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Original article

Effect of parenteral administration of Selenium and vitamin E on health status of mammary gland and on selected antioxidant indexes in blood of dairy cows

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Abstract

This study focuses on the effect of parenteral administration of Selenium (Se) and vitamin E on concentration of Se in plasma and the activity of glutathione peroxidase (GPx) in the blood of dairy cows during peripartal period and their effect on the reduction of clinical mastitis. From a 220 individuals Holstein herd in a two-four lactation-gestation cycle the control group (C), 1st (D1) and 2nd (D2) experimental group were selected. Every group consisted of 15 cows in the last phase of the pregnancy. All cows were fed with the diet containing 0.1 mg of Se per kg/DM. The blood samples from *vena jugularis* were collected approximately 21 days before calving (control sampling), 3 days, 12 days and 21 days after calving. On the day of control sampling and 12 days before calving in D1 group, cows were injected subcutaneously in the sprescapular region with preparation Selevit inj. a.u.v. at the doses of 48.4 mg/head of Se, and 550 IU/head of α-Tocoferol (α-Toc). In D2 group, cows were injected by the same preparation only on 21th day before calving with the same doses of Se and α-Toc. The increase in the concentration of Se in the plasma and activity GPx in blood in D1 group on the 3rd day and 12th day after calving were determined. Increase in plasmatic concentrations α-Toc on 3rd day after calving and reduction of occurrence of clinical mastitis (13.3%) as compared with control group were found.

Key words: dairy cows, glutathione peroxidase, mastitis, selenium, vitamin E

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Introduction

The most famous, economically challenging and the most problematic disease in dairy cattle throughout the world is an infection of the mammary glands (mastitis). This disease reduces the production of milk, increases the Somatic Cell Count, Total Number of Microorganisms and causes changes in the mammary gland of different scale (Vasil 2007a,b, Vasil et al. 2012).

Among the preventive measures to reduce morbidity and the incidence of mastitis during lactation, an important issue is a balanced nutrition sufficient to ensure the optimum progress of the dose which support the natural defence mechanisms of the organism and the metabolism of the mammary gland. Among the most important nutrients but often deficient in compound feeding stuffs, involved in the biological functions and antioxidative activity are selenium compounds (Se) and vitamin E (vit. E) (O'Rourke 2009).

One of the most important selenoproteins which contributes to antioxidant protection is the enzyme glutathione peroxidase (GPx). Different forms of this enzyme are present in all tissues which are subject to oxidative stress. Glutathione peroxidise and vit. E co-operate against peroxide radicals and this relationship is described as "sparing effect". Vitamin E acts on the level of lipid membranes and is part of the first line of the antioxidant protection of the polyens acids. Cytoplasm is functional site for GPx, which catalyzes the reduction of hydrogen peroxide and organic peroxides (Zigo et al. 2012).

On the other hand, sufficient or excessive intake of vit. E and Se with the feed must be determined order to exert a positive impact on their plasma levels, which is a common problem in the feeding of cows at different lactation stages. In the dry period in most of the holdings of the cattle the changes in the ration play an important role. Especially in the peripartal period to the total supply of feed is limited, what negatively impacts the supply of the body with the Se and h-Toc. In addition, at the end of lactation and dry period a minimum of grain feed is used, in which the content of Se is higher compared with contents in other feeding stuffs (Slavik et al. 2006).

According to Pavlata et al. (2005), diets containing under 0.3 mg of Se/kg of DM, and 500 IU of vitamin E/cow per day are deficient in antioxidants and decrease immunostimulation of organism in the dry period. In our study, this situation is partially resolved by parenteral administration of seleno-vitamins preparation which is also used in the treatment of deficiencies. Application of synthetic injectable forms of Se and α-Toc seems to be the most effective solution of the problem of the requirements of the organism to both antioxidants, especially in the peripartal period, when the oral supplementation fails to increase their reduced concentration in the blood plasma of dairy cows.

