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Antioxidant status and mammary health of dairy cows fed on diets supplemented with selenite

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ABSTRACT

The peripartum period, which comprises the three weeks before and after parturition in dairy cattle, is characterized by marked physiological, metabolic and endocrine changes as the cow transitions from the metabolically demanding states of pregnancy to lactation. The dairy cows are at risk, and they are prone to various diseases (mastitis, metritis, ketosis) that could affect their productivity associated with immune suppression. The study aimed at evaluating the effect of peroral supplementing selenite at various concentrations on the selenium concentration (Se), glutathione peroxidase (GPx) activity and malondialdehyde (MDA) level of blood on the occurrence of mastitis in dairy cows during peripartum period. Forty five multiparous Holstein cows averaging 550 ± 50 kg of body weight in 2 to 4 parity were divided into three equal groups (n=15) at the end of gestation. The first experimental group (D1) was fed with addition of selenite at a dose of 0.3 mg.kg^{-1} of dry matter (DM). The second group (D2) was added the same form of selenium as in group D1 at a dose of 0.5 mg.kg^{-1} of DM. The control group (C) of cows) was fed with only selenium from native sources in content 0.2 mg per kg of DM. The blood samples for Se detection and activity of GPx were collected from the *jugular vein* of all tested cows month before the expected time of calving, on parturition day and at 14th day after calving. In group (D2) with addition of selenium at a dose of 0.5 mg/kg of DM in diet, were increased the plasmatic concentration of selenium in blood plasma and colostrum as well as reduced levels of MDA. As a result, antioxidative protection was observed decreased occurrence of mastitis by 13.3% in D2 group during the first two weeks of lactation. The application of selenium in feed have economically significant impacts such as the reduction of mastitis and is one of the ways how to increase selenium in animal food and products.

KEY WORDS: Selenium, Peroral supplementation, Cows, Peripartal period, Mastitis.

1. INTRODUCTION

Antioxidants play a role in preventing cell damage by reducing free radicals. There is a balance between free oxygen radicals and the preventive antioxidant system in healthy animals. The shift in the balance between free radicals and antioxidant system in favour of oxidants is known as oxidative stress (Bendich, 1990; Cheng, 2014).

The antioxidant defense system can be accomplished by enzymatic mechanisms such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and non-enzymatic mechanisms such as reduced glutathione, vitamins C and E, β-carotene, ceruloplasmin, bilirubin and total antioxidant capacity (TAC) (Fig. 1) (Zengin, 2017).

The occurrence of oxidative stress is often detected with increased levels of malondialdehyde (MDA), which is the last product of lipid peroxidation in diseases such as mastitis, enteritis, respiratory and hoof diseases, endoparasitic diseases, transportation, and pregnancy in ruminants. In clinical practice for accurate diagnosis, the determination of oxidative stress has become important part as a complementary component (Witko-Sarsat, 1999).

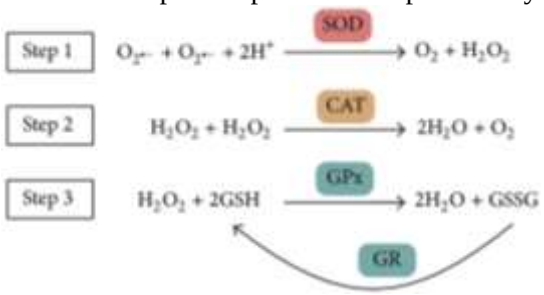

Figure.1. Main enzymatic antioxidant defense system in vivo and their reactions on scavenging free radicals and hydrogen oxide

Legend: SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; and GR, glutathione reductase.

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 (Gong, 2016).

Prepartum immune suppression is multifactorial but is associated with endocrine changes and decreased intake of critical nutrients (Horky, 2015). Adequate trace mineral nutrition during the pregnant period is essential for

an effective antioxidant defence system. It plays an important role in optimizing the immune responses and in helping the dairy cows to cope with the stress of early lactation. Micronutrient components, especially selenium is often deficient in compound feeding stuffs during dry period in dairy herds. Is one of the essential micronutrients that plays a specific role in terms of antioxidant defence of organism before oxygen radicals (Castillo, 2005; Pavlata, 2004).

Selenium forms part of the enzyme GPx, which participates in eliminating free radicals from the organism. High levels of free oxygen radicals lead to oxidation stress during which cells and tissues may suffer damage (Mohri, 2014).

