

INFLUENCE OF SELENIUM AND VITAMIN E SUPPLEMENTATION DURING PREGNANCY ON UDDER HEALTH AND MILK QUALITY IN DAIRY COWS AT PARTURITION

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ABSTRACT

Selenium and vitamin E ranks among very important antioxidant agents protecting the organism from the effect of reactive oxygen forms. The deficiency of both nutrients during pregnancy in cows often result in metabolic disorders and increased of cases of related diseases (mastitis, retained placenta and other reproductive disorders). The aim of the present work was to study the influence of different dose of parenteral administration selenium and vitamin E in dairy cows prior to parturition on selected metabolic parameters, udder health and milk quality. A total in herd of 270 Holstein cattle in east of Slovakia in a two-four lactation-gestation cycle the control group (C) and 2 experimental groups (D, D1) were selected. All groups were similarly housed, managed and fed with the diet containing from 36 to 42 mg vitamin E and 0.2 mg.kg⁻¹ Se of DM through the study period. In group D a products containing vitamin E and selenium were administered IM four weeks prior to the expected date of parturition in total dose of 1000 mg of dl- α -tocopherol acetate and of 44 mg sodium selenite per cow, respectively. In group D1 the same products were administered twice, four and two weeks prior to parturition. Blood samples were 4 weeks prior to predicted calving date (the time of treatment), on parturition day and at 14th day after calving for assessment of plasma vitamin E and selenium concentrations. Blood samples of the calves were drawn from *jugular vein* at birth and first colostrum was also collected. The occurrence of the mastitis and retained placenta during the first 14th day after calving were evaluated in all groups. Higher plasmatic and colostrum concentrations of selenium and vitamin E were found only in group with repeat application of Se and vitamin E (D1) collected on the day of parturition. At the 14th day of postpartal period a trend of lower occurrence of mastitis was observed in group D1 compared to D group, administered IM once and control group. Parenteral supplementation of selenium and vitamin E during pregnancy had no impact on their transmission into the milk and on the presence of bacterial agents in raw milk obtained from dairy cows diagnosed with mastitis.

Keywords: dairy cows; injection; milk; mastitis; vitamin E; selenium

INTRODUCTION

Most diseases in dairy cows occur at or just after calving, which is a period associated with immune suppression, resulting in an increased susceptibility to infections. Parturition immune suppression is multifactorial but is associated with endocrine changes and decreased intake of critical nutrients. Among the most important nutrients but often deficient in compound feeding stuffs, involved in the biological functions and antioxidative activity are vitamin E, and selenium (Se) compounds (Persson et al., 2007; Kafizadeh et al., 2014).

The vitamin E (α -tocopherol) 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 (Meglia et al., 2006; Persson et al., 2007).

Along with Se, vitamin E ranks among very important antioxidant agents protecting the organism from the effect of reactive oxygen forms. As an extinguisher of peroxidation reactions in membranes, vitamin E is probably the most important antioxidant in cell membranes (Pavlata et al., 2004; Balicka and Jastrzębski, 2014).

The antioxidant effect of Se depends mainly on glutathione peroxidase (GPx), in which selenium is contained (Horký et al., 2013).

Vitamin E and GPx operate at different sites in the cell. The function-site for GPx is cell cytosol and vitamin E operates within lipid membranes (Meglia et al., 2006; Mehri et al., 2013).

According to Pavlata et al. (2005), diets containing under 0.3 mg of Se.kg⁻¹ of DM, and 500 IU of vitamin

E/cow per day are deficient in antioxidants and decrease immunostimulation of organism in the dry period.

The status of both these nutrients in the blood is essential for the health and performance of cows during the periparturition period as well as the offsprings (Lacetera et al., 1996; Pavlata et al., 2012).

Application of synthetic injectable forms of Se and vitamin E seems to be the most effective solution of the problem of the requirements of the organism to both antioxidants. Especially in the periparturition period, when the oral supplementation fails to increase their reduced concentration in the blood plasma of dairy cows and is one of the ways how to increase selenium and vitamin E in functional foods from animal sources (Horký, 2014; Kafizadeh et al., 2014).

