

# Influence of parenteral administration of selenium and vitamin E during pregnancy on selected metabolic parameters and colostrum quality in dairy cows at parturition

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**ABSTRACT:** 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 and colostrum quality. A total of 19 dairy cows from a farm with selenium deficiency were included in the study. The cows were divided in 3 groups (C, E1, and E2). In group E1 a product containing selenium and vitamin E (Selevit inj. a.u.v.) was administered IM four weeks prior to the expected date of parturition. In group E2 the same product was administered twice, eight and four weeks prior to parturition. Group C consisted of control animals to which no product was administered. On the day of parturition samples of blood and first colostrum were collected for laboratory examination. Concentrations of selenium were determined in blood and that of vitamin E, thyroid hormones (T3 and T4) and activities of enzymes detecting muscular damage (CK, AST, LD) were determined in serum. Colostrum was analysed to determine the concentrations of selenium, vitamin E, immunoglobulins, as well as to determine its density. The occurrence of the disease during the first month after parturition was evaluated in all groups. Higher concentrations of selenium and vitamin E were found in the samples (experimental groups E1 and E2) collected on the day of parturition. Group E2 showed a significantly ( $P < 0.05$ ) higher T3 concentration compared to groups C and E1 ( $3.05 \pm 0.42$  nmol/l vs  $1.88 \pm 0.71$  and  $1.81 \pm 0.30$  nmol/l, respectively). The same pattern was confirmed for immunoglobulins concentrations in colostrum ( $34.08 \pm 5.93$  U ZST vs  $22.87 \pm 5.41$  and  $21.38 \pm 8.33$  U ZST, respectively). Compared to group C, cows in group E2 also showed significantly ( $P < 0.05$ ) higher concentrations of selenium in colostrum ( $45.43 \pm 10.56$  vs  $29.29 \pm 8.42$  µg/l). The administration of selenium and vitamin E did not influence other parameters evaluated in the study. During the first 30 days of the postpartum period a trend of lower occurrence of mastitis was observed in group E2 compared to both group C and E1 (no case of mastitis compared to 5 and 4 cases of treated mastitis, respectively).

**Keywords:** cattle; immunoglobulins; thyroid hormones; T3; mastitis

Selenium is present in all tissues of the organism. Physiological functions of selenium are mediated in particular by selenoproteins (Underwood and Suttle, 1999). Glutathione peroxidases (GSH-Px 1-4) belong among the most important selenoproteins, whose main function coincides with their antioxidative effect. Iodothyronine deiodinase is another important selenoprotein. This enzyme regulates thyroxine (T4) conversion to biologically active 3,3',5-triiodothyronine (T3) or to re-

verse triiodothyronine (rT3). The latter represents a thyroid hormone in an inactive form. The following substances belong among other known selenoproteins: thioredoxin reductases, selenoproteins P, W, R, T, N, selenium binding proteins (58, 56 and 14 kDa), sperm capsule selenoprotein, rat prostate gland glandular epithelial cells protein, and others (Arthur et al., 1990; Larsen and Berry, 1995; Fuchs, 1996; Kohrle, 2000; Zimmermann and Kohrle, 2002; Birringer et al., 2002).

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Insufficient selenium intake by the organism is manifested by numerous biochemical changes, such as reduced selenium concentration and GSH-Px activity in blood and tissues, and increased activity of creatine kinase (CK), aspartate aminotransferase (AST) and lactate dehydrogenase (LD) in serum due to muscular damage, increased production of reactive forms of oxygen and final products of lipoperoxidation in blood and tissues (malonyl dialdehyde, thiobarbituric acid reactive substances (TBARS),  $F_2$ -isoprostanes), as well as other possible secondary changes like shift in T3 : T4 ratio (Underwood and Suttle, 1999).

Biological functions of selenium are complemented by vitamin E, which also shows the effects of a cellular antioxidant. Therefore some clinical manifestations of hypovitaminosis E and selenium deficiency are very similar. Therapy and prophylaxis of these disorders usually consist of the administration of both substances together (Smith, 1996; Smith et al., 1997; Weiss, 1998; Allison and Laven, 2000). Deficiencies of selenium and vitamin E are frequently diagnosed also in the Czech Republic (Pavlata et al., 1999, 2002a), which requires treatment by administration of products containing selenium and vitamin E (Pavlata et al., 1999, 2001a, 2002b). The aim of the present work was to study selected metabolic parameters and colostrum quality after the administration of such product to dairy cows in the final period of pregnancy.