The aim of the work was to monitor the impact of parenteral application of Se and α-Toc during peripartal period on the occurrence of inflammation of the mammary glands and their influence on changes of plasma concentrations of Se, α-Toc and GPx activities in the blood of dairy cows.

Materials and Methods

Animals, feeding and milking

Study was carried out in herd of 220 Holstein dairy cows in east of Slovakia. Dairy cows were kept freely housed in three cowsheds and milked twice a day. In farms seasonal feeding was used with total mix ration (TMR) according to actually needs during dry period and lactation (Table 1). Milking took place in the tandem milking shed Boumatic 2 x 10 Xpressway (Wisconsin, USA). Before drying intramammary antibiotic preparation Orbenin Dry cow a.u.v. (Pfizer, IT) to every quarter of udder was applied.

Experimental design and sampling

During dry period there were selected 45 dairy cows between 2nd and 4th lactation in last phase of pregnancy. Selected dairy cows were divided into 2 experimental and one control group of 15 cows each.

On 21 days before the time of expected delivery from selected cows blood was taken from *vena jugularis* to test tubes: 15 ml with the addition of 0.3 ml of lithium heparin and 2 ml into test tubes with the addition of 0.1 ml of lithium heparin. After the withdrawal and mixing the samples are transported at a temperature of +4°C, in a cool box to the laboratory. Blood plasma was obtained by high-speed centrifugation of heparinised blood at 3000 rpm (Centro – Intertec, SR) for 15 min. and divided into two 3 ml tubes, from which the samples for assaying concentrations of Se and α-Toc were taken. All samples of blood plasma, together with 2 ml of heparinised blood samples were frozen at a temperature of -54°C untill the analyses were performed.

After the withdrawal of blood 21 days before expected calving, selected groups of dairy cows were treated as follows:

– The first group of dairy cows (D1) was on 21st and 12th day before calving, administered subcutaneously in one site behind the scapula Selevit. inj.

Table 1. Ingredients and chemical composition of the prepartal and postpartal rations.

Ingredients	Ration	
	prepartum	postpartum
Grass hay (kg/day/cow)	1.0	0.5
Corn silage (kg/day/cow)	10.0	20.0
Clover-grass silage (kg/day/cow)	7.0	14.0
Triticale grain (kg/day/cow)	2.16	3.66
Soybean meal (kg/day/cow)	0.6	1.5
Rapeseed extracted scrap (kg/day/cow)		1.2
Urea (kg/day/cow)		0.03
The limestone ground (kg/day/cow)		0.06
[a] Trace mineral lick		
	[b] Composition	
DM (g/kg)	481	460
CP (g/kg DM)	131.05	145.75
Fat (g/kg DM)	26.1	29.93
NDF (g/kg DM)	342.64	336.6
ADF (g/kg DM)	227.04	210.86
NSP (g/kg DM)	381.16	413.33
Starch (g/kg DM)	255.45	304.3
NDP (g/kg DM)	23.1	14.7
NE MJ/kg	6.26	6.55
Se µg/kg DM	97	110
[c] Vitamin E m.j./kg	38	49

DM – dry matter, CP – crude protein, NDF – neutral detergent fibre, ADF – acid detergent fibre, NSP – non-starch polysaccharides, NDP – non-degraded protein, NE – net energy

[a] Composition of mineral lick (g/kg) without Se: Ca 45.32, P 33.3, Mg 76.0, Zn 6.0, Mn 3.0, I 0.2, NaCl ad 1000g; [b] Composition – analysed values; [c] Vitamin E (IU) – international unit of Vitamin E defined as1 mg (±) – α-tokoferolacetate

a.u.v. (Biotika a.s., SR) at a dose of 22 ml/PCs, (48.4 mg/Se and 550 IU/α-Toc/cow). Effective substances in v 1 ml of Selevit inj. are tocopheryl acetate 25 ml and sodium selenate 2.2 mg.

– The second group of dairy cows (D2) was on 21st day before calving given, subcutaneously once administered of Selevit inj. (Biotika a.s. SR) a.u.v. on the same dose of Se and α-Toc as the group D1.

