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Original article

Comparison of effect of parenteral and oral supplementation of Selenium and vitamin E on selected antioxidant parameters and udder health of dairy cows

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Abstract

The aim of this study was to compare the effect of parenteral and oral supplementation of Selenium (Se) and vitamin E (VTE) on selected antioxidant parameters in blood and colostrum as well as their effect on the incidence of mastitis in dairy cows during the final phase of gravidity (6 weeks) and first two weeks after calving. For the practical part of the study 36 dairy cows of Slovak pied breed in the second to fourth lactation-gestation cycle were selected. The animals weredivided into three groups: the control (C) and two experimental groups (D1 and D2). The selected groups were treated as follows: in group D1 products containing Se (Selevit inj.) and vitamin E (Erevit sol. inj.) were administered intramuscularly twice, six and three weeks prior to parturition; in group D2 a vitamin-minerals supplement in the form of sodium selenite ($Na_2SeO_{3)}$ and dl-α-tocopherol acetate were supplemented orally for six weeks calving. The blood samples were collected from the *vena jugularis* in dairy cows approximately 42 days before calving (control sampling), on parturition day, and the 14th day after calving. Higher concentrations of Se and VTE were found in the blood plasma samples of both experimental groups collected on the day of parturition. In addition, the orally supplemented group (D2) showed higher Se and α-tocopherol concentrations in blood plasma on the14th day after calving as well a reduction of occurrence of mastitis by about 25 % compared to the control group. The relationship between inflammatory response and oxidative stress was also confirmed. The concentrations of milk malondialdehyde indicating lipid peroxidation during mastitis were significantly higher in milk samples from infected cows than in milk samples from healthy animals in each monitored group. In order to prevent oxidative stress and moderate inflammatory response in dairy cows it is very important to optimally balance their nutritive needs with an appropriate ratio of Se and VTE supplements. Therefore we still recommend supplementation of the cows' postpartum dietwith 0.5 mg of Se/kg dry matter (DM) and 102 mg of dl-α-tocopherol acetate/kg DM to stabilize their optimal blood levels, stimulate the activity of glutathione peroxidase and reduce the incidence of mastitis.

Key words: dairy cows, calving, colostrum, glutathione peroxidase, milk malondialdehyde, mastitis

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Introduction

Healthy cows are the foundation of sustainable milk production. However, mastitis and other infectious diseases are common problems in dairy herds, resulting in increased costs and decreased production. Most diseases in dairy cows occur at, or just after, calving, which is a period associated with immune suppression, resulting in increased susceptibility to infections. Prepartum immune suppression is multifactorial but results from endocrine changes and decreased intake of critical nutrients. Among the most important nutrients often deficient in compound feeds, involved in the biological functions and antioxidative activity are vitamin E (VTE) and selenium (Se) (Waller et al. 2007, Kafilzadeh et al. 2014).

Vitamin E is a term used to describe all tocol and tocotrienol derivatives that exhibit biological activity similar to a-tocopherol. There are eight naturally occurring VTE isomers. Of these eight isomers, a-tocopherol has been proven to be most effective in preventing VTE deficiency syndromes, and is the most biologically active isomer (O'Rouke 2009).

Selenium is one of the essential trace elements which protect organisms from oxidative damage. The presence of Se in soil on the territory of the EU is very variable, from an average of 0.05 to 0.1 ppm. Se deficient soils are found on the territory of Nordic countries, France, the Balkans and England. In recent years, it has been confirmed that the Central Europe is an area with very low concentrations of Se in the soil as well (Gresakova et al. 2013).

There are many biological functions of Se, mainly as the component of various selenoproteins. The most important of these is glutathione peroxidase (GPx). Different forms of this enzyme are present in all tissues which are exposed to oxidative stress. The biological functions of selenium are complemented by VTE, which also functions as a cellular antioxidant. Antioxidants are involved in the prevention of many disorders in female reproduction, such as mastitis, retained placenta, infertility, endometritis, and ovarian cysts (Table 1). The target site of GPx activity is cell cytosol, and VTE is corporated into lipid membranes. Both systems participate in the protection of membrane polyunsaturated fatty acids (PUFA), which are very sensitive to the effects of reactive oxygen species activity. Vitamin E deficiency in animals is associated with PUFA metabolism alteration, which may subsequently lead to impaired function of many cells, among others polymorphonuclear cells, which provide the main protective mechanisms against infections (Pechová et al. 2008, Hoque et al. 2016).