The receiving adequate level of selenium in the diet is essential for the maintaining of good health and reproduction parameters. The nutritional requirements of Se in cattle are estimated at 100 μg/kg DM for beef cattle and at 300 μg/kg DM for dairy cows. Diets containing under 200 μg Se/kg of DM, are deficient for antioxidant effect and immunostimulation of organism in transition period. Selenium deficiency provokes an inflammatory process due to reduced antioxidant activity in tissues when there is an accumulation of immune cells in response to prolonged inflammation; therefore, the concentration of selenium negatively correlated with the degree of cellular infiltration in the parenchyma of the udder. A reduction in mastitis after dietary selenium intakes occurs as a result of enhanced activities of GPx (Pavlata 2004; Hall, 2014).

The aim of the present study was to examine the effect of peroral supplementing selenite at various concentrations on the changes in the activities of erythrocyte glutathione peroxidase and its functional component, selenium and malondialdehyde levels as stress marker on the occurrence of mastitis in dairy cows after calving.

2. MATERIAL AND METHODS

Animal management: The experiment was carried out in herd of 270 Holstein cattle on a farm in east of Slovakia. Cows were kept in a free housing system with a separate calving barn and were fed the diets based on a total mixed ration (TMR) that is required (NRC, 2001) 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, with Se content 0.2 mg per kg of DM in both diets. Water was offered ad libitum. Cows were milked twice a day in the parallel parlor Boumatic 2 x 10 Xpressway (Wisconsin, USA) with the average milk yield of 7,800 ± 46 kg per lactation. Before drying was applied intramammary antibiotic preparation Orbenin Dry cow a.u.v. (Pfizer, IT) to every quarter of udder.

Experimental groups and peroral supplementation of Se: The experiment included 45 healthy, multiparous cows in the last stage of pregnancy, which were randomly divided into three groups (n=15). Month before expected calving were cows in groups D1 and D2 peroral supplemented as follows:

The first group (D1) of cows 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 second group (D2) of cows was supplemented in the dose 0.5 $mg.kg^{-1}$ of DM in form of sodium selenite (Centralchem, SR) in total dose of 7.0 mg Se/cow per day.

The control group (C) of cows was without the addition of selenium with the natural Se content 0.2 mg per kg of DM. Selenium was mixed to the basic ration (TMR) and fed in the morning dose.

Collection of samples and laboratory examination: Month before the expected calving, on parturition day and at 14^{th} day after calving were collected blood samples from the *jugular vein* into 12 ml heparinized test tubes to determine Se, the activity of GPx and MDA levels. We also collected colostrum into 10 ml tubes immediately after the parturition. On the basis of the comprehensive examinations which consisted of a clinical examination, California mastitis test (CMT), and laboratory examination was analysed milk from each quarter of the udder on the 14^{th} day according to commonly accepted rules Malinowski (2006) and Jackson and Cockcroft (2002).

For the purpose of determining nutrient composition and Se values was taken 1 kg comprehensive sample of TMR from precalving and postcalving feed according to Van Soets (1991).

Laboratory analysis: The samples of heparinized blood were centrifuged for 10 minutes at 3000 rpm and plasma were separated into two 5 mL tubes. 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 by atomic absorptive spectrometer Zeman 4100 (Perkin Elmer, USA) according to the analytical procedure standardised by Pechova (2005).

The GPx activity in heparinized whole blood was measured photometrically using a set supplied by Ransel (Randox RS 505) on 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). MDA levels from plasma samples were measured spectrophotometrically according to Witko-Sarsat (1999).

Statistical analysis: Tukey´s post tests were used to compare all three 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. The results were expressed as mean (M) ± standard deviation (SD).

3. RESULTS AND DISCUSSION

Selenium (Se) is a trace element that plays an important role in the health and performance of animals. In cattle, selenium deficiency can have economically significant impacts such as reduced fertility, placental retentions, and the incidence of mastitis and metritis. The content of Se in the organism changes depending upon the amount of that element in the ration. The Se content in the soils of EU countries is very low, and that is why this microelement must be added into the diet of farm animals (Kommisrud, 2005; Horký, 2014).

The most common criterion for direct evaluation of Se status in the body is the concentration of this element in the blood (Pavlata, 2004).

According to Dargatz (1996), the rate of adequate selenium in the blood of cattle is between 80 and 160 mg/mL. In plasma, the selenium is mainly found in the albumin fraction. According to Villard (2002), the appropriate level in plasma selenium is between 51 and 85 μg/L. To achieve this adequate rate, additional dietary intake of 0.5 mg/kg DM may be enough.

Selenium plasma concentrations of cows is shown in Tab. 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 75.1 – 76.3 μg/L, which can be considered as adequate 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.

The differences in Se concentrations in cows from different groups were not, associated with changes in GPx activity (Tab. 1).

