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Effect of Peroral Supplementation of Selenium and Vitamin E on Udder Healthin Dairy Cows

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Abstract:*Intrammamary infections – mastitis and prepartum immune suppression are almost associated with endocrine changes and decreased intake of critical nutrients. Among the most important antioxidant nutrients but often deficient in compound feeding stuff are vitamin E (VTE), and selenium (Se) compounds.The aim of the experiment was to study Seand VTE sources in the diet of dairy cows in late phase of pregnancy and their effects on udder health and milk quality during the first two weeks after calving. The experiment included 40 cows of Slovak Pied cattle divided into two equal groups (n = 20). The first group (D)was peroral supplemented with addition of 0.3 mg Se/kg in anorganic form of sodium selenite and 50 mgVTE in form of dl-α-tocopherol acetate/kg of DM given from 6 weeks pre partum to parturition (total dose of 5.0 mg Se/kg and 1020 dl-α-tocopherol acetate/kg of DM per day). The control group (C) was without peroral supplementation of Se and VTE with the diet containing 0.2 mg of Se and 56 mg VTE/kg of DM.In supplemented group were observedincreased plasmatic concentrations of Se and VTEon the day of parturition and on the 14th day of lactationas well as increased activity of glutathione peroxidaseimmediately after calving.In addition to increased plasma levels, a 12.5% reduced incidence of clinical mastitis was observed in the supplemented group during the first two weeks after calving.*

I. INTRODUCTION

One of the main factors limiting the use of productive abilities of dairy cows is the level of nutrition and ensuring the appropriate quantity and particularly the quality of feed, its regular intake and thus sufficient energy and necessary nutrients. From the spectrum of nutrient requirements, it is important to pay attention to individual antioxidant nutrients which create optimum conditions for metabolic processes and development of the natural defense mechanisms against the action of infectious agents from the external environment. One of the most critical periods for dairy cows is the peripartum period when immunosuppression is associated with endocrine changes and decreased intake of critical nutrients [1].

Selenium (Se) and vitamin E (VTE) compounds are among the most effective antioxidant nutrients, but they

are often deficient in compound feed during the dry period and in the postpartum stage [2,3]. Selenium is one of the essential trace elements which protect organisms from oxidative damage. The presence of Se in soil on the territory of EU is very variable, from an average of 0.05-0.1 ppm. How to Se deficient soils are given territory of Nordic countries and territories of France, the Balkans and England. In recent years, it is confirmed that the Central Europe is area with very low concentrations of selenium in the soil [4]. Many biological functions of this element, which is present in various selenoproteins, have been described. The most important of them is glutathione peroxidase (GPx).Different forms of this enzyme are present in all tissues which are subject to oxidative stress. Biological functions of selenium are complemented by vitamin E, which also shows the effects of a cellular antioxidant.The function-site for GPx is cell cytosol and vitamin E operates within lipid membranes. One important function of both systems is protection of polyunsaturated fatty acids in membranes, which are very sensitive to the effect of reactive oxygen forms [1].

Their long-term nutritional deficiency can cause enlargement of the heart muscle, increased incidence of mastitis, degenerative bone and joint involvement, myodystrophy, conception disorders, placental retention, miscarriages and others. In recent years, Se and VTE have been widely discussed in relation to their role in reaching of optimal reproductive parameters or the occurrence of reproductive disorders at their latency [5,6].

The aim of this study was to evaluate the effect of oral supplementation of Se and vitamin E on the incidence of mastitis in dairy cows during the peripartum period.

II. Materials and Methods

2.1 Cows and milking

This study was carried out in herd of 270 Slovak Pied cattle in east of Slovakia. The animals were kept in a free housing system with a separate calving barn and equipped with individual boxes with bedding. The cows were fed a total mixed ration (TMR) according to actually request during dry period and lactation. The mean daily intake for the dry period and at 5th day after calving under study was 10 kg and 18 kg of DM respectively. During the drying and post partum periods, all cows on the farm received feed rations with the same level of Se (0.2 mg.kg^{-1}of DM). The average milk yield of the cows was 7,700 ± 50 kg of milk per lactation.Milking took place in the parallel parlour Boumatic 2 x 10 Xpressway (Wisconsin, USA). Before drying was applied intramammary antibiotic preparation Orbenin Dry cow *a.u.v.* (Pfizer, IT) to every quarter of udder.