Selenium and VTE supplementation given to cows around calving has been reported to prevent suppression of blood neutrophil and macrophage function during the early post-parturition period. Vitamin E supplementation also enhances the rise in specific antibodies titres after vaccination, and increases *in vitro* T and B cell mitogenesis, interleukin production, and phagocyte activity (Khatti et al. 2016).

According to the nutrient requirements of dairy cattle (NRC; 2001), dietary recommendations for VTE and Se intake are 1000 IU VTE/head/day and 0.3 mg Se/kg of DM for dry cows. Diets containing less than 0.2 mg Se/kg of DM and 500 IU of VTE/head/day do not provide antioxidant and immunostimulating effects in the transition period. Fresh green forages are excellent sources of VTE, usually containing 80-200 IU VTE/kg of DM. However, concentrates and stored forages (hays, haylages, and silages) are generally deficient in VTE.

Deficiencies of Se and VTE are frequently diagnosed also on farms in central and northern Europe, which requires treatment by administration of products containing Se and VTE. Generally, farmers apply Se and VTE products by giving dietary supplements, injections, salt licks, drenches and bolus. There are two different sources of Se in nutritional supplements available: mineral, such as sodium selenite or selenate, and organic present in selenium-rich yeast (selenomethionine, selenocysteine) (Mehdi and Dufrasne 2016).

Application of synthetic injectable forms of Se and a-tocopherol seems to be the most effective way to meet the organism needs for both antioxidants, especially in the case of dry cows, when oral supplementation fails to increase the reduced concentration in the blood plasma of dairy cows (Pavlata et al. 2002, Spears and Weiss 2008).

The aim of this study was to compare the effect of parenteral and oral supplementation of Se and VTE on selected antioxidant parameters in the blood and colostrum of dairy cows and indicate their effect on the occurrence of mastitis during the peripartum period.

Materials and Methods

Description of farm and animals

This study was carried out in a herd of 280 Slovak Pied cattle in the 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) containing grass hay, corn silage, clover-grass silage, triticale grain, soybean meal and concentrate according to actual demand during the dry period and lactation (Table 1). The mean daily intake during the dry period and on the 5th day after calving was 10 kg and 18 kg of DM,

Table 1. Overview of Se and vitamin E deficiency syndromes in ruminants.

Species	Syndrome	Affected system, resp. organ
Cattle	Nutritional myodystrophy of calves	Skeletal muscle, myocardium
	Retained placenta	Placental connection with the uterus
	Fetal death, reabsorption Ovarian cysts	Embryonic vascular system Ovaries
	Decreased production, mastitis	Udder, mammary gland
	Immune system disorders	Decreased Th lymphocyte production and phagocytic activity
	Anemia	Erythrocytes
Sheep/goat	Nutritional myodystrophy	Skeletal muscle, myocardium
	Stiff lamb disease Infertility Fetal death, reabsorption	Striated muscle Loss of uterine tone Embryonic vascular system
	Decreased production, mastitis	Udder, mammary gland
	Immune system disorders	Decreased Th lymphocyte production and phagocytic activity

Source: modified table according to Zigo et al. (2021).

respectively. Milking took place in a parallel parlor Boumatic 2 x 10 Xpressway (Wisconsin, USA). The average daily milk production was 19.3±1.06 kg. Before drying, intramammary antibiotic preparation Orbenin Dry cow *a.u.v.* (Pfizer, IT) was applied to every quarter of the udder. The calves were separated immediately after birth (within 15 minutes) and received 2 L of colostrum followed by feeding of another 2 L in 8-10 hours via a nursing bottle tube.