The aim of the experiment was to study the influence of different dose of parenteral administration selenium and vitamin E in dairy cows prior to parturition on selected antioxidant parameters, occurrence of mastitis and milk quality.

MATERIAL AND METHODOLOGY

Animal management

The experiment was carried out in herd of 270 Holstein cattle in east of Slovakia. Dairy cows were kept in a free housing system with a separate calving barn and equipped with individual boxes with bedding and were allowed *ad libitum* access to water. All animals received the diets based on a total mixed ration (TMR) that is required for the cows during the dry period and the beginning of lactation. The pariparturition cows were fed with the diets containing grass hay (3.8%), corn silage (45.0%), clover-grass silage (33.3%), grass haylage (3.5%), triticale grain (10.2%), soybean meal (2.8%) and concentrate (1.4%) as presented in Table 1. During *pre partum* and *post partum*, all cows received the diets containing 36 and 42 mg of vitamin E per kg of DM, respectively, but with the same amount of Se (0.2 mg.kg⁻¹ DM) in both diets. The calves were separated from the dams immediately after the birth and were artificially fed with 2 L of colostrum using a calf nursing bottle with nipple for the next 8 – 10 hours.

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. The average milk yield of the dairy cows was

7,500 ±40 kg 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.

Parenteral administration of selenium and vitamin E

In total 45 cows (aged 2.5 – 5 years) in the final period of pregnancy an average weight of 628 ±19 kg were randomly assigned into three groups (C, D, D1). Four weeks prior to the expected parturition were the cows in groups C, D and D1 treated as follows:

D – experimental group of 15 animals 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 IM once during the dry period (4 weeks prior to expected parturition) in the dose 20 mL *pro toto* of Selevit inj. *a.u.v.* and 1.7 mL *pro toto* of Erevit sol. inj. (total dose of 44 mg of sodium selenite and 1000 mg of dl- α -tocopherol acetate per cow, respectively).

D1 – experimental group of 15 animals to which the injectable products Selevit inj. *a.u.v.* and Erevit sol. inj. were administered twice during the dry period (4 and 2 weeks prior to expected parturition), on the same dose of sodium selenite and dl- α -tocopherol acetate as the group D (total dose of 88 mg of sodium selenite and 2 000 mg of dl- α -tocopherol acetate).

The control group (C, n = 15) was without parenteral supplementation of vitamin E and Se.

Collection of samples and laboratory examination

Blood samples were collected into 12 mL heparinised test tubes from the *jugular vein* of cows four weeks before the expected time of calving, on parturition day and at 14th day after calving. Blood samples of the calves were drawn from *jugular vein* at birth (before suckling). We also collected colostrum into 10 mL tubes immediately after the parturition.

On the basis of the comprehensive examinations on the 12th day according to Jackson and Cockcroft (2002)

Table 1 Nutrient composition of the pre partum and post partum rations fed.

Item	Composition	
	Pre partum	Post partum
DM (g.kg ⁻¹)	475	460
CP (g.kg ⁻¹ DM)	123.1	147.05
Fat (g.kg ⁻¹ DM)	24.7	27.9
NDF (g.kg ⁻¹ DM)	339.6	328.2
ADF (g.kg ⁻¹ DM)	222.1	209.1
NSP (g.kg ⁻¹ DM)	371.2	415.1
Starch (g.kg ⁻¹ DM)	252.2	309.1
NDP (g.kg ⁻¹ DM)	23.4	16.1
NE, MJ.kg ⁻¹	6.2	6.7
Se mg.kg ⁻¹ DM	0.2	0.2
* Vitamin E IU.kg ⁻¹	36	42

Note: *Composition – analysed values; 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, *IU – international unit of vitamin E defined as 1 mg (\pm) α -tocopherol acetate.

which consisted of a clinical examination, examination of milk from each quarter of the udder and California mastitis test (CMT). From the sampling 10 mL of the milk sample at a 45° angle to the microbiological examination was assessed the health status of the mammary gland of dairy cows and were detected different forms of mastitis (latent, subclinical, subacute and acute). 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 Van Soets et al. (1991).

