Original paper

THE EFFECT OF SUPPLEMENTATION ON SELENIUM AND ZINC CONTENT IN BLOOD AND MILK OF DAIRY COWS

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Abstract

Milk is an important source of microelements for calves during the suckling period as well as in human nutrition. Concentration of trace elements in blood and their secretion via milk can significantly change depending on food intake and composition. Proper control of selenium and zinc content in blood and in milk can improve the status of these microelements, so that the occurrence of deficiency or excessive quantity due to their increased intake can be prevented. This paper presents the results of the study on the concentration of selenium and zinc in the blood and milk of diary cows whose rations have been supplemented by organic forms of selenium (0.2 mg/kg DM) and zinc (40 mg/kg DM) during the last ten days of dry period and early lactation. Supplemented cows in trial groups A and B achieved significatly higher concentrations of these microelements in blood (Se 186.70±8.50 μg/L vs. 118.80±7.05 μg/L), blood serum (Zn 1204.70±109.5 μg/L vs. 1095.40±130.2 μg/L) and milk (Se 57.30±8.05 vs. 21.30±4.60 μg/L; Zn 2893.90±120.15 μg/L vs. 1952.10±130.50 μg/L) on 60th day postpartum compared to non-supplemented control.

Key words: *blood, dairy cows, milk, selenium, zinc, supplementation*

Introduction

Microelements (selenium, zinc, copper, manganese and iodine) have an important role in preserving dairy cows health, immune system functions and optimal reproductive and production characteristics (Joksimović and Davidović, 2006 and 2007; Siciliano-Jones et al., 2008; Spears and Weiss, 2008). The concentration of microelements in milk varies depending on the content of these elements in soil and in dairy cow feeds (Malbe et al., 2010). Supplementation of animal feeds with critical microelements has become a common practice in order to prevent defficiency disorders and secure maximum productivity. However, the range of increase of their concentration in milk is limited by biochemical homeostatic mechanisms (Pechová et al., 2008).

Selenium (Se) is an essential microelement for human and animal health. Selenomethionine (Se-Met) is the most important nutritional form of organic selenium in different cereals (Weiss and Hogan, 2005). The main advantage of organic selenium is its improved retention in tissues which thereby increases the selenium body reserves. These reserves are particularly important in stress conditions, during disease and gestation when the need for selenium increases and food supply is usually decreased due to lower food consumption (Gunter et al., 2003). In stress conditions, Se-Met is being released through protein catabolism and used as selenium source for newly synthesized selenoproteins such as GSH-Px, thioredoxin reductase and methionine sulphoxide reductase. These enzymes extinguish a variety of free radicals contributing to maintaining animals health and high productive and reproductive in stress conditions (Joksimović Todorović et al., 2012).

Recommendations of the National Research Council (2001) are that inorganic Se be added into the dairy cow rations in the quantity from 0.1 to 0.3 mg/kg dry matter (DM); however FDA (2003) ammended the regulation by approving the use of up to 0.3 ppm daily selenized yeast in the nutrition of dairy and fattening animals. Maximum tolerant level can amount to 0.5 ppm (Steven and Elliott, 2007). Concentration of selenium in milk increases linearly with the intake of Se in the form of selenized yeast (SY) or other high-Se food, but only to a certain level. Bioactivity of selenium from selenized yeast is estimated to be 20% higher than in inorganic selenium (Weiss, 2005).

It is confirmed that addition of organic forms of Se increases the Se concentration in milk (Juniper et al., 2006; Knowles et al., 2006; Muňiz-Naveiro et al., 2006). Small Se anions are rapid transported through digestive tract membrane (Pechová et al., 2008). Ran et al. (2010) proved that on day 60, 75 and 90 of lactation the selenium concentration in blood of cows supplemented by selenized yeast was 10, 11 and 14% higher in comparison to the cows supplemented inorganic selenium. Many authors indicate that the concentration of microelements is generally higher in colostrum than in ripe milk (Pavlata et al., 2004). After the colostral period, selenium concentration decreases (Hosnedlová et al., 2005). Negative correlation of Se content in relation to lactation days, can be caused by emptying the Se body reserves because of its gradual loss via milk. Weiss and Hogan (2005) determined that Se concentration in colostrum was about 3.8 times higher than in milk ($P<0.01$) in dairy cows supplemented by 0.3 mg/kg DM selenized yeast or sodium selenate during the dry period and first 28 days of lactation. Selenium concentration in colostrum and milk were 1.5 to 2 times higher ($P<0.01$) in cows fed SY compared to those supplemented with selenate.