In total, 36 cows (between the 2nd and the 4th lactation) in the final period of pregnancy based on e sonographic examination were randomly assigned into three groups (C, D1, and D2). Six weeks prior to the expected parturition cows in groups D1 and D2 received one of the treatments:

D1 – an experimental group of 12 animals with a median age of 4.3±0.3 years to which the injectable products Selevit inj. a.u.v. (sodium selenite 2.2 mg, dl-α-tocopherol acetate 25 mg in 1 ml of the solution), and Erevit sol. inj. (dl-α-tocopherol acetate 300 mg in 1 ml of the solution) were administered intramuscularly (IM) twice during the dry period (6 and 3 weeks before expected parturition). The totals dose of sodium selenite and dl-α-tocopherol acetate were 88 mg and 2 000 mg, respectively.

D2 – an experimental group of 12 animals with a median age of 3.9±0.2 years was orally supplemented with 0.3 mg of Se/kg as Na_2SeO_3 and 50 mg of dl-α--tocopherol acetate/kg of DM given daily for six weeks before parturition (total doses: 5 mg of Se/head and 1020 mg of dl-α-tocopherol acetate/head per day).

The control group (C) with a median age of 4.6±0.3 years did not receive antioxidants. The animals' diet contained 0.2 mg of Se and 56 mg of VTE/kg of DM.

Collection of samples and laboratory examination

Blood samples were collected into 12 ml heparinized test tubes from the jugular vein of cows six weeks before the expected time of calving (before the supplementation period), on parturition day, and on the 14th day after calving. Samples of the first colostrum were also collected into 10 ml tubes. The health status of the mammary gland was assessed based on comprehensive examination on the 14th day according to the National Mastitis Council (2001). It consisted of a clinical examination, evaluation of milk from each quarter of the udder by California mastitis test (CMT, Jackson and Cockcroft, 2002), and collection of two 10 ml milk samples at a 45° angle for the microbiological examination and determination of malondialdehyde (MDA) concentration. The TMR nutritional values as well as concentrations of selected mineral elements were assessed in a 1 kg comprehensive sample of TMR from feed troughs according to Bujnak et al. (2011).

The blood plasma was obtained by high-speed centrifugation of heparinized blood at 3000 rpm for 15 min. Plasma from each sample was divided into two 3 ml tubes for determination of Se and a-tocopherol concentrations. All samples of blood plasma, milk and colostrum, and 2 ml of heparinised whole blood samples were stored at -54 °C until analysis.

The concentrations of Se in the samples of feed, plasma and colostrum were determined, after wet mineralization in a closed system using a microwave (Milestone MLS 1200) and digestion with HNO_3 and H_2O_2, by atomic absorptive spectrometer Zeman 4100 (Perkin Elmer, USA) equipped with a generating system, according to the procedure Pechova et al. (2005).

The GPx activity in the samples was measured using the method developed by Paglia and Valentine

Table 2. Composition of the feed rations fed pre partum and post partum.

Parameter	Composition	
	Pre partum	Post partum
DM (g/kg)	473	456
CP (g/kg DM)	128.03	148.15
Fat (g/kg DM)	25.4	28.62
NDF (g/kg DM)	348.12	332.3
ADF (g/kg DM)	229.02	211.16
NSP (g/kg DM)	374.12	418.03
Starch (g/kg DM)	257.25	307.5
NDP (g/kg DM)	24.2	15.6
NE, MJ/kg	6.18	6.67
Se mg/kg DM	0.2	0.2
[b]Se mg/kg DM	0.5	-
Vitamin E IU/kg	56	60
[c]Vitamin E IU/kg	102	-

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 – analysed values; [b]Se – analysed value with addition of 0.3 mg Se/kg of DM in form of Na_2SeO_3, [c] Vitamin E – analysed value with addition of 50 IU vit. E/kg of DM in form d-l-α-tocopherol acetate (IU) – international unit of vitamin E defined as 1 mg (±) α-tocoferol acetate.

(1967), using a commercial kit (Randox RS 505) and an automatic analyzer (Cobas Mira), and expressed as units per gram of hemoglobin (U/g of Hb). Hemoglobin was analyzed using Drabkin's method using a commercial reagent **(**Randox, UK).

