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EFFECT OF PERORAL SUPPLEMENTATION WITH SELENIUM AND VITAMIN E DURING LATE PREGNANCY ON UDDER HEALTH AND MILK QUALITY IN DAIRY COWS

Milan Vasil', Fratišek Zigo, Juraj Elečko, Martina Zigová, Zuzana Farkašová

ABSTRACT

The aim of the experiment was to study selenium and vitamin E 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 48 cows of Holstein breed divided into four equal groups (n = 12). The first experimental group (D1) was fed with addition of vitamin E in total dose of 1020 dl-a-tocopherol acetate Se/cow per day. The second group (D2) was added the selenium at a dose of 0.3 mg.kg^{-1} of DM in form of sodium selenite. The third group (D3) was supplemented with addition of vitamin E in combination with sodium selenite in total dose of 1020 dl-a-tocopherol acetate Se/cow per day and of 5.0 mg Se/cow per day, respectively. The control group (C) was without the addition of selenium and vitamin E. In group (D2) with addition of selenium at a dose of 0.3 mg.kg^{-1} of DM and vitamin E a dose of 50 mg dl-a-tocopherol acetate/kg of DM in diet, increased the plasmatic concentration of selenium and vitamin E and reduced the incidence of mastitis by 13.3% and number of somatic cells during peripartal period in comparison with other groups.

Keywords: dairy cows; selenium; vitamin E; mastitis; SCC

INTRODUCTION

Cows that are especially highly efficient are predisposed to metabolic, infectious and reproductive diseases during the periparturient period because of immune system suppression, rapid hormonal changes during birthing, and metabolic stress associated with lactation. The periparturient period is a time during which dairy cows are at risk, and they are prone to diseases that could affect their productivity. Mastitis, hypocalcemia, fatty liver syndrome, retained placenta, metritis, ketosis and other related diseases occur frequently in this period. Due to immune system suppression during the periparturient period, such diseases cause decreases in productivity and even culling of cows **(Kafilzadeh et al., 2014; Horký, 2015)**.

Vitamins and minerals are micro-nutrient components that play important functions in all organisms, especially during reproduction. Selenium (Se) and vitamin E (VTE) are often deficient in compound feeding stuffs during dry period. Biological functions of selenium are complemented by VTE, which also shows the effects of a cellular antioxidant **(Mohri et al., 2005; Meyer et al., 2014)**.

It has been reported that the supplementation of Se and VTE during late pregnancy provides increased fertilization in the number of service per conception and pregnancy rate, decreased open days, ovarian cysts, incidence of mastitis and retained placenta **(Lacetera et al., 1996)**.

According 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 under 0.2 mg Se/kg of DM, and 500 IU of VTE/head/day are deficient for antioxidant effect and immunostimulation of organism in transition period. Fresh green forages are excellent sources of VTE, usually containing 80-200 IU VTE/kg of DM. Diets of cows in total confinement housing depend on ensiled forages and hay as a source of roughage. These forages contain only one-fifth to one-sixth the amount of vitamin E in freshly cut forages in the vegetative state.

This study aimed to determine the effects of Se and VTE application on occurence of mastitis and the quality of the produced milk in terms of functional food into the diet of dairy cows.

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.

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 ±48 kg per lactation. Milking took place in the parallel parlor 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.

The experiment included 48 Holstein dairy cows divided into four equal groups received the diets based on a total mixed ration (TMR). All animals received the diets based on a TMR that is required for the cows during the dry period and the beginning of lactation containing grass hay, corn silage, clover-grass silage, grass haylage, triticale grain, soybean meal and concentrate.

During pre partum and post partum, all cows received the diets containing 31 and 36 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.

Peroral application of Se and VTE

Four weeks prior to the expected parturition were the cows in groups D1, D2 and D3 peroral supplemented as follows:

- the first group (D1) of cows (n = 12) was supplemented with addition of Hydrovit E forte (PharmaGal, SR) in the dose 50 mg dl-a-tocopherol acetate/kg of DM in total dose of 1020 dl-a-tocopherol acetate Se/cow per day).
- the second group (D2) of cows (n = 12) was added the selenium at a dose of 0.3 $mg.kg^{-1}$ of DM in form of sodium selenite (Centralchem, SR) in total dose of 5.0 mg Se/cow per day.
- the third group (D3) of cows (n = 12) was supplemented with addition of Hydrovit E forte (PharmaGal, SR) in the dose 50 mg dl-a-tocopherol acetate/kg of DM and of 0.3 mg Se/kg in form of sodium selenite (Centralchem, SR) in total dose of 1020 dl-a-tocopherol acetate and 5.0 mg Se/cow per day, respectively).
- the control group (C) of cows (n = 12) was without the addition of selenium and vitamin E (this group of animals received only selenium and vitamin E from native sources). Selenium and vitamin E were mixed to the basic ration (TMR) and fed in the morning dose.