Zinc (Zn) is an essential microlement and represents an integral component of over 300 enzymes. It influences reproductive functions, secretion of gonadotropin, androgen, prostaglandin and prolactin (Suchý et al., 1998) and plays a role of antioxidant (Arthur, 2001). Natural sources of zinc for farm animals can be found in brans, cereals and animal yeast (Suchý et al., 1998). Average concentration of zinc in food is 36 mg/kg DM. The dairy cow requirement for zinc is 50 mg/kg DM (min 45, max 250 mg/kg DM), for lactating dairy cows 850-1200 mg zinc daily (per consumption of 17-24 kg dry matter daily), and for cows in dry period 600 mg daily (per consumption of 12 kg dry matter) (Kruczyńska, 2004). The addition of chelate forms of zinc increases its utilisation by microorganisms in rumen and increases its transport to the blood or tissues (Strusińska et al., 2003). The National Research Council (2001) recommends that dairy cow feeds should be zinc supplemented in the quantity from 40 to 60 mg/kg DM.

Zinc cations are accumulated in blood, tissues and in a great degree in milk independently on the intake what is associated with regulation of intestinal absorption and change of metabolic demands (Windisch, 2002). Zinc absorption is an active process, in which zinc is partly retained in intestine mucosa from where it is slowly released (Pechová et al., 2008). Absorbed zinc can link with albumin and be transported through liver towards tissues. The greatest part of zinc in the organism is situated in muscular tissue, liver, bones and mammary gland (Suchý et al., 1998). In blood, zinc in plasma is 75% linked primarily to proteins, 33% to erythrocytes and 3% to leukocytes (Bencko et al., 1995). Zinc concentration in plasma is about 15 µmol/L, out of which 84% is linked to albumin, 15% to α_2-macroglobulin and 1% to amino acids (Tapiero and Tew, 2003). Zinc content in blood can be reduced by 50% in the states of high stress, traumas or inflammatory processes (Zadák, 2002). After the colostral period, zinc concentration does not change significantly (Hosnedlová et al., 2005).

Kellogg et al. (2004) suggest that zinc methionine significantly increases lactation performances, milk quantity and milk fat, and improves health state of mammary gland since it reduces the somatic cell count (SSC) by 33.3% improving the structure of keratin in teat ducts. Cope et al. (2009) observed that addition of recommended levels of zinc chelate form in the feeds for dairy cows significantly increases the milk quantity (37.6 kg/day) in relation to inorganic zinc (35.2 kg/day) or lower levels of organic chelate zinc (35.2 kg/day). Yang et al. (2011) report that exchanging 30% inorganic copper, zinc and manganese by chelate forms of these nutrients 6 weeks prepartum up to 305 days of lactation in dairy cows leads to increase of milk production by 6.5% and reduces SCC by 34%.

Dairy cow milk contains 2-6 mg/L zinc and this value is higher than Se concentration which is 2-60 µg/L (Illek et al., 2000; Knowles et al., 2006). Zinc in milk is primarily bound to colloid calcium phosphate in casein micelles (Silva et al., 2001). Almost 90% zinc is bound to casein in ripe milk, while 60% zinc is bound to casein in colostrum (Kincaid and Cronrath, 1992). Rodriguez et al. (2001) determined that concentrations of selenium and zinc in milk vary significantly throughout the season decreasing from March to September and being significantly higher during winter and spring than during summer and autumn. These seasonal differences are probably caused by various contents of microelements in food.

The aim of this paper was to determine the effect of supplementation of organic selenium (selenized yeast, SY) and organic zinc (zinc methionine, ZnMet) into bovine rations on the concentration of these microelements in blood and milk of Holstein-Friesian cows.