The content of α-tocopherol in plasma and colostrum was determined using the HPLC method developed by Hess et al. (1991) after extraction of the samples in N-heptane, their evaporation, and subsequent dissolution in methanol. Determination of α-Toc from the homogenized sample of TMR after saponification and extraction was carried out using the HPLC method according to Smith et al. (1997).

Milk samples (0.05 ml) were inoculated onto blood agar (Oxoid, UK) and cultivated at 37°C for 24 h. Based on the colony morphology, *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 onto blood agar, cultivated at 37°C for 24 h and identified biochemically using the STAPHY-test, STREPTO-test, ENTERO-test, and TNW Pro 7.0 sotfware (Erba-Lachema, CZ). Dry matter was acquired by drying the samples at 105°C for 48 h. The nutritional values of TMR were determined using AOAC methods (2001).

Malondialdehyde (MDA) determination

The selection of milk samples from all groups for the measurement of MDA concentration was based on the veterinary medical record, CMT results, and clinical examination. In each experimental group two subgroups of quarter milk samples were chosen. In the first subgroup milk came from udder quarters without clinical signs of mastitis or other illnesses, milk samples from the second subgroup were positive in CMT (score 1-4) and bacteriological cultures. Lipid oxidation products expressed as MDA concentration were measured using the method based on a reaction of lipid peroxides with thiobarbituric acid and analyzed by UV-VIS spectrophotometry at 532 nm, as described by Andrei et al. (2016). The results were expressed in $nmol \cdot ml^{-1}$ of milk.

Statistical analysis

One-way analysis of variance (ANOVA) with the *post hoc* Dunett´s Multiple Comparison Test was used to compare the experimental groups with the control group. Comparison of MDA in healthy and mastitis milk samples in the individual experimental groups was done using a paired 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 the tables are expressed as means (M) and standard deviation (SD).

Results

Plasma and colostrum Se concentrations are shown in Tables 3 and 4. Plasma Se and VTE concentrations in cows were similar before oral and repeated parenter-

Table 3. Effect of oral and parenteral supplementation of selenium and vitamin E on selenium concentrations in blood plasma and colostrum (μg/L).

Period		C	D1	D2
		M±SD	M±SD	M±SD
42th day a.p.	Cows	75.5±6.8	74.1±6.5	72.1±6.8
Parturition	Cows	69.4±6.7[a]	81.3±6.7[b]	88.1±9.1[b]
	Colostrum	30.5±4.4[a]	38.2±6.8	44.7±5.6[b]
14th day p.p.	Cows	71.6±6.1[a]	75.7±8.7	82.3±8.1[b]

Note: D1 – parenterally supplemented group on the 42nd and the 21st day before parturition; D2 – group after 42 days of oral supplementation of Se and vitamin E, C – control group; a. p. – ante partum; p.p – post partum; M±SD – mean ± standard deviation; [a,b] significance level $p<0.05$.

Table 4. Effect of oral and parenteral supplementation of selenium and vitamin E on α-tocopherol concentrations in blood plasma and colostrum (μg/mL).

Period		C	D1	D2
		M±SD	M±SD	M±SD
42th day a.p.	Cows	5.5±0.58	5.1±0.62	5.3±0.56
Parturition	Cows	4.4±0.76[a]	7.4±0.86[b]	8.2±0.82[b]
	Colostrum	9.8±1.74[a]	12.4±3.1	18.1±2.3[b]
14th day p.p.	Cows	4.6±5.8[a]	5.1±0.76	6.4±0.68[b]

Note: D1 – parenteral supplemented group on the 42nd and the 21st day before parturition; D2 – group after 42 days of oral supplementation of selenium and vitamin E, C – control group; a. p. – ante partum; p.p – post partum; M±SD – mean ± standard deviation; [a,b] significance level $p<0.05$.

al supplementation. In both experimental groups, plasma concentrations of Se and VTE directly after calving were higher compared to the control group. Moreover, in the plasma of animals supplemented orally (D2) both parameters (Se and VTE) had higher values on the 14th day after calving. Similarly, the colostrum of cows from the D2 group was richer in Se and VTE compared to D1 and C groups.

