = one day after calving; +2 = 1 week after calving

The statistical model for calculation of multiple analysis of variance of mean iP levels among the groups of cows showed a significant difference (P=0.001), which means that the dynamics of iP level in blood was significantly affected by administering vitamin D.

In Table 7 mean serum Mg level in blood samples is presented according to timing of sampling. Data processing revealed that only in KatAn® group there was a statistically significant difference which confirmed that the anionic diet in our experiment affected the absorption and metabolism of Mg as the mean levels significantly (P<0.01) increased.

Calculation of multiple analyses of variance of mean Mg value among groups of cows showed a statistically significant (P=0.001) difference, so, the dynamics of serum Mg level was significantly affected by the anion diet.

Considering literature data on normal serum Mg values which are from 0.69 and 1.23 mmol/L we can conclude that supplementation of cows with this element was throughout the experiment at an adequate level and did not affect the mechanisms that balance the absorption of Mg from the intestines and mobilization from bones (Oetzel, 1993).

DISCUSSION

Balancing the cation-anions in the diet is a relatively new method to prevent MF, to improve health and production. Alkalogenic diets (>+200 mEq/kg DMI) tend to cause MF, whereas acidogenic diets tend to prevent it (Dishington, 1975; Oetzel, 1993; Block, 1994). In the literature various approaches for the calculation

of DCAD in feed intake are described (Goff, 1992; Tucker *et al*., 1992; Sanchez and Blauwickel, 1994). Most authors in their research work calculated DCAD on the basis of the difference among the summing up of Na, K, Cl, and S quantities so we used this formula, as well. According to this calculation cows from the control group and cows from the group that received the vitamin D_3 injection were fed a diet with mean DCAD +95.99 mEq/kg DMI, and KatAn group mean –99.00 mEq/kg DMI. Researches report that increased anionic diet contributes only to a lower incidence of clinical form of MF (Ender *et al*., 1962; Dishington and Bjørnstad, 1982; Block, 1984), while the others claim that the anionic diet affected also the degree of Ca level in the blood of cows during the puerperal period (Vagg and Payne, 1970; Gaynor *et al*., 1989; LeClerc and Block, 1989). It turned out that the processes of retention, absorption, and balancing of Ca, iP, 1,25-dyhidroxyvitamin D_3 and parathyroid hormone concentration during the puerperal phase of cows were more expressed when pregnant cows were on anionic diet not withstanding an apparent calcinuria (Verdaris and Evans, 1976; Lomba *et al*., 1978; Oetzel *et al*., 1991). Thus the anion diet protected the cows from pathological processes of hypocalcaemia because the 1,25-dyhidroxyvitamin D_3 level in the blood increased.

In our case the control group and the group of cows which was 7 days before the expected calving administered i/m Duphafral® Vit. D_3 1000, received the same feed and thus the same DCAD +95.99 mEq/kg DMI. The results of previous research works show that feed intakes with DCAD values between +50 to +300 and more mEq/kg DMI generally increase the risk for MF (Block, 1984; Oetzel, 1993; Block, 1994). Many nutritionists are of the opinion that for the prevention of MF is necessary to add anions salts only when DCAD is higher that +200 mEq/kg DMI (Oetzel, 1993; Block, 1994; Rajčevič *et al*, 1999). In our case the addition of 300 g/day of anion salt KatAn® caused the change of DCAD value of feed intake to –99.00 mEq/kg DMI. Sanchez and Blauwickel (1994) claim that only values of DCAD in the feed intake of dry cows between –100 to –150 mEq/kg DMI contribute to successful MF prevention. Goff, 1992; Goff and Horst (1993) reached a similar conclusion, namely, only values between –100 and –200 mEq/kg DMI efficiently prevent MF.