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. We also collected colostrum into 10 ml tubes immediately after the parturition.

On the basis of the comprehensive examinations on the 14th day according to **Jackson and Cockcroft (2002)** which consisted of a clinical examination, California mastitis test (CMT) and laboratory examination was analysed milk from each quarter of the udder. For the purpose of determining the values selected vitamin-mineral elements, was taken 1 kg comprehensive sample of TMR from feed according to **Van Soets et al. (1991)**.

Laboratory analysis

The blood plasma obtained by high speed centrifugation of heparinised blood at 3000 rpm during 15 min. The concentration of the Se in samples of feed, plasma, colostrum were determined by atomic absorptive spectrometer Zeman 4100 (Perkin Elmer, USA) according to the analytical procedure standardised by **Pechova et al. (2005)**.

The concentration of α-tocopherol in the samples of feed, plasma and colostrum were analysed by HPLC method according to **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)**. The SCC were analysed in a commercial laboratory using a MilkoScan FT2 (Foss Electric, Hillerod, Denmark).

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 *Enterobacteriacae* 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.

Statistical analysis

Tukey´s post tests were used to compare all four experimental groups and significant effect of peroral treatment 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

Selenium and vitamin E are important nutrients in animal and human areas. The receiving adequate level of selenium and vitamin E in the diet is essential for the maintaining of good health and reproduction parameters. Selenium is a part of the enzyme GPx transforming hydrogen peroxide to water and molecular oxygen. The food is low on selenium content and the total amount of antioxidants, which are associated with civilization diseases in many cases **(Horký, 2015)**.

Selenium plasma concentrations of cows is shown in Table 1. In assessing the blood selenium status we can use three basic stages of evaluation: adequate (<100 μg of $Se.L^{-1}$), marginal (70 – 100 $\mu g.L^{-1}$) and deficient (>70 $\mu g.L^{-1}$) **(Pavlata et al. 2004)**.

At the beginning of the period considered, the measured values of Se in the blood plasma of dairy cows were in the range of 72.1 – 77.3 $\mu g.L^{-1}$, which can be considered as marginal concentration of this element. The animals of the supplemented groups D2 and D3 had significantly higher blood Se concentrations at day of parturition and 14th day after than the groups D1 and C (Tab.1).

It is well accepted that vitamin E supplementation during the dry period has a positive effect on the udder health of dairy cows in early lactation as various studies reported a

Table 1 Effect of peroral supplementation of selenium and vitamin E on the concentrations of α-tocopherol ($\mu g.mL^{-1}$) and Se ($\mu g.L^{-1}$) in blood plasma, milk and colostrum.

Period			Groups C M ±SD	D1 M ±SD	D2 M ±SD	D3 M ±SD
28th day a. p.	cows	VTE	5.5 ±0.58	5.2 ±0.50	5.8 ±0.72	5.0 ±0.56
		Se	75.1 ±6.8	75.2 ±6.1	77.3 ±5.5	72.1 ±6.8
parturition	cows	VTE	4.4 ±0.76^{a}	4.3 ±0.48^{a}	8.4 ±0.76^{b}	8.2 ±0.82^{b}
		Se	69.4 ±6.7a	68.1 ±6.9a	82.5 ±5.8b	88.1 ±9.1b
	colostrum	VTE	9.8 ±1.7^{A}	10.2 ±2.1^{A}	19.7 ±2.5^{B}	18.1 ±2.3^{B}
		Se	30.5 ±4.4^{a}	35.1 ±3.1^{a}	43.1 ±4.7^{b}	44.7 ±5.6^{b}
14th day p. p.	cows	VTE	4.6 ±0.58^{a}	4.2 ±0.48^{a}	6.9 ±0.57^{b}	6.4 ±0.67^{b}
		Se	71.6 ±6.1^{a}	70.4 ±7.1^{a}	85.7 ±8.8^{b}	82.3 ±8.1^{b}

Note: *a. p. – ante partum; p. p. – post partum*; Se – selenium, VTE – vitamin E, a, b significance level p <0.01 or A, B significance level p <0.001 is presented by different superscribes in a row.