Table.1. Effect of peroral supplementation diet on the concentrations of Se (μg.L^{-1}) **in blood plasma and activity of GPx** (U.g^{-1} of Hb) **in blood**

Period	C		D1		D2	
	Se	GPx	Se	GPx	Se	GPx
	M ±SD	M ±SD	M ±SD	M ±SD	M ±SD	M ±SD
month *a. p.*	75.1 ± 6.8	402 ± 36.1	76.3 ± 7.2	405 ± 36.7	75.7 ±6.2	398 ± 34.2
parturition	70.2 ± 6.2^{a}	446 ± 36.8	75.2 ± 5.5^{a}	493 ± 46.2	82.3 ± 7.7^{b}	431 ± 40.8
14th day *p.p.*	70.6 ± 6.1^{a}	443 ± 42.4	74.9 ± 6.8	428 ± 37.4	76.7 ± 8.7^{b}	430 ± 35.6

Note: D1 – peroral supplemented group of Se at a dose of 0.3 mg.kg^{-1} of DM; D2 – peroral supplemented group of Se at a dose of 0.5 mg.kg^{-1} of DM; C – control group; par. – parturition, *a. p. - ante partum; p. p. - post partum*; $^{a, b}$ significance level p <0.05 is presented by different superscribes in a row.

GPx activity in experimental groups remained the same as in the control group. These results seem to confirm the theory that GPx activity is an indication of a long-term level of selenium intake by animals, while blood selenium levels reflect immediate selenium intake levels more promptly. Selenium is being incorporated into erythrocyte GPx during erythropoiesis, which means that the enzyme activity depends on the presence of utilizable selenium during the production of erythrocytes. Increased GPx activity after selenium supplementation is usually observed for a period of 90 to 120 days (Hogan, 1993; Kruze, 2007).

Table 2. show that after peroral supplementation of the selenium supplements in the dose 0.5 mg.kg^{-1} of DM in group D2 was observed decreased occurrence of mastitis and infected quarters by 13.3%. In control and D1 groups were observed the occurrence of mastitis on the level 26.7 % and 20.0 %, respectively.

Table.2. Influence of peroral supplementation of Se (μg.L^{-1}) **on occurrence of mastitis and MDA levels**

groups	Σ^{h}		Σ^{i}		Inf. quar.	MDA* μmol/L	Se in colostrum	Milk prod.*
	n	%	n	%		M ±SD	M ±SD	
C	11	73.3	4	26.7	10	3.70 ± 0.41^{b}	31.6 ± 3.7^{a}	33.4 ±5.8
D1	11	80	3	20.0	7	3.12 ± 0.27	34.5 ± 4.8	32.6 ±7.9
D2	13	86.6	2	13.3	5	2.65 ± 0.52^{a}	37.4 ± 4.1^{b}	34.5±6.4

Note: Σ^{h} – number of healthy dairy cows, Σ^{i} – number of infected dairy cows, Inf. quar. – infected quarters, D1 – peroral supplemented group of Se at a dose of 0.3 mg.kg^{-1} of DM; D2 – peroral supplemented group of Se at a dose of 0.5 mg.kg^{-1} of DM; C – control group, MDA* - malondialdehyde level detected from blood plasma, Milk prod.* – milk production in the first month, $^{a, b}$ significance level p <0.05 is presented by different superscribes in a collum.

According to Hall (2014) is a relationship between selenium content in the diet and mastitis frequency in cows, knowing that the phagocytic activity of neutrophils is the primary defense mechanism against mastitis. Selenium affects the innate and the adaptive immune responses of the mammary gland through humoral and cellular activities.

According to Finch (1996), several researchers have demonstrated a significant reduction in the incidence of mastitis in dairy cows after they were supplemented with selenium.

According to Eulogio (2012) the performance and economic feasibility of the use of selenium allowed to obtain a profit margin of 0.21 $ per animal per day. The addition selenium sources to the diet of ruminants can also improve the increasing of Se concentration in milk and reduces the amount of somatic cells, which are the indicators of inflammatory diseases of the mammary gland

4. CONCLUSION

Blood selenium concentration plays an important role in maintaining mammary gland health. The selenium addition at a dose of change to 0.5 $mg.kg^{-1}$ of the diet increased the concentration of selenium in the blood plasma, colostrum and leads to a reduction of MDA levels and incidence of mastitis during peripartal period.

Therefore, assessing Se status of the body, especially during the period of transition, is very important to establish nutritional strategies early enough in the dry period that could improve transition cow health. The application of Se in feed doses is one of the ways, how to increase Se in colostrum and animal products.

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