Several blood analyses in cows with MF were done (Kronfeld, 1971; Ramberg *et al*., 1984; Oetzel, 2000). It was established that serum Ca concentration decreased below 2.0 mmol/L, usually below 1.2 mmol/L and sometimes below 0.5 mmol/L. The clinical picture of MF is sometimes to a certain degree associated with the concentration of the Ca measured in the serum, however this is not to be taken for granted. It is important to know that it is a case of physiological occurrence because serum Ca concentration is immediately after calving often decreased below the critical level, which is from 1.75 to 2.25 mmol/L. The line between subclinical hypocalcaemia and clinical form of MF has not been clearly defined yet. Larsen, *et al*. (2001) report that characteristic clinical signs (muscle weakness, depression of the cardiovascular system, hypothermia, recumbency, depression of consciousness) begin to show when Ca concentration in the blood is decreased below 1.60 mmol/l. Jazbec *et al*. (1970) report that 84% of cows (n = 120) with the clinical form of MF had in the blood

≤1.84 mmol/L Ca, 75% of cows ≤1.13 mmol/L iP and 70% of cows >0.82 mmol/L Mg. Serum iP is usually low, values are between 0.48 to 0.97 mmol/L. Besides hypocalcaemia we can often find concurrent hypophosphataemia. Serum Mg usually slightly increases immediately after calving from 1.65 to 2.06 mmol/L (Jazbec *et al*., 1971; Kronfeld, 1971).

Serum Ca

As it is evident from Table 5 the injection of vitamin D_3 and supplementation of KatAn® salt to dry cows did not significantly affect the dynamics of serum Ca, as no significant ($P>0.05$) differences were displayed among groups of cows. The dynamics of Ca concentration during the immediate post calving period was significantly ($P<0.05$) affected by the timing of blood sampling. According to our hypothesis we assumed that the differences in dynamics of serum Ca among the groups of cows would occur. We expected that vitamin D_3 application and supplementation of anion salts would to a certain degree stop the postpartal drop of serum Ca, and our expectations were realized. Mean Ca values were in all three groups on the day of calving, and one day after it, above the low physiological level 1.75 mmol/L. The established low hypocalcaemia was clinically not expressed to the extent that cows would show recumbency after calving. Serum Ca level directly before and after calving decreases between 1.76 and 2.25 mmol/L (Jazbec *et al*., 1970; Pherson and Jonsson, 1991; Zepperitz *et al*., 1994).

Many researchers report that one i/m dose of 10 million IU vitamin D_3 administered 2 to 8 days before calving was an efficient prevention against MF (Jazbec *et al*., 1970; Bar *et al*., 1980, 1985). However, more exact studies revealed that the application of one million units of vitamin D_3/45 kg B.W. produced the best preventive results (Seekles *et al*., 1958; Jazbec *et al*., 1970; Reinhardt and Conrad, 1980). In our experiment we did not take into account the recommended dose. The cows were administered s.c. the lump dose of vitamin D_3 which according to the opinion of researchers guarantees an effective prevention of above 80% (Gürtler *et al*., 1977; Bar *et al*., 1985). A lump dose of 10 million IU Vit. D_3 is recommended also by the producer Fort Dodge. Maybe we can seek here the answer why in our experiment the i/m application of vitamin D_3 in a dose 10 million IU did not yield full prevention and that one cow from this group was affected by MF (Table 3 and Table 4).

From Table 5 it is evident that during the puerperal period Ca level in Duphafral® and KatAn® groups did not drop below the physiological level (1.76 mmol/L). Below this concentration the initial signs of hypocalcaemia cardiovascular, muscular and neurological signs of MF began to show. All such cows displayed progressive muscle weakness, hypothermia and disturbed consciousness. In experimental hypocalcaemia blood flow is reduced by about 60% to all tissues except kidney, heart, lung and bladder in which the reduction is not as high. During periods of prolonged hypocalcaemia in cows, blood flow at skeletal muscles and the alimentary tract may be reduced to 60–70 % of normal for a long period (Barzanji and Daniel, 1987, 1988).

In the published literature findings are somewhat different compared to ours. The positive affect of anion diet (Cl and S), and the i/m application of vitamin D_3 respectively, were in most cases more effective compared to our experiment (Gregorović *et al*., 1975; Goff *et al*., 1986; Sanchez and Blauwickel, 1989).

Serum iP

Our results show that mean iP concentration in the control group was slightly higher compared to KatAn® group yet still slightly above the physiological level (1.61 mmol/L). We assumed the mean serum iP to be higher in cows on anion diet compared to cows in the control group. However, the concentrations were even slightly below the physiological level. On the other hand the result is valid, as iP concentrations were in positive correlation with Ca concentration during the puerperal period. If Ca concentration decrease so did iP concentration. The anion diet did not affect the increase of iP in the blood of cows. In our case such diet even caused the decrease of iP concentration in the serum. The same results were established also in our previous article (Gašperlin *et al*., 2002).