A comparison of enzymatic antioxidant activity in the examined groups is shown in Table 5. A significant decrease of GPx activity was detected in the control group after calving in comparison with the orally supplemented group D2. When comparing the activity of GPx on the 14th day after calving, no statistical differences were observed among the studied groups of cows.

The evaluation of the effect of oral and parenteral supplementation of Se and VTE on the incidence of mastitis is shown in Table 6. On the 14th day after calving, a 25% reduction in mastitis incidence was observed in the orally supplemented dairy group (D2) compared to the control group. Both subclinical and clinical mastitis incidence decreased. The number of infected udder quarters was lower by 10.

Bacteriological milk cultures from the infected quarters revealed coagulase-negative staphylococci, *Staphylococcus aureus*, and *Streptococcus uberis*, which are most often associated with subclinical and clinical mastitis. There were no differences in pathogens isolated from various disease courses among groups.

Figure 1 compares milk MDA concentrations from healthy and infected quarters in the examined groups. The milk MDA concentrations were evaluated in samples taken on the 14th day postpartum. In all groups, MDA concentration was higher in mastitic milk compared to milk from healthy udders. No statistical differences in milk MDA concentrations were observed when comparing the three groups of cows.

Discussion

Assessment of oral and parenteral supplementation of Se and VTE

The peripartum period is one of the most critical phases of the reproductive and lactation cycles of cows. During this period, it is important to supply the cows with mineral-vitamin supplements containing Se and VTE for proper immunostimulation and antioxidative protection. Estimated daily nutritional requirements of dairy cows for Se and VTE are 300 μg/kg DM and 20 to 60 international units (IU), respectively. By analyzing the feed ration during the dry period, the Se content 0.2 mg/kg DM and 56 IU/VTE per kg/DM were determined (Table 2). Although the content was too low according to the recommendations of NRC (2001) both antioxidant nutrients have similar functions and dietary Se consumption is influenced by VTE. The amount of Se should be increased when a diet low in VTE

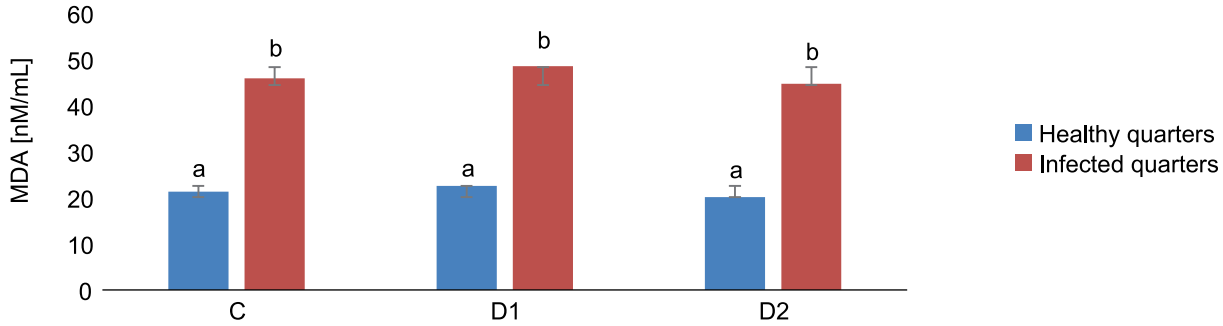

Figure 1. Comparison of milk malondialdehyde (MDA) concentrations (nM.mL^{-1}) in healthy and infected quarters
Note: D1 –group supplemented parenterally on the 42nd and the 21st day before parturition; D2 – group after 42 days of oral supplementation of Se and vitamin E, C – control group; ^{a,b} significance level between columns at $p < 0.05$.

is consumed. Therefore, Se deficiency could be partially compensated by an adequate intake of VTE and vice versa (Liu *et al*. 2016). Pavlata et al. (2004a) recommend determining Se and VTE not only in the TMR, but also in the blood of animals, because those concentrations often do not correlate. In assessing the blood Se status the same authors recommend three basic ranges: adequate (>100 µg of Se/L), borderline (70-100 µg/L) and deficient (<70 µg/L).