– The third group of dairy cows (C) was not treated.

Monitoring of plasma concentration of Se, α-Toc and GPx activities in the blood of dairy cows was carried out on 3rd, 12th and 21st day after calving by sampling blood from the *vena jugularis*. On the basis of the comprehensive examinations on the 21st day according to Vasil (2009), which consisted of a clinical examination, examination of milk from each quarter of the udder, California mastitis test (Jackson and Cockcroft 2002), and the sampling 10 ml of the milk sample at a 45° angle to the microbiological examination the health status of the mammary glands of dairy cows was assessed.

For the purpose of determining the nutritional values as well as selected mineral elements and vitamins, 1 kg comprehensive sample of total mixed ration (TMR) from feed troughs was taken according to Bujnak et al. (2011).

Laboratory analyses

Glutathione peroxidase activity per gram of haemoglobin in the erythrocytes was determined by the RANSEL-RANDOX RS 505. Haemoglobin was analyzed by Haemoglobin kits (RANDOX-RANSEL, UK). The concentration of the Se in the sample of feed was determined after wet mineralization in a microwave oven 1200 LS module (Milestone) by atomic absorptive spectrometer Zeman 4100 (Perkin Elmer, USA) equipped with generating device system, according to procedure by Bouwstra et al. (2010). The concentration of Se in the blood plasma was evaluated in duplicate using the fluorimetric methods according to Rodriguez et al. (1994).

After the extraction of the sample of blood plasma in N-heptane, its evaporation and subsequent dissolution in methanol the content of α-Toc was determined by analysis according to the HPLC method of Hess et al. (1991). Determination of α-Toc from the homogenized sample after saponification and extrac-

Table 2. Effect of parenteral application of Se and vit. E in dairy cows on the concentrations of Se (μg/L) in blood plasma before and after calving.

Period		Groups		
		C M ± SD	D2 M ± SD	D1 M ± SD
Se	21st day *a. p.*	64.8 ± 3.33	63.6 ± 5.42	65.2 ± 5.39
	3rd day *p. p.*	62.08 ± 4.54^{A}	64.60 ± 4.50	72.53 ± 5.54^{B}
	12th day *p. p.*	69.0 ± 4.04^{A}	68.8 ± 2.47	75.0 ± 3.84^{B}
	21th day *p. p.*	79.9 ± 2.04	78.6 ± 2.63	80.7 ± 3.29

D1 – experimental group treated with Selevit inj.a.u.v. on 21st and 12th day before parturition at the doses 48.4 mg of Se and 550 IU of α-Toc *pro toto*, D2 – experimental group treated with Selevit inj.a.u.v. on 21st day before parturition at the doses 48.4 mg of Se and 550 IU of α-Toc *pro toto*, C – control group without parenteral application, a. p. – ante partum, p.p – post partum; ^{A,B} significance level of P<0.001

Table 3. Effect of parenteral application of Se and vit. E in dairy cows on the activity of GPx (U/g of Hb) in blood before and after calving.

Period		Groups		
		C M ± SD	D2 M ± SD	D1 M ± SD
GPx	21st day *a. p.*	397 ± 25,3	401 ± 28,6	398 ± 30,2
	3rd day *p. p.*	386 ± 38,8^{a}	413 ± 37,5^{b}	441 ± 34,8^{B}
	12th day *p. p.*	393 ± 34,0^{a}	399 ± 37,8	446 ± 40,4^{B}
	21st day *p. p.*	415 ± 33,1	406 ± 34,0	438 ± 49,1

D1 – experimental group treated with Selevit inj.a.u.v. on 21st and 12th day before parturition at the doses 48.4 mg of Se and 550 IU of α-Toc *pro toto*, D2 – experimental group treated with Selevit inj.a.u.v. on 21st day before parturition at the doses 48.4 mg of Se and 550 IU of α-Toc *pro toto*, C – control group without parenteral application, a. p. – ante partum, p.p – post partum; ^{a,B} significance level of P<0.01, ^{a,b} significance level of P<0.05

tion by HPLC method was carried out according to Smith et al. (1997).