Material and methods

The trial was conducted on 30 cows of Holstein-Friesian breed (13 secundipara and 17 third calving), in the intensive keeping system and on 10^{th} day before expected calving randomly divided into three equal groups consisting of ten cows each. Control group was fed basic ration with no supplements of selenium and zinc in food. Trial groups were fed rations supplemented by microelements starting from 10^{th} day before expected calving (-10 d) up to 60^{th} day of lactation (60 d). Trial group A was supplemented by 0.2 mg/kg DM organic selenium in the form of selenized yeast (Sel-Plex 50, Alltech, Inc), and trial group B received 40 mg/kg DM organically bound zinc in the form of zinc methionine (Bioplex Zn, Alltech, Inc). Average consumption of DM was about 20 kg. Cows were fed individually and water was available *ad libitum*. Blood for analysis (20 mL) was taken from each animal in heparinized test tubes by punctioning the *v. jugularis* on 10^{th} day prepartum and 60^{th} day postpartum, in the morning before feeding. Milking was performed twice daily and milk samples were taken on 60^{th} day of lactation during the morning milking. Determination of selenium concentrations in blood, zinc in milk serum, selenium and zinc in milk was performed by hydrid procedure of atomic absorption spectrophotometry (AAS). Results were statistically processed by means of software package SPSS 8.0. Intergroup comparisons were analyzed using Student t-test.

Results and discussion

Table 1 presents the results of the study on average concentration of selenium in whole blood and milk on 10th day prepartum and 60th day of lactation.

Table 1. *Selenium concentration (Mean±SD) in whole blood and in milk on 10th day prepartum and 60th day of lactation*

Period	Concentration of selenium in blood (µg/L)			Concentration of selenium in milk (µg/L)		
	Control group (n=10)	Trial group A (n=10)	Statistical significance between the groups in the same period	Control group (n=10)	Trial group A (n=10)	Statistical significance between the groups in the same period
-10 d	120.10±10.02	119.10±8.40	NS	20.10±4.12	25.50±6.62	NS
60 d	118.80±7.05	186.70±8.50	**	21.30±4.60	57.30±8.05	**
Statistical significance within each group in different periods	NS	**	-	NS	**	-

NS non-significant, P>0.05; **P<0.01

Blood and milk selenium concentration in cows from Trial A and Control did not differ on the tenth day prepartum. On the sixtieth day of lactation selenium concentration in whole blood (186.70±8,50 µg/L) and milk (57.30±8.05 µg/L) of supplemented cows was significantly higher (P<0.01) compared to control. In Trial group A the addition of selenised yeast into dairy cow ration increased selenium concentration in blood and milk by 50% and 100%, respectively.

The results obtained correspond to the results of Ortman and Pehrson (1999), Knowles et al. (2006), Slavik et al. (2008) and Ran et al. (2010), who determined that addition of different forms of selenium in dairy cow rations can increase the concentration of selenium in milk. Ran et al. (2010) proved that addition of selenized yeast in the nutrition of dairy cows in prepartum period and period of early lactation significantly increases the concentration of selenium in milk within first three months of lactation. The results of our research corroborate the results of Ceballos et al. (2009) who determined that addition of 6 mg selenium into bovine rations on a dialy basis within the period of 75 days satisfies the needs of individual and enables the increase of intake of selenium in consuming dairy products. Ortman and Pehrson (1999) added 3 mg selenium daily, and Gunter et al. (2003) added 2.8 mg selenium in feed daily and found that selenized yeast more efficiently increases the concentrations of selenium in the blood of dairy cows in relation to inorganic selenium.

In the trial carried out by Pavlata et al. (2000) the content of selenium in bovine blood serum was not lower than 100 µg/L. Weiss and Hogan (2005) reported that in cows whose ration was supplemented by 0.3 mg/kg DM selenized yeast from the 60th day prepartum up to the 28th lactation day the blood serum selenium concentration at calving (0.059 mg/L) and on the 28th lactation day (0.074 mg/L) was about 1.4 times higher compared to cows supplemented by the same quantity of sodium selenat (0.043 mg/L and 0.054 mg/L). The results obtained by Maus et al. (1980) and Weiss et al. (1990) show that the concentration of selenium in dairy cows plasma ranges from 1016-1397 nmol/L (or 0.08-0.11 μg/mL) after addition of 0.25 - 0.3 mg/kg DM dietary selenium. Jukola et al. (1996) state that the optimal preventative action against mastitis can be obtained when the whole blood selenium concentration is higher than 0.18 μg/mL and in blood plasma 0.08 μg/mL. Similar values were obtained in our trial by adding selenized yeast to cows ration.

Table 2 displays the results of the research on average concentration of selenium in whole blood and milk on 10th day prepartum and on 60th day of lactation.