Scholz and Stober (2002) also considered a blood concentration of Se greater than 100 µg/l as adequate in cows. At the beginning of the studied period, the concentration of Se in the blood plasma of dairy cows ranged from 72.1 to 75.5 µg of Se/L, which can be considered the borderline concentration. The animals from the D1 and D2 groups had significantly higher blood Se concentration on the parturition day than the control animals ($p < 0.05$). However, increased plasma concentration ($p<0.05$) on the 14th day after parturition was recorded only in the orally supplemented group (D2) (Table 3).

The VTE status of dairy cows is one important component of a well-functioning immune system because of its antioxidant effects on cows and young dairy calves (Pavlata *et al*. 2004b).

NRC (2001) recommends 40-60 IU/kg DM of VTE for optimal antioxidant action of this vitamin in feed and to ensure sufficient plasma concentrations in dairy cows during the peripartum period. In our analysis, the content of VTE in TMR was 56 IU/kg DM, which may be considered adequate for dairy cows during the drying period.

According to the recommendation of Cohen et al. (1991) on optimal VTE supplementation in TMR, serum a-tocopherol concentration should be higher than 4.0 mg/mL. Canadian researchers tested ten clinically normal cows from five different herds and found mean serum VTE concentrations to range from 3.19-5.3 mg/mL when cows were fed a diet with a VTE dose in TMR up to 50 IU/kg DM. Our findings indicate that plasma levels of VTE (5.1 - 5.5 mg/mL) at the beginning of the study were adequate in all monitored groups (Table 4).

Bouwstra et al. (2010) reported that long-term oral administration of VTE to cows in the dry period has a better effect on alpha-tocopherol plasma concentration than parenteral supplementation. Similarly, in our study, in parenterally supplemented cows (group D1), the plasma concentration of VTE was increased only on the day of parturition while no changes were observed in the colostrum and blood plasma VTE on the 14th day after calving compared to the control group. Nutrient deficiencies during pregnancy in cows often result in metabolic disorders and increased incidence of related diseases (mastitis, nutritional myopathy, retained placenta, and other reproductive disorders). The lowest plasma concentrations of Se and a-tocopherol are typically detected between one week prepartum and two weeks postpartum (Kafilzadeh et al. 2014).

Meglia et al. (2006) and Pavlata et al. (2004a) found decreased plasma concentrations of VTE and Se on the parturition day, because of the reduction in DM intake at calving, and an increased need for antioxidants during this time. We observed a similar phenomenon in our study. The concentrations of Se and VTE were lower in the control group after calving compared to the supplemented groups D1 and D2.

Pregnant cows with a low intake of Se and VTE in the feed during the drying period will likely have low concentrations of these antioxidants in the colostrum and give brith to calves deficient in these elements. Se and VTE deficiencies in calves are associated with a significantly increased risk of myodegeneration and illness, mainly due to immune function compromise. Therefore, determining the concentration of these antioxidants in TMR and colostrum should be a part of preventive diagnostics of increased calve morbidity (Pavlata et al. 2004b).

In our study, colostrum examination revealed a trend towards higher Se and VTE concentrations ($p<0.01$) in the D2 group after 42 days of oral supplementation of Se and VTE during the dry period.

Table 5. Effect of oral and parenteral supplementation of selenium and vitamin E on the activity of glutathione peroxidase (U/g of Hb) in blood of dairy cows

Period	C	D1	D2
	M±SD	M±SD	M±SD
42nd day a. p. cows	497± 45.3	481 ± 41.6	475 ± 46.7
Parturition	384 ± 32.8[a]	406 ± 37.5	452 ± 46.2[b]
14th day p. p. cows	357 ± 36.4	364 ± 41.2	382 ± 37.4

Note: D1 – parenterally supplemented group on the 42nd and the 21st day before parturition; D2 – group after 42 days of oral supplementation of selenium and vitamin E, C – control group p; a. p. – ante partum; p.p – post partum; M±SD – mean ± standard deviation; [a,b] significance level $p<0.05$