2.2 Peroral supplementation of Se and VTE

A total 40 dry cows in the final period of pregnancy were randomly assigned into two groups (S and C). Six weeks prior to the expected parturition were cows in group S peroral supplemented with addition of 0.3 mg Se/kg in form of sodium selenite (Na_2SeO_3)and 50 mg/VTE in form of dl-α-tocopherol acetate/kg of DM in total dose of 5.0 mg Se and 1020 dl-α-tocopherol acetate of DM per cow/day). The control group (C) was without peroral supplementation of Se and VTE with the diet containing 0.2 mg of Se and 56 mg vitamin E/kg of DM.

2.3 Collection of samples and laboratory examination

Blood samples were collected into 12 ml heparinised test tubes from the jugular vein of cows six weeks before the expected time of calving (before supplementation period), on parturition day and at 14th day after calving. First colostrum was also collected into 10 ml tubes. On the basis of the comprehensive examinations on the 14th day according to National Mastitis Council [7]which consisted of a clinical examination, examination of milk from each quarter of the udder, California mastitis test[8], and two sampling 10 ml of the milk samples at a 45° angle to the microbiological examination were assessed the health status of the mammary gland of each selected dairy cow. For the purpose of determining the nutritional values as well as selected mineral element,

were sampled 1 kg comprehensive sample of TMR from feed troughs was taken according to Bujnak et al. [9]. The nutritional values of TMR were determining by the AOAC methods [10].

The blood plasma obtained by high speed centrifugation of heparinised blood at 3000 rpm during 15 min. Plasma from each sample was divided into two 3 ml tubes, from which the later setting concentrations of Se and a-tocopherol. All samples of blood plasma, milk and colostrums together with 2 ml (detection of GPx) of heparinised blood samples were stored at -54 °C until analysis.

The concentration of the Se in samples of feed, plasma and colostrum were determined after wet mineralization in a closed system using a microwave (Milestone MLS 1200) digestion technique with HNO_3 and H_2O_2 by atomic absorptive spectrometer Zeman 4100 (Perkin Elmer, USA) equipped with generating device system, according to procedure Pechova et al. [11]. The GPx activity in the samples was measured by the method developed by Paglia and Valentine [12], using a set supplied by Ransel (Randox RS 505) and the automatic analyser Cobas Mira, and expressed in terms per gram of haemoglobin in the erythrocytes (U/g of Hb).Determination of α-tocopherol from the blood serum and colostrum samples after evaporation in N-heptane and subsequent dissolution in methanol was analysed according to the HPLC method of Hess et al. [13].

Milk samples (0.05 ml) were inoculated onto blood agar (Oxoid, UK) and cultivated at 37 °C for 24h. Based on the colony morphology, bacteria *Staphylococcus* spp. were selected for the tube coagulase test (Staphylo PK, ImunaPharm, SR). Suspect colonies *Staphylococcus* spp., *Streptococcus* spp. and *Enterobacteriacae* spp. were isolated on blood agar, cultivated at 37°C for 24h and identified biochemically using the STAPHY-test, STREPTO-test, resp. ENTERO-test and identification by software TNW Pro 7.0 (Erba-Lachema, CZ).Dry matter was acquired by 48 h drying sample at 105 °C.

2.4 Statistical analysis

The concentration of selenium measured in the samples was evaluated by ANOVA analysis of variance and the differences between the individual groups were analysed by t-test. Differences between the mean values of the different treatment groups were considered assuming significance levels of 0.05 and 0.01. Values in tables are means (M) and standard deviation (SD).