In cows fed anion diet (DCAD –99.00 mEq/kg DMI) and KatAn® group (DCAD +95.99 mEq/kg DMI) mean iP values were at calving and the day after it significantly lower compared to one week before it (Table 6, $P<0.001$). Delaquis and Block (1995) report that reduction of DCAD in feed intake from +481.8 to +327.2 mEq/kg DMI did not affect iP concentration in plasma.

The dynamics of serum iP of the affected cow shows the positive effect of the anion diet as iP concentration in the cow named Švica (Table 4) was, notwithstanding hypocalcaemia (Ca=0.165 mmo/L), the highest (iP= 1.40 mmol/L) with regard to other cows with MF in the control and Duphafral group (Table 4). The concurrent hypophosphataemia did not occur.

Most researchers report that the application of vitamin D_3 to a large extend contributed to the stabilization of iP in the blood of cows during the puerperal period which also ensured a more reliable prevention of MF. Jazbec *et al*. (1970) report that mean iP before application of vitamin D_3 varied from 1.73 to 2.06 mmol/L. Two days after application the concentration increased between 2.0 and 2.29 mmol/L and it decreased at calving from 1.58 to 1.85 mmol/L and rose again to the initial level 4 to 6 days after calving. Similar findings that are reported by Jazbec *et al*. (1970), Bar *et al*. (1985) and Zepperitz *et al*. (1994) were also established in our experiment. Cows that received vitamin D_3 injection had in the blood at calving and one day after it a much higher ($P<0.05$) mean iP concentration compared to cows from control and KatAn® group (Table 6).

Serum Mg

The dynamics of mean Mg values in our experiment matches quite well with the results of other authors (Jazbec *et al*., 1971; Gaynor *et al*., 1989; Tucker *et al*., 1988, 1991). As evident from Table 7, the dynamics of mean Mg concentration in the blood of cows was significantly ($P<0.01$) affected by the anion diet during the puerperal period. Mg concentrations increased to the level, which was established even in cows with clinical signs of MF. Some researchers found that the level of serum Mg was not significantly changed by anion and cation diets

(Tucker *et al*., 1988, 1991; Goff, *et al*., 1991). Delaquis and Block (1995) report that DCAD reduction in feed intake from +481.8 to + 327.2 mEq/kg DMI had no effect on Mg concentration in plasma.

Administration of Duphafral® Vit. D_3 1000 affected the stabilization of serum Mg during the critical postpartal period. Similar occurrences were detected also in the control group. Jazbec *et al*. (1971) report that in cows treated with only 5 million IU of vitamin D_3 serum Mg began to increase 4 days before calving. At calving the concentration was 1.23 mmol/L. The highest level 1.30 mmol/L was reached the second day after calving. By applying of 10 million IU of vitamin D_3, Mg level in the serum was during the time of parturition, at calving and after it stable within the range of 0.92 to 1.06 mmol/L. Very similarly as in our experiment. Zepperitz *et al*. (1994) established an after the injection of vitamin D_3 Mg concentration, with regard to the control group, significantly ($P<0.001$) decreased immediately after calving.

Our results showed that the anion diet stimulated the regulative mechanisms in cows so that serum iP concentration decreased and that of Mg increased. Gaynor *et al*. (1989) report that after a anion diet serum Mg concentration was 1.16 mmol/L, while after a cation diet only 0.83 mmol/L. We can conclude that the anion diet positively affected metabolism of Mg, which is shown by significantly higher ($P<0.001$) Mg values in the blood of cows from KatAn group compared to mean Mg values from control and Duphafral® group.

CONCLUSIONS

On the basis of our experiment we can conclude that the calculations of feed intakes during the close-up period in the control and Duphafral® Vit. D_3 1000 groups showed that DCAD was within the positive range. Respectively, cows on low cation diet. DCAD +95.99 mEq/kg DMI were within the range that did not present a greater risk for the clinical occurrence of MF. The group of cows that orally received 14 days before expected calving 300 g KatAn® salts per day had a negative DCAD, which means that cows were on anionic diet. With regard to the experience of other researchers we are of the opinion that DCAD –99.00 mEq/kg DMI was insufficient for an effective prevention of MF and with it related diseases.