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 vitamin E. All samples of blood plasma 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, colostrum were determined after wet mineralization in a closed system using a microwave (Milestone MLS 1200) digestion technique with HNO₃ and H₂O₂ by atomic absorptive spectrometer Zeman 4100 (Perkin Elmer, USA) equipped with generating device system, according to the analytical procedure standardised by Pechova et al. (2005).

The GPx activity in heparinized whole blood was measured photometrically 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⁻¹ of Hb). Haemoglobin was analyzed by Haemoglobin kits (Randox-Ransel, UK).

After the extraction of the samples of plasma, colostrum and milk in N-heptane, its evaporation and subsequent dissolution in methanol was determined in duplicate by the content of α-tocopherol analysis according to the HPLC method of Hess et al. (1991). Determination of vitamin E from the homogenized sample from TMR after saponification and extraction by HPLC method was carried out by Politis et al. (1996).

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, bacteria *Staphylococcus* spp. were selected for the tube coagulase test (Staphylo PK, ImunaPharm, SR). Suspect colonies *Staphylococcus* spp., *Streptococcus* spp. and *Enterobacteriaceae* spp. were isolated on blood agar, cultivated at 37 °C for 24 h 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. The nutritional values of TMR were determining by the AOAC methods (1995).

Statistical analysis

Tukey's post tests were used to compare all three experimental groups was indicated by ANOVA. 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).

RESULTS AND DISCUSSION

Improved intake of selenium and vitamin E is important for dairy cows because of a positive effect of these substances in prophylaxis of many health disorders, which frequently occur in cows and calves already in the early postpartum period. Such disorders consist of nutritional muscular dystrophy, reproductive disorders (retained placenta, increased incidence of endometritis and ovarian cysts), increased somatic cell count, higher occurrence of clinical forms of mastitis, immunity disorders, frequent occurrence of respiratory and gastrointestinal infections in calves (Bouwustra et al., 2010; Meyer et al., 2014).

The lowest plasma of Se and α-tocopherol concentrations are generally observed between 1 week prepartum and 2 weeks postpartum (Kafilzadeh et al., 2014).

In experimental group (D1) higher concentration ($p < 0.01$) of selenium and α-tocopherol (μg.mL⁻¹) in blood plasma and colostrum was found in comparison to D group, administered IM once during the dry period and control group (C) on the day of parturition (Table 2 and 3). Mean α-tocopherol concentrations in blood plasma in all groups were to range from 5.1 – 5.8 μg.mL⁻¹ four weeks before the expected time of calving. Tables 2 and 3 furthermore shows that in all groups not was found the differences in concentration of selenium and vitamin E in the milk at 14th day after calving.

Plasma levels of α-tocopherol up to 4.0 mg.mL⁻¹ have been reported to be adequate in cattle. Canadian researchers testing 10 clinically normal cows from 5 different herds found mean serum vitamin E concentrations in the 5 herds to range from 3.2 – 5.3 mg.mL⁻¹ (Lacetera et al., 1996).

Table 2 Effect of parenteral supplementation of selenium and vitamin E on the concentrations of α-tocopherol (μg.mL⁻¹) in blood plasma, milk and colostrum.

Period		C	D	D1
		M ±SD	M ±SD	M ±SD
28 th day <i>a. p.</i>	cows	5.5 ±0.58	5.8 ±0.54	5.1 ±0.62
	cows	4.4 ±0.76 ^a	4.1 ±0.62 ^a	6.4 ±0.86 ^b
parturition	calves	3.7 ±0.42	3.3 ±0.68	4.1 ±0.74
	colostrum	9.8 ±1.7 ^a	10.8 ±2.6 ^a	14.4 ±2.1 ^b
14 th day <i>p. p.</i>	cows	4.6 ±0.58	4.8 ±0.52	5.1 ±0.76
	milk	0.56 ±0.17	0.63 ±0.18	0.71 ±0.23

Note: D – parenteral supplemented group on 28th day before parturition; D1 – parenteral supplemented group on 28th and 14th day before parturition; C - control group; *a. p.* - *ante partum*; *p. p.* - *post partum*; ^{a, b} significance level $p < 0.05$ is presented by different superscribes in a row.