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). Suspected colonies of *Staphylococcus* spp., *Streptococcus* spp. and *Enterobacteriaceae* spp. were isolated on blood agar, cultivated at 37°C for 24h and identified biochemically using the STAPHYtest, STREPTOtest, resp. ENTEROtest and identification by software TNW Pro 7.0 (Erba-Lachema, CZ).

Dry matter was determined by 48h drying sample at 105°C. The nutritional values of TMR were determining by the AOAC methods (2001) as content of crude protein, neutral detergent fibre, acid detergent fibre, non-starch polysaccharides, non-degraded protein, fat, and netto energy.

Statistical Analysis

One-way analysis of variance (ANOVA) with the *post hoc* Dunnett's Multiple Comparison Test was subsequently used to compare the control group to the parenterally injected groups. The results are presented as mean ± SD. Differences were considered as significant when P<0.05. The statistical software GraphPad Prism, Version 4.00 (2003) was used.

Results

The effect of parenteral application of Selevit inj. a.u.v. on concentration of Se (μg/L) in blood plasma of dairy cows during peripartal period is described in Table 2. In D1 group, with repeated parenteral application on 21st and 12th day before calving increased concentration of Se on 3rd and 12th (P < 0.001) day

Table 4. Effect of parenteral application of Se and vit. E in dairy cows on the concentrations of α-Toc (fg/ml) in blood plasma during peripartal period.

Period		Groups		
		C M ± SD	D2 M ± SD	D1 M ± SD
α-Toc	21st day *a. p.*	3.10 ± 0.41	3.08 ± 0.39	3.11 ± 0.33
	3rd day *p. p.*	2.12 ± 0.31[A]	2.27 ± 0.24	3.23 ± 0.50[B]
	12th day *p. p.*	2.83 ± 0.24	2.91 ± 0.33	3.20 ± 0.36
	21st day *p. p.*	3.63 ± 0.31	3.52 ± 0.34	3.83 ± 0.23

D1 – experimental group treated with Selevit inj.a.u.v. on 21st and 12th day before parturition at the doses 48.4 mg of Se and 550 IU of α-Toc *pro toto*, D2 – experimental group treated with Selevit inj.a.u.v. on 21st day before parturition at the doses 48.4 mg of Se and 550 IU of α-Toc *pro toto*, C – control group without parenteral application, a. p. – ante partum, p.p – post partum; [A,B] significance level of P<0.001

Table 5. The occurrence of individual forms of mastitis in the studiet dairy cows.

Group	Σ^s		Σ^i		Reject	Iq quar.	Mastitis forms from infected quarters							
							L		SC		SA		A	
	n	%	n	%			n	%	n	%	n	%	n	%
C	**10**	66.7	**5**	33.3	4	14	1	7.1	4	28.6	7	50	2	14.3
D2	**10**	66.7	**5**	33.3	3	12	0	0	5	41.7	5	41.7	2	14.3
D1	**12**	80	**3**	20	3	7	1	14.2	2	28.6	4	57.1	0	0
Total	**32**	71.1	**13**	28.9	10	33	2	6	11	33.3	15	45.5	5	15.2

D1 – experimental group treated with Selevit inj.a.u.v. on 21st and 12th day before parturition at the doses 48.4 mg of Se and 550 IU of α-Toc *pro toto*, D2 – experimental group treated with Selevit inj.a.u.v. on 21st day before parturition at the doses 48.4 mg of Se and 550 IU of α-Toc *pro toto*, C – control group without parenteral application, Σ^s – number of healthy dairy cows, Σ^i – number of infected dairy cows, reject – rejected quarters, Iq – infected quarters, L – latent mastitis, SC – subclinical mastitis, SA – subacute mastitis, A – acute mastitis

after calving was seen compared to control group (C). D2 group did not show increased plasmatic concentration during the following period.