Statistically significant ($P>0.05$) differences in Ca concentration one week before calving (2^{nd} sampling) and at calving (3^{rd} sampling) were established without regard to the group of cows. At calving and 24 hours after it the lactation stress caused a greater decrease of serum Ca level than the anion diet or the vitamin D_3 injection (Table 5).

Cows, that received an injection of Duphafral® Vit. D_3 1000 had at calving and a day after it a much higher ($P<0.05$) mean iP concentration in the blood compared to cows in the control and KatAn® group (Table 6).

After i/m administration of 10 M IU Vit. D_3 the level of serum Mg was before calving, at calving and after it stable within the range of 0.82 and 1.33 mmol/L.

The anion diet positively affected Mg metabolism as mean Mg values in the blood were significantly ($P<0.001$) higher in KatAn® group compared to the control or Duphafral® group, respectively.

On the basis of the analyses of feed intake, and the fact that all cows were affected by MF during the immediate puerperal period, we may with confidence assume that the most important cause for an unexpected occurrence of the disease in both trial groups was a deficient Ca concentration in the feed intake immediately after calving when the requirements for this element are suddenly elevated.

Statistical analyses of clinical-laboratory data supported our opinion that the best prophylactic measure for the prevention of MF and with it related diseases on small and medium large farms in Slovenia is the i/m administration of 10 ml (10 million IU cholecalciferol) Duphafral® Vit. D_3 1000.

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DUPHAFRAL® VIT. D_3 1000 I KATAN® ANJONSKE SOLI U PREVENTIVI MLEČNE GROZNICE KOD MLEČNIH KRAVA

ZADNIK T, SORŠAK B, KLINKON MARTINA i STARIĆ J

SADRŽAJ

Na tri mlečne farme sa sličnim krmnim obrokom testiran je, na 30 crno-belih krava, efekat intramuskularno (i.m.) aplikovanog preparata Duphafral® Vit. D_3 1000 i peroralno (p.o) aplikovanih KatAn® anjonskih soli u preventivi mlečne groznice (MF). Prvoj grupi krava (n=10, katjonsko-anjonska razlika obroka (DCAD) = 95.99 mEq/kg suve materije) je bilo aplikovano 10 ml Vit D3 nedelju dana pre očekivanog termina teljenja. KatAn grupi (n=10; DCDA=-99.00 mEq/kg suve materije) je peroralno aplikovano 300 g anjonskih soli na dve nedelje pre očekivanog termina teljenja. Treća grupa krava (n=10; DCAD= +95.99 mEq/kg suve materije) je bila kontrolna. Tokom studije je kontrolisan klinički status krava i vršeno je višekratno uzorkovanje krvi (dve i jednu nedelju pred teljenje i zatim prvi, drugi i sedmi dan po teljenju) radi određivanja koncentracije Ca, iP i Mg u serumu.

Najbolji rezultat je postignut pri aplikaciji 10 miliona I.U. holekalciferola (Vit. D_3) nedelju dana pre očekivanog termina teljenja. U ovoj grupi je samo jedna krava obolela od MF drugi dan nakon teljenja. U KatAn grupi je došlo do pojave tipičnih znakova mlečne groznice kod jedne krave prvi dan nakon telenja, a druga je imala znakove retencije posteljice. Incidenca MF u kontrolnoj grupi je bila znatno veća. Jedna krava je razvila MF, druga je imala retenciju posteljice a kod jedne se razvila dislokacija sirišta. Zbog odbijanja silaže sa umešanim KatAn solima u ishrani, svakodnevno je bilo potrebno individualno tretirati krave pripremljenim rastvorom (300 g) putem ručnog drenčera. Na osnovu rezultata izvršenih analiza, kliničko-laboratorijskih podataka i činjenice da je aplikacija anjonskih soli komplikovana, smatramo da je najbolja profilaktična mera za snižavanje incidence MF, kao i bolesti povezanih sa njom, na malim i srednje velikim farmama mlečnih krava u Sloveniji, i.m. aplikacija preparata Duphafral® Vit D_3 1000.