Table 3 Effect of parenteral supplementation of selenium and vitamin E on the concentrations of selenium ($\mu\text{g.L}^{-1}$) in blood plasma, milk and colostrum.

Period		C M \pm SD	D M \pm SD	D1 M \pm SD
28 th day <i>a. p.</i>	cows	75.1 \pm 6.8	76.3 \pm 7.2	74.1 \pm 6.5
	cows	69.4 \pm 6.7 ^a	67.2 \pm 7.8 ^a	81.3 \pm 6.7 ^b
parturition	calves	62.3 \pm 5.1	61.8 \pm 5.8	65.3 \pm 6.9
	colostrum	30.5 \pm 4.4 ^a	33.4 \pm 4.8 ^a	41.2 \pm 5.7 ^b
14 th day <i>p. p.</i>	cows	70.6 \pm 6.1	69.9 \pm 7.7	75.7 \pm 8.7
	milk	15.6 \pm 3.8	16.8 \pm 2.7	18.1 \pm 3.2

Note: D – parenteral supplemented group on 28th day before parturition; D1 – parenteral supplemented group on 28th and 14th day before parturition; C – control group; *a. p.* - *ante partum*; *p. p.* - *post partum*; ^{a, b} significance level $p < 0.01$ is presented by different superscribes in a row.

According to Mohri et al. (2005) calves are born with physiologically low stores of vitamin E, a fat-soluble vitamin that crosses the bovine placenta in limited amounts. Low plasma levels below 4.0 mg.mL⁻¹ in the present study have been reported in calves from the control and D groups.

Studies on vitamin E supplementation in late gestation dairy cattle have focused on enhancing cow immunity and performance, whereas such studies in beef cattle have focused on the benefits to the calf. The effects of vitamin E supplementation in pregnant beef cows have varied across experiments. Parenteral administration of 3000 IU of vitamin E to crossbred beef cows approximately one month prior to parturition increased plasma vitamin E concentrations in calves and enhanced their passive immune status (Gunter et al., 2003).

Scholz and Stöber (2002) described as also adequate a

blood level of selenium greater than 100 $\mu\text{g.L}^{-1}$. At the beginning of the period considered, the measured values of Se in the blood plasma of dairy cows were in the range of 74.1 – 76.3 $\mu\text{g.L}^{-1}$, which can be considered as marginal concentration of this element. The animals of the supplemented group D1 had significantly higher ($p < 0.01$) blood Se concentrations at day of parturition than the controls but the persistence of increased plasma levels at 14th day after parturition was not recorded.

It has been reported that blood Se concentrations below 50 $\mu\text{g.L}^{-1}$ are considered diagnostic of frank deficiency, and clinical signs, such as nutritional myodegeneration, can occur at these levels. Se is known to cross the placenta to the calf easily however it is not transferred well through colostrum or milk. Therefore, the Se status of the cow before calving is an important determinant in the Se status of calves (Grunter et al., 2003; Pavlata et al., 2004).

Table 4 Effect of parenteral supplementation of selenium and vitamin E on the activity of GPx (U.g^{-1} of Hb) in blood of dairy cows and calves.

Period		C M \pm SD	D M \pm SD	D1 M \pm SD
28 th day <i>a. p.</i>	cows	397 \pm 35.3	405 \pm 36.7	398 \pm 34.2
	cows	406 \pm 36.8	493 \pm 46.2	431 \pm 40.8
parturition	calves	274 \pm 24.5	289 \pm 26.2	317 \pm 30.4
	cows	443 \pm 42.4	428 \pm 37.4	438 \pm 35.6

Note: D – parenteral supplemented group on 28th day before parturition; D1 – parenteral supplemented group on 28th and 14th day before parturition; C – control group; *a. p.* - *ante partum*; *p. p.* - *post partum*.