In Table 3 the effect of parenteral application of Selevit inj. a.u.v. on activity of GPx (U/g of Hb) in blood of dairy cows during peripartal period is described. In D1 group we determined increased activity as compared to C group on 3rd and 12th ($P < 0.01$) day after calving. In D2 group activity was increased on 3rd ($P < 0.05$) day after calving as compared to C group.

The effect of parenteral application of Selevit inj. a.u.v. on changes in plasmatic concentration of α-Toc (μg/ml) in blood plasma during peripartal period is described in Table 4. Increased concentrations of α-Toc were seen in D1 group ($P < 0.001$) on 3rd day after calving as compared to C group. In D2 group no changes in comparison to control group were seen.

The effect of parenteral application of Selevit inj. a.u.v. on occurrence of mastitis in dairy cows during 21 days after calving is described in Table 5. The lowest occurrence of mastitis (20%) was in D1 group. In groups D2 and C equal occurrence of mastitis (33.3%) was determined. The least infected quarters (7) were in D1 group. From the clinical forms of mastitis subacute in C groups (7) and in D2 group (5) were determined and acute forms in both groups in 2 cases. In D1 group only subacute forms (4) were recorded.

Microbiologically examination identified *S. uberis* (7), *S. dysgalactiae* (6), *S. chromogenes* (6) a *S. xylosus* (5) in all monitored groups. In groups with parenteral treatment isolated *S. dysgalactiae* (4), *S. chromogenes* (4), *S. uberis* (3) a *S. xylosus* (3), while in control group *S. uberis* (4), *S. dysgalactiae* (4) a *S. chromogenes* (2) were found.

Discussion

Due to to a low content of Se in the soil on the territory of the Member States of the EU the deficiency of this element in the production dairy herds occurs, but it is also very common and discussed the problem for other ruminants. The content of Se in feed for dairy cows of less than 0.1 mg/kg/DM occure often during dry period, which is insufficient for proper antioxidant and immunostimulation functions of the organism (Gresakova et al. 2013).

Kommisrud et al. (2005) in their study, carried out on 254 individuals of Norvegian Red cattle highlights the importance of determining Se in ration for dairy cows. They found that diets low in Se (Se < 0.06 μg/g of DM), fed to dairy cows for 30 days after the birth caused 1.3 to 1.4 times higher probability to develop intramammary infections than diets with higher Se concentrations (Se > 0.11 μg/g of DM). Cow with reduced activity of GPx were fed with the low-dose of Se during the dry period.

In our study, we found a lower incidence of mastitis (13.3%) in dairy cows from D1 group with parenteral supplementation compared to control group. In addition to the reduction in the incidence of mastitis in groups D1 with parenteral supplementation, we have seen increased plasma concentration of Se as well as the increase in activity of GPx on the 3th and 12th day after calving compared with the control group.

Pavlata et al. (2002) set reference values for Se in the cattle, which are subdivided into three groups. Concentrations below 30 μg/L tend to be measured in animals with clinical symptoms of nutritional muscular dystrophy and is referred to as a deficient value. The value of 30 μg/L to 50 μg/L is described as marginal value, but for dairy cows in the dry period the marginal value is up to 75 μg/L. Values above 75 μg/L are considered to be adequate for the proper function of the selenoproteins. At the beginning of the period considered, the measured values of Se in the blood plasma of dairy cows were in the range of 63.6 – 40.5 μg/L, which can be considered as liminal concentrations of this element. Marginal values were also found on the 3th day after calving in the control group (C) (69.0 μg/L) as well as inparenterally treated group D2 (42.8 μg/L). An adequate supply of Se in the post-natal period, were found in the group D1, that was treated with parenteral on 21st and 12th day before calving.

In addition to determining Se in the feed dose and in the blood of dairy cows, the authors recommend a diagnostic assay for GPx activity, the enzyme which removes the excess of peroxide radicals in the cytoplasm. They also determined the limits of the activity of GPx, which correspond to the deficiency of Se as it is its integral component. The activity of GPx being 175-190 U/g of Hb was rated as deficient, and value in the range 190-225 U/g of HB was rated as a border value for the supply of Se. of The activity of GPx over 225 U/ g of Hb is reguarded as a sufficient level.