Table 6. Occurrence of mastitis in the studied groups two weeks after calving

Group	Total/healthycows			Mastitic cows		Heathy/ infected quarters	Mastitis forms in infected quarters %		
	n_T	n	%	n	%		SC	SA	A
C	12	5	41.7	7	58.3	30/18	14.6	18.8	4.7
D_1	12	6	50.0	6	50.1	34/14	12.5	12.5	4.2
D_2	12	8	66.7	4	33.3	40/8	6.3	10.4	1.0
Total	36	19	52.7	17	47.3	104/40	16.4	14.6	6.94

Note: n_T – number of cows included in the group, n – number of healthy/infected dairy cows from each group, SC – subclinical mastitis, SA – subacute mastitis, A – acute mastitis, D1 – parenterally supplemented group on the 42nd and the 21st day before parturition; D2 – group after 42 days of oral supplementation of Se and vitamin E, C – control group.

The orally supplemented group tended to have higher blood plasma Se and VTE concentrations than the control group, which probably translated to higher transfer ($p<0.05$) to colostrum. It shows that oral supplementation is more efficient and stabilizes the monitored antioxidants in blood plasma and colostrum. Our data are similar to those found in Czech Red-and-White cattle, in which there was a very close correlation between colostral and blood Se and VTE contents. Concentrations of both antioxidants in colostrum may be higher in combined breeds than in dairy breeds due to lower colostrum production (Pavlata et al. 2004a).

Evaluation of glutathione peroxidase activity

Synthesis of reactive oxygen species (ROS) and their accumulation during parturition or inflammation are controlled by antioxidant enzyme systems. Several defense mechanisms prevent oxidative damage in living organisms. These include scavenging enzymes such as GPx, superoxide dismutase (SOD), and catalase (CAT) (Andrei et al. 2011). Blood GPx activity reflects long-term Se supply since the regeneration of the enzyme is related to the erythrocyte lifespan. However, it is a matter of debate how fast the changes in GPx activity follow alterations in Se status.

For practical use, Pavlata et al. (2002) recommend the lower limit of the reference value of GPx in whole blood of cattle of 250 U/g of Hb. In our study, the activity of GPx throughout the study period in all experimental groups of cows was adequate. During calving, increased ROS accumulation results in a faster decrease in GPx activity. We observed this effect as significantly lower GPx activity in the control group after calving than in orally supplemented group D2 (Table 5). Decreased GPx activity in the control group is also closely related to the long-term reduced intake of Se in the TMR at a level of 0.2 mg/kg DM. In contrast, with a sufficient long-term supplementation of Se in the feed ration, it is possible to incorporate this element into the erythrocyte GPx and thus ensure sufficient antioxidant protection against ROS, which was also seen in our study in the orally supplemented group.

The antioxidant status does not only depend on the long-term Se supply and current status of GPx activity. Glutathione peroxidase action is complemented by vitamins and other redox systems in the elimination of ROS. Consequently, the consumption of one antioxidant may affect the concentration of the others, since the action of antioxidant enzymes also depends on their sparing effect and target tissue.

Evaluation of the occurrence of mastitis

The current content of Se and VTE in the feed ration and blood in dairy cows are closely linked to the optimal immune function and mammary gland health. Selenium and VTE deficiencies in dairy cows have been often associated with intramammary infection (Mehdi and Dufrasne 2016).

Table 6 shows that after oral supplementation of the selenium-vitamin supplements in group D2 a 25% reduction of mastitis was observed and 10 less infected quarters, compared to control cows. Table 6 shows that in our study oral supplementation of the selenium-vitamin supplements led to a 25% reduction of mastitis incidence and ten fewer infected udder quarters than in control cows. Kommisrud et al. (2005) carried out their study on 254 dairy cows of the Norwegian Red breed to highlight the importance of determining Se in the feed ration. They found that offering diets low in Se <0.1 μg/g of DM for a long time was associated with an increased incidence of mastitis by 1.3 to 1.4 times 30 days after calving.

Pavlata et al. (2004a) compared the influence of different doses of parenteral Se and VTE in dairy cows prior to parturition on selected metabolic parameters, colostrum quality, and occurence of mastitis. Cows injected two times during the dry period, 8 and 4 weeks before expected parturition, (88 mg of sodium selenite and 1 000 mg of a-tocopherol acetate) had significantly ($p<0.05$) higher concentrations of Se and VTE in the colostrum collected on the day of parturition. Moreover, no clinical cases of mastitis were reported in the parenterally supplemented group compared to 5 incidents of treated mastitis in the control group during the first month of lactation. In our study, repeated injections increased a-tocopherol acetate and Se concentrations in blood plasma compared to the control group. However, we did not observe statistically significant differences in the occurrence of mastitis 14 days after calving.