Table 2. *Zinc concentration (Mean±SD) in blood serum and in milk on 10th day prepartum and on 60th day of lactation*

Period	Concentration of zinc in blood serum (μg/L)			Concentration of zinc in milk (μg/L)		
	Control group (n=10)	Trial group B (n=10)	Statistical significance between the groups in the same period	Control group (n=10)	Trial group B (n=10)	Statistical significance between the groups in the same period
-10 d	1070.50± 150.2	1055.50± 112.1	NS	1970.20± 140.8	1962.30± 135.1	NS
60 d	1095.40± 130.2	1204.70± 109.5	*	1952.10± 130.5	2893.90± 120.1	*
Statistical significance within each group in different periods	NS	*	-	NS	*	-

NS non-significant, P>0.05; *P<0.05

There was no significant difference in the concentration of zinc in blood serum and milk between Control and Trial group B on the tenth day prepartum. Supplementation of organically bound zinc resulted in significant increase (P<0.05) of zinc concentration in blood serum (1204.70±109.5 μg/L) and milk (2893.90±120.1 μg/L) compared to control on the sixieth day of lactation. Statistically significant increase of zinc concentration in blood serum and milk was confirmed also in Trial B group on the 60th day postpartum.

Our results correspond with the research of Pechová et al. (2008), who during the first half of lactation supplemented their rations with 0.4 mg/kg DM selenium and 105 mg/kg DM zinc, the average consumption of DM being 20.5 kg. Authors determined that the concentration of selenium in whole blood (183.75±29.63 μg/L) was higher than in milk (28.59±7.12 μg/L), while the content of zinc in blood serum (1063.92±181.13 μg/L) was lower than in milk (3855.2±814.7 μg/L). In this respect, Pechová et al. (2008) did not determine significant correlation between the concentrations of these microelements in milk and blood nor between milk production and concentration of these microelements in milk. Zinc content in milk in our trial was within usual values for consumable milk 2-6 mg/L (Knowles et al., 2006).

Transport of zinc into milk is an active process (Kelleher and Lonnerdal, 2003), and in the milk of dairy cows zinc is linked primarily to casein (Kincaid and Cronrath, 1992). Dobrzańsi et al. (2005) determined an average zinc content of 2.575±644 μg/L in full blood of clinically healthy cows in full lactation and in the age of 4-12 years. Kruczyńská

(1992) confirmed that the level of zinc in blood serum of cows has an average value of 13.0-48.7 µmol/L, and in calves 10.7-39.0 µmol/L. Average value of zinc in dairy cows plasma is 10.5-17 µmol/L (Masoero et al., 1998), while Strusińska and Iwańska (1994) established the value of 15.7 µmol/L zinc in blood plasma in cows 60 days postpartum.

Campillo et al. (1998) determined that the concentration of zinc in fresh cows milk is 3±0.2 µg/mL and in dried milk 23.5±0.3 µg/g.

Hosnedlová et al. (2005) analysed 50 samples of milk of dairy cows collected in the period between May and June and determined an average value of zinc to be 4.67±0.64 mg/L milk. These authors, on all examined farms, obtained higher values than those obtained in our research. The concentration of zinc in milk was highest on the farms with milk production of over 7000l daily (4.96±0.21 mg/L) and lowest on the farms with the production of up to 1000l daily (4.18±1.30 mg/L).

Conclusion

Supplementation of dairy cow rations with organic selenium (selenized yeast, SY) and organic zinc (zinc methionine, ZnMet) from the tenth day before expected calving up to the sithieth day of lactation has a positive effect on the concentrations of these nutrients in cow blood and milk.

Compared to the control group receiving no such supplementation the cows in trial groups on the sixtieth day postpartum had:

- Significantly higher blood selenium concentration (186.70±8.50 µg/L vs 118.80±7.05 µg/L);
- Significantly higher blood serum zinc concentration (1204.70±109.5 µg/L vs 1095.40 µg/L ± 130.2);
- Significantly higher selenium (57.30±8.05 vs 21.30±4.60 µg/L) and zinc concentrations in milk (2893.90±120.15 µg/L vs 1952.10±130.50 µg/L);
- Established differences were at the level of significant (P<0.05) for zinc and very significant (P<0.01) for selenium.

Within the trial groups the addition of microelements into dairy cow rations resulted in:

- The increase of selenium blood and milk concentrations in Trial group A cows by approximately 50% and over 100%, respectively, the established differences at the beginning and at the end of trial being at the level of very significant (P<0.01);
- Statistically significant increase of zinc concentrations in Trial group B cows blood serum and milk on the sixtieth day postpartum compared to the tenth day prepartum (P<0.05).

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