In our study, the activity of GPx throughout the period under review in all groups was higher than 386 U/g of Hb, what we can consider in terms of its activity as adequate. In the parenterally treated group D1, we have seen increased activity compared to control group on 3rd and 12th (P < 0.001) day after calving, as well as on the 3rd (P < 0.01) day after calving in the group D2.

Zigo et al. (2012) showed the average daily intake of vit. E in production dairy cows in the first phase of lactation when the daily fodder intake is 20 kg dry weight being 400 IU/cow with a predominance of hay, to 1500 IU/cow with a predominance of silage and about 2500 IU/cow in grazing feeding. In order to achieve a positive effect on reducing the risk of mastitis in primiparous dairy cows and dairy cows in the post-natal period a daily supplementation with vit. E at the level of 1000-1800 IU/cow should be ensured.

According to Bouwstra et al. (2009) deficiency of vit. E in the ration occurs due to the fact that the compound feed for dairy cows, is mostly all year composed of preserved feed with the content of vit. E reduced in comparison with the fresh green fodder plants. Its sufficient supplementation is secured primarily through the vitamin-mineral additives to the grain compound feeding stuffs which in the dry period is, however, considerably reduced. Injecting vitamin-mineral accessories based on the Se and vit. E at the dairy cows has in dry period a pronounced effect on the increase in their plasma levels compared to the oral application. After parenteral administration of Selevit inj a.u.v. we have seen increased plasma levels of α-Toc in the D1 at 3rd (P < 0.001) day after calving compared to the control group. In spite of the parenteral supplementation, were plasma levels of α-Toc in D2 and D1 groups to low physiological values ranging from 2.27-3.83 μg/ml, which were can be in the peripartal period deficient in the peripartal period.

In order to obtain a higher positive effect of vit. E and Se on the health status of the mammary gland, it is necessary to repeat the parenteral application as shown in D1 group which reduced the incidence of mastitis by 13.3% compared with the D2 and control group.

Hogan et al. (1993) showed decreased (37%) incidence of mastitis after calving as well as the reduced prevalence of bacterial agents on the 21st day in dairy cows which were given parenterally before calving a high dose of vit. E (2 IU/kg) and Se (2 μg/kg).

Our results show that a single parenteral application of Se and vit. E on the 21st day before calving had no impact on reducing the incidence of mastitis and prevalence of bacterial agents in comparison with the control group. A repeat doses of Se and vit. E on the 21st and 12th day before calving in D1 group had an impact on reducing acute clinical mastitis forms.

Hawari and Al-Dabbas (2008) point to the various forms of mastitis and bacterial agents isolated from the udders for cows in 10 farms of Holstein Friesian cattle in Jordan. Up to 15.7% reported cases of clini-

cal mastitis with macroscopic changes of the mammary gland and with excretions or secretions were reported. An additional 31.4% of reported cases of subclinical mastitis was also reported. Increased incidence of mastitis was seen in older cows. *S. aureus* was the most common cause of clinical and subclinical mastitis. In addition to the *S. aureus* (40.6%), the clinical mastitis was caused by *Streptococcus spp.* (26.1%), coliforms (8.7%), *Proteus spp.,* 1.4%, *Corynebacterium spp.* (5.8%) and *Pseudomonas spp.* (4.3). With our analysis of the quarters samples we confirmed the presence of bacteria: *Streptococcus dysgalactiae, Streptococcus uberis* and coagulase-negative staphylococci, which is most often associated with the formation of the subacute (15), subclinical (11), and acute (5) forms of mastitis.

We can conclude that the repeated parenteral application of Se and vit. E in doses of 48.4 mg/Se and 550IU/ α-Toc per head during the peripartal period has a preventive effect on the the incidence of mastitis however, it does not affect the presence of bacterial agents in milk obtained from mastitis suffering cows.

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