Similar results were described by Smith et al. (1997) after repeated intramuscular injection of 2 mg/kg body weight of α-tocopherol acetate and 0.1 mg/kg body weight of Se on the 42nd and the 21st day prepartum in combination with oral supplementation of 740 IU of vitamin E and 3 mg of Se per day. In early lactation, the occurrence of intramammary gland infections decreased from 63% to 37% compared to the control group supplemented with 100 IU of vitamin E/day and on a low selenium diet (<0.10 mg/kg DM).

Intramammary infections are most commonly of microbial origin, as up to 95% of mastitis is caused by pathogenic bacteria that penetrate the mammary gland through the teat canal. The bacteria causing the most common forms of mastitis may be divided into two groups. The first group consists of contagious pathogens (e.g. *Staphylococcus aureus, Streptococcus agalactiae, and Streptococcus dysgalactiale*). They survive and grow within the mammary gland and so transmission of infection from infected to uninfected quarters and from cow to cow most likely occurs during milking (Zigo et al. 2021). In the second group, we are environmental pathogens found in the environment, such as *Streptococcus uberis*, *E. coli*, non-aureus staphylococci (NAS), and *Corynebacterium spp.* (Hawari et al., 2008).

Analyzing the milk samples from the infected udder quarter, we confirmed NAS (S. *epidermidis, S. chromogenes* and *S. xylosus)* bacteria *Streptococcus uberis* and *Staphylococcus aureus,* which are most often associated with the subclinical and clinical forms of mastitis. There were no differences in the bacteria species or course of mastitis among the studied groups of cows.

Comparison of milk malondialdehyde concentrations

Intramammary infections are among to the main causes of depletion of antioxidant enzymes resulting in excess ROS accumulation and lipid peroxidation. Malondialdehyde (MDA) is generated during lipid peroxidation and is considered a biomarker of oxidative stress. The results of our study show that MDA concentrations from affected quarter milk samples were increased compared to samples from healthy cows in each monitored group. The comparison of infected milk samples among individual groups did not reveal changes in concentration of MDA (Fig. 1).

The changes in MDA concentration from affected quarter milk samples and changes in blood GPx in the present study are in accordance with the previous report (Turk et al. 2017) that showed a significant increase in MDA level in mastitis cows as compared to healthy cows.

Castillo et al. (2006) and Sharma et al. (2011) observed a higher MDA concentration in the milk samples from healthy udders and those with mastitis, as well as in the blood of cows in early lactation as compared to the cows during the dry period or mid-lactation. The activity of GPx has been confirmed to closely correlate with the antioxidant capability of the organism. Our results indicate a close relationship between inflammatory response and oxidative stress in mastitis, which can be interpreted as an important role of the antioxidant defense system in maintaining the health of the mammary gland. In order to prevent oxidative stress and moderate inflammatory response as well as to improve the immune status of dairy cows, it is important to meet their nutritive needs and balance them with an appropriate ratio of vitamin-mineral supplements involved in antioxidative defense mechanisms.

Conclusion

Both routes of Se and VTE administration to pregnant dairy cows positively influence the concentration

of a substances in the blood plasma of animals on the parturition day. However, only prolonged oral supplementation is associated with high concentrations of Se and α-tocopherol in colostrum and the blood plasma of cows on the 14th day after calving. Therefore, we conclude that oral supplementation of Se and VTE is more efficient and has a better stabilizing effect on the antioxidant enzymes in blood plasma and colostrum, thus leading to the reduced incidence of mastitis. The data obtained in this study also show that the duration of higher plasma α-tocopherol and Se concentrations is relatively short after parenteral injections. Therefore we recommend a combination of the injectable and oral vitamin E supplements to quickly increase and then maintain a high concentration of VTE and Se in cows during the postpartum period.

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