Table 2 Occurrence and aetiology of mastitis at 14th day after calving.

groups	Σ^h n	%	Σ^i n	%	Infected quarters	Mastitis forms from infected quarters (%) L	SC	SA	A	Milk produc.*	SCC* $_x10^3$
C	11	73.3	4	26.7	12	16.7	33.3	25.0	25.0	33.4 ±4.7	235 ±46^{b}
D1	11	73.3	4	26.7	11	9.1	18.2	45.5	27.3	32.6 ±5.1	229 ±38^{b}
D2	11	73.3	4	26.7	9	-	22.2	55.5	22.2	32. ±5.9	216 ±31
D3	13	86.7	2	13.3	5	-	20	60	20	35.7±6.1	174 ±54^{a}

	pathogens CPS[1]	CNS[2]	*Str. spp.*	other[3]
C	3	5	2	2
D1	2	3	4	2
D2	4	4	1	
D3	2	2		1

Note: Σ^h – number of healthy dairy cows, Σ^i – number of infected dairy cows, nIq – 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, Milk produc.* – milk production in the first month, SCC* – in the first month of lactation, CPS – (coagulase-positive staphylococci) S. aureus, S. hyicus, CNS[1] – (coagulase-negative staphylococci) S. *epidermidis, S. chromogenes, S. xylosus* and *S. schleiferi*, Str. spp. – *S. uberis*,S. agalactiae, other – *E. coli, Bacillus* spp., a, b – significance level p <0.05 is presented by different superscribes in a colum.

reduced incidence of (sub)clinical mastitis after supplementation **(Bouwstra et al. 2010)**.

Over the last 10 year, feeding strategies may have changed due to positive reports and new recommendations for supplementation VTE might only have a positive effect in studies where cows started with a marginal or deficient VTE status, which then improved during the trial because of the high level of VTE supplementation. Serum a-tocopherol concentrations (>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 $mg.mL^{-1}$ **(Batra et al., 1992)**.

Low plasma levels (<4.0 $mg.mL^{-1}$) in the present study have been reported in calves from the control and D1 groups (Table 2).

Table 2 shows that after oral administration of the selenium-vitamin supplements in group D3 was observed the reduction of cases of mastitis and infected quarters. In D1, D2 and C groups were observed the same occurence of mastitis on the level 73.3%.

Similar results were found by **Smith et al. (1997)** who supplied dairy cows with addition of 0.3 ppm selenium to all classes of cattle and feeding 1000 $IU.day^{-1}$ of supplemental vitamin E to dry cows and springing heifers and 500 $IU.day^{-1}$ to lactating cows improves immunity, reduces the incidence of clinical mastitis, and reduces SCC.

Staphylococci are the main aetiological agents of ruminant IMI and *Staphylococcus aureus* with coagulase-negative species (CNS) is the most frequent isolate from subclinical and clinical cases IMI. 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)**.

By our analysis of the quarter samples we confirmed CPS, CNS, bacteria *Streptococcus uberis*, *Streptococcus agalactiae*, which is most often associated with the formation of the subacute and acute forms of mastitis.

CONCLUSION

Supplemental vitamin E and selenium improve immune function of dairy cattle, especially during the peripartum period. An inadequate intake of selenium and vitamin E is related with an increased their plasmatic concentrationand reduced the incidence of mammary gland infections and

number of somatic cells. The application of selenium and vitamin E in feed doses is one of the ways how to increase their intake in animal food and products.

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Contact address:

Doc. MVDr. Milan Vasiľ, CSc., University of Veterinary Medicine and Pharmacy, Department of Animal Husbandry, Komenského 73, 041 81 Košice, Slovakia, E-mail: milan.vasil@uvlf.sk

MVDr. František Zigo, PhD., University of Veterinary Medicine and Pharmacy, Department of Animal Husbandry, Komenského 73, 041 81 Košice, Slovakia, E-mail: frantisek.zigo@uvlf.sk

MVDr. Juraj Elečko, CSc., University of Veterinary Medicine and Pharmacy, Department of Animal Husbandry, Komenského 73, 041 81 Košice, Slovakia, E-mail: juraj.elecko@uvlf.sk

MVDr. Martina Zigová, PhD., Pavol Jozef Šafárik University of Košice, Department of Pharmacology, Šrobárova 1014/2, 040 01 Košice, Slovakia, E-mail: chripkova.martina@gmail.com

MVDr. Zuzana Farkašová, PhD., University of Veterinary Medicine and Pharmacy, Department of Animal Husbandry, Komenského 73 041 81, Košice, Slovakia, E-mail: zuzana.farkasova@uvlf.sk