Table 5 Influence if injectable supplementation of selenium and vitamin E on occurrence of mastitis, milk yield and retained placenta in multiparous dairy cows.

groups	Σ^h		Σ^i		Rejected quarters*	Infected quarters	Mastitis forms from infected quarters in % (n^{iq})				Milk production*	Retained placenta
	n	%	n	%			L	SC	SA	A		
C	11	73.3	4	26.7	2	11	9.1	18.2	18.2	36.4	33.4 \pm 6.8	0
D	11	73.3	4	26.7	0	10	0	30.0	50.0	20.0	32.6 \pm 7.9	1*
D1	13	86.6	2	13.3	1	7	0	0	57.1	42.9	34.5 \pm 6.4	0

Note: Σ^h – number of healthy dairy cows, Σ^i – number of infected dairy cows, n^{iq} – infected quarters, rejected quarters – dairy cows with atrophy or fibrosis in the mammary gland, L – latent mastitis, SB - subclinical mastitis, SA – subacute mastitis, A - acute mastitis, D –parenteral supplemented group on 28th day before parturition; D1 – parenteral supplemented group on 28th and 14th day before parturition; C – control group, Milk production* – milk production in the first month, 1* – a cow with the retained placenta was not included into the evaluation of the milk production in the first month.

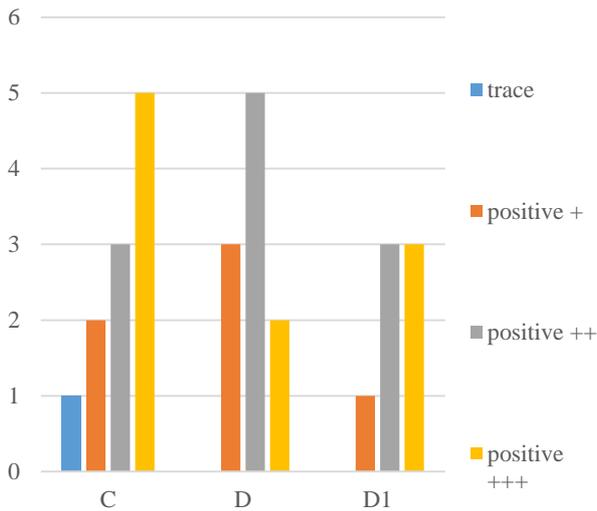


Figure 1 Number of infected mammary quarters detected by CMT at 14th day after calving in selected groups.

Legend: trace – in somatic cell up to 400×10^3 ; positive (+) - somatic cell range 400×10^3 to 1200×10^3 ; positive (++) - somatic cell range 1200×10^3 to 5000×10^3 ; positive (+++) - somatic cell range over 5000×10^3 .

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 (Pilarczyk et al., 2011; Pechová et al., 2012).

Glutathione peroxidase activity in erythrocytes from cows during the experiment did not differ between all both groups injected with Se and cows without Se. Also, GPx activity did not differ in calves from injected groups in compared with control (Table 4).

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^{-1} of Hb. In our study, the activity of GPx throughout the period under review in all experimental groups of cows and calves that is considered to be adequate for optimal GPx activity.

Table 5 shows that after repeat parenteral administration of the selenium-vitamin supplements in group D1 was observed the reduction infected quarters and cases of mastitis. In D1 group was observed reduction in the incidence of mastitis by 13.3% compared to control group. Smith et al. (1997) observed reduction of intramammary infection after repeat intramuscular injection of 2 mg of α -tocopherol acetate and 0.1 mg selenium.kg⁻¹ of body weight at 42nd and 21st days prepartum. In early lactation was determined decrease occurrence the intramammary gland infections from 37% compared to control group supplemented with 100 IU of vitamin.day⁻¹ and with low selenium diet ($<0.10 \text{ mg.kg}^{-1} \text{ DM}$). Figure 1 shows the number of infected mammary quarters detected by CMT at 14th day after calving in selected groups. The repeat parenteral supplemented group (D1) had 13.4% positive quarters and in the control group had a 11.2% increase of infected quarters (24.6%).