III. Results and Discussion

During the drying period, the main source of nutrients for dairy cows is bulk feed. The Se content in this feed depends on its concentration and form in the soil and the cumulative ability of the plants to absorb this element. According to the NRC [13], the Se content in dairy feeds during drying is often lower than 0.1 mg/kg DM, which is insufficient for proper antioxidant and immunostimulatory function of the organism. Insufficient concentrations of Se in feed rations together with increased degradation of VTE during drying and canning of feed often lead to the decrease of these compounds in the blood plasma of dairy cows as well as in colostrum and milk.

Pavlata et al. [6,14] determined reference values of Se and VTE in cattle and divided them into three groups. Concentrations below 30 µg/L and vitamin E below 4.0 µg/mL are found in animals with clinical signs of nutritional muscular dystrophy and are referred to as deficient values. Selenium values from 30 µg/L to 50 µg/L and vitamin E values between 4-8 µg/mL are considered as borderline. Se values above 75 µgL and vitamin E above 8 µg/mL are considered adequate for proper selenium protein function and antioxidant defense levels. The Se blood plasma values measured in our study in dairy cows at the beginning of the observed period were in the range of 83.8 - 85.7 µg/L and those of vitamin E reached 5 - 5.5 mg/mL, which can be considered adequate. Marginal Se values were determined on the day of calving and on the 14th day after calving in the control group. Elevated levels of Se as well as of VTE after calving and on the 14th day of lactation, which can be considered adequate, were observed in the supplemented group of dairy cows. Although elevated plasma levels of Se and VTE in the supplemented group of dairy cowswas insignificant their cumulative effect in the colostrum (Table 1).

Table 1. Effect of peroral supplementation of selenium and vitamin E on mean plasma and colostrum concentrations

Period	Category	Control		Supplemented	
		Se(µg/L)	**VE**(µg/mL)	**Se**(µg/L)	**VE**(µg/mL)
		M±SD	M±SD	M±SD	M±SD
42th day a.p.	cows	85.7 ± 7.8	5.5±0.58	83.8 ± 6.3	5.0±0.56
parturition	cows	70.0 ± 6.9[a]	4.4±0.76[a]	81.7 ± 7.1[b]	7.2±0.82[b]
	colostrum	31.1 ± 4.6	9.8±1.74	35.7 ± 3.3	12.1±2.3
14th day p.p.	cows	72.1 ± 6.5[a]	4.6±0.8[a]	78.0 ± 5.1[b]	6.4±0.68[b]

Note: Se – selenium, VE – vitamin E; a. p. – ante partum; p.p – post partum; [a,b] significance level$P<0.05$.

Synthesis of reactive oxygen species and their accumulation during the parturition or inflammation are controlled by antioxidant enzyme systems. Several defence mechanisms are available to prevent oxidative damage including scavenging systems as the enzymes glutathione peroxidase (GPx), superoxide dismutase or catalase [16].Glutathione peroxidase activity is considered to be an indicator of long-term Se supply, as it depends on the erythrocyte life cycle. However, it has been discussed how rapidly the GPx activity reflects changes in the Se status. For practical use, Pavlata et al. [6]recommend the lower limit of the reference value of GPx in whole blood of cattle of 250U/g of Hb. In our study, the activity of GPx throughout the period under review in experimental group of cows is considered to be adequate. The significant decrease of GPx activity we detected in the control group after calving (Figure 1). The antioxidant status depends on the current antioxidants activity of GPx when the inflammation of mammary gland starts. Consequently, the consumption of one antioxidant may possibly affect the concentrations of the others, since the action of antioxidant enzymes also depends on their sparing effect and tissue site.

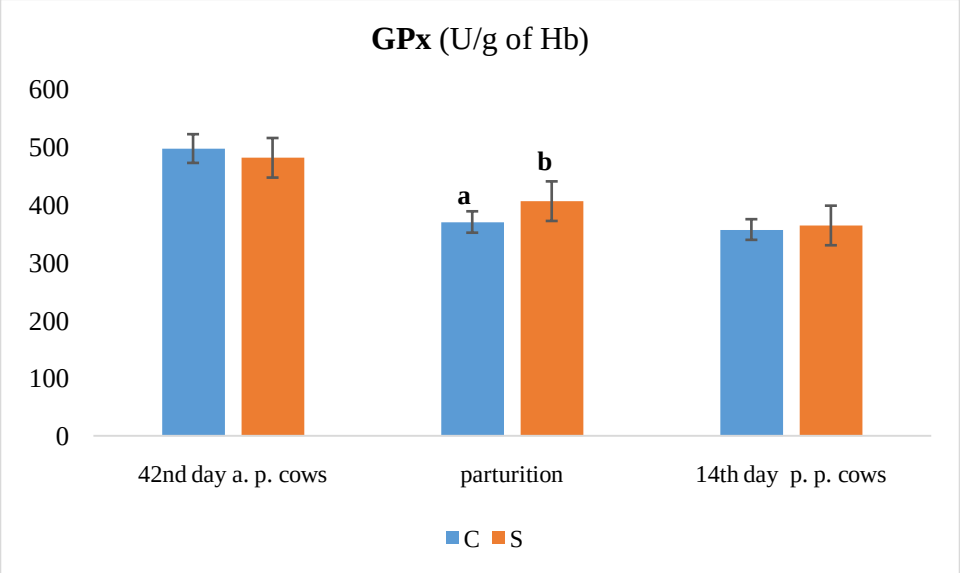

Figure 1. Effect of peroral supplementation of selenium and vitamin E on the activity of GPxinblood of dairy cows
Note: C – control group; S – supplemented group; [a,b] significance level$P<0.05$.

Table 2 shows the incidence of mastitis in the monitored groups at 14th day after calving. In the control group, we observed 16 infected quarters, which exceeds the number detected in the supplemented group by 4 quarters. Within the monitored groups, the incidence of clinical mastitis was decreased by 12.5% in the supplemented group. The administration of selenium and vitamin E brought about a change in the prevalence of

clinical form of mastitis. While clinical mastitis was detected in 7 quarters in the control group, only 2 quarters were clinically affected in the supplemented group.

Table 2. Occurrence of mastitis in the monitored groups of dairy cows at 14th days after calving

group	$\sum^h$		$\sum^i$		Infected quarters	Mastitis form and % of infected quarters			Milk production*
	n	%	n	%		L	SC	CM	
C	13	65.0	7	35.0	16	7.5	15.0	17.5	31.5 ±3.5
S	15	75.0	5	25.0	12	10.0	12.5	5.0	32.2 ±2.8

Note: $\sum^h$ – number of healthy cows, $\sum^i$ – number of mastitic cows, L – latent mastitis, SC – subclinical mastitis, CM – clinical mastitis, Milk production* - milk production after first month of lactation; C – control group; S – supplemented group.

By our analysis of the infected quarter samples we confirmed bacteria as are *S. aureus*, non-aureus staphylococci and *Streptococcus uberis* which are most often associated with the formation of the subclinical and clinical forms of mastitis. In isolated bacteria caused subclinical and acute forms of mastitis were not detected differences in occurrence of mastitis in following groups of dairy cows.

IV. Conclusion

It can be therefore concluded that the peroral administration of the product containing Se and VTE to dairy cows during last 6 weeks before calving showed a positive effect on the increase of Se and a-tocopherol concentrations in blood plasma on the day of parturitionand on the 14th day of lactation. In addition, in peroral supplemented group was incidence of clinical mastitis a 12.5% lower but it does not affect the presence of bacterial agents in milk obtained from mastitis suffering cows. The data obtained in this study also suggest that duration of higher plasma of α-tocopherol and Se concentrations is relatively short and therefore we still recommend their postpartum supplementation to stabilize their optimal levels.

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