Positive (+) CMT were the most frequently recorded in the three cases of infected quarters group D. Positive

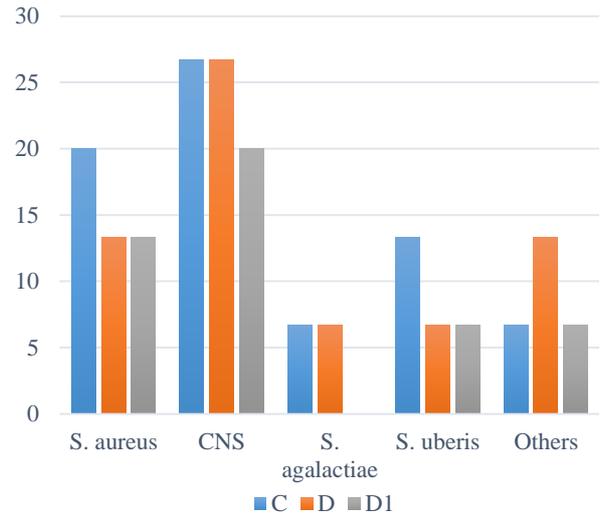


Figure 2 Aetiology of mastitis at 14th day after calving in multiparous dairy cows (%).

Legend: CNS – (coagulase negative staphylococci) - *S. epidermidis*, *S. chromogenes*, *S. xylosus* and *S. schleiferi*, Others - *Bacillus* spp. and *Enterococcus* spp.

quarters (++) were recorded in five quarters in group D and in three cases in control and D1 group. The most quarters with positive scores (+++) were recorded in control and D1 group. This explains the increase in the incidence of subacute and acute forms of mastitis in all selected groups.

Staphylococci and streptococci are the main aetiological agents of ruminant IMI. *Staphylococcus aureus* with coagulase-negative species (CNS) are the most frequent isolates from subclinical and clinical cases IMI (Vasil' et al., 2012).

CNS infection is generally seen as an increase in the SCC in milk of the infected quarter. Milk SCC usually remains below $500,000 \text{ cells.mL}^{-1}$ (Djabri et al., 2002). In a study in which dairy cows were followed-up throughout the whole lactation, the geometric mean SCC was over $600,000 \text{ cells.mL}^{-1}$ in quarters with persistent CNS infection, but only $60,000 \text{ cells.mL}^{-1}$ in healthy quarters (Taponen et al., 2007).

The annual incidence of clinical IMI in dairy herds is generally lower than 5%, but in a small percentage of herds the incidence may exceed 30 – 50% of the animals, causing mortality (gangrenous mastitis) or culling of up to 70 % of the herd (Vautor et al., 2009).

In figure 2 by our analysis of the quarter samples were mainly confirmed CNS in all experimental groups. Further been confirmed *Staphylococcus aureus* *Streptococcus uberis*, *Streptococcus agalactiae*, which are most often associated with the formation of the subacute and acute forms of mastitis. CNS and mixed infection caused subclinical and latent forms of mastitis in all experimental groups.

In a study carried out in the US and Canada, 15% of new intramammary infections post-partum were due to CNS (Dingwell et al., 2004). In an earlier Canadian study, quarter prevalence of CNS infections ranged from 5% to 6% during early lactation and increased from 14% to 17%

towards the end of lactation (Davidson et al., 1992). In a survey from Estonia, 16% of the quarters positive for bacterial growth harbored CNS (Haltia et al., 2006). The highest prevalence of intramammary infections with CNS and *S. aureus* was reported in Finland, where CNS and *S. aureus* were isolated from 50% of the positive quarters.

CONCLUSION

It can be therefore concluded that repeat parenteral administration of the product containing vitamin E and Se to pregnant dairy cows showed a positive effect on the increase of α -tocopherol and Se concentrations in blood plasma and in colostrum on the day of parturition as well as the reduction of clinical forms of mastitis 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 Se and α -tocopherol concentrations after repeat administration is relatively short and do not affect on their transmission into the milk.

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