## MATERIAL AND METHODS

This work goes out from a part of an experiment realised in a framework of an investigation of selenium metabolism in the maternal transfer of selenium to newborn calves (Pavlata et al., 2003). The method in this work is expanded and results are evaluated from the view of an influence of selenium and vitamin E on selected metabolic parameters, colostrum quality and state of health.

### Description of farm and animals

The study was carried out on a dairy farm FADOM at Dolni Mesto, district Havlickuv Brod, Czech Republic in autumn. The herd consisted of Red-and-White dairy cows (Czech Red-and-White dual purpose breed crossed with Red Holsteins up to 80%). Total capacity of the farm was 400 animals. Selenium deficiency was repeatedly diagnosed in

this herd. The last examination prior to start of the study revealed mean selenium concentration in whole blood of  $45.3 \pm 10.7 \mu\text{g/l}$  in 8 cows from the group of late pregnant animals. Increased occurrence of mastitis was also detected in the herd (862 treated cases during the last year). The same applies to retained placenta (13% of all cows that calved during the year) and metritis (18.5% of all cows that calved during the year). Isolated cases of clinical forms of nutritional muscular dystrophy were found in calves (10 cases during the last year). The animals were kept in a free housing system with a separate calving barn and equipped with individual boxes with bedding. The cows were divided in groups according to the lactation and reproduction stage. A total mixed ration (TMR) wagon was used for feeding the animals. The ration of dry cows included in the study consisted of 15 kg of preserved forage (5 kg of maize silage, 10 kg of grass haylage) and 4 kg of meadow hay. Three weeks prior to the expected parturition a grain mixture in the quantity of 3.5 kg per animal (wheat, barley, extracted soya meal, rapeseed cake, limestone, salt) was added to the feed. In total 19 animals (aged 2.5–10 years) in the final period of pregnancy were included in the study. On the same day the animals were randomly divided in 3 groups:

C – control group of 5 animals to which no product containing selenium and vitamin E was administered prior to parturition;

E1 – experimental group of 7 animals to which the injectable product Selevit inj. a.u.v (sodium selenite 2.2 mg, alpha tocopherol acetate 25 mg in 1 ml of the solution) was administered once during the dry period (4 weeks prior to expected parturition) in the dose recommended by manufacturer, i.e. 20 ml IM (44 mg of sodium selenite and 500 mg of alpha tocopherol acetate);

E2 – experimental group of 7 animals to which the injectable product Selevit inj. a.u.v was administered twice during the dry period (8 and 4 weeks prior to expected parturition), i.e. in the total dose of 88 mg of sodium selenite and 1 000 mg of alpha tocopherol acetate.

### Collection of samples and laboratory examination

Blood samples from all the cows were collected on the day of parturition. First colostrum was also collected.

The following values were measured in the samples: whole blood treated with heparin – selenium; blood serum – activity of AST, CK, LD, concentration of vitamin E, T3, T4; colostrum – density, content of immunoglobulins (IG), concentration of selenium and vitamin E.

The method of selenium determination consisted of the following steps: samples of whole heparinised blood and colostrum were mineralised in a closed system using a microwave (Milestone MLS 1200) digestion technique with HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>. Samples were evaporated and the mineral residue was dissolved in water to which 20% HCl was added. Selenium was then determined by Unicam 939 AA Spectrometer using a hydride AAS technique. Catalytic activity of AST and LD was determined by an automatic analyser Cobas Mira using standardised photometric methods with BioVendor sets (<sup>L</sup>AST – catalogue No. 10351, and <sup>L</sup>LDH – catalogue No. 12353). Catalytic activity of CK was determined using the same machine and the set Kreatinkinasa NAC 105 (Lachema a.s.). Vitamin E (alpha tocopherol) was determined by fluorometry according to Bouda et al. (1980) using a fluorescence spectrophotometer 204 Perkin-Elmer. Thyroid hormones were determined using Immulite machine by means of chemiluminescence sets by BioVendor (Total T3 – LKT 31 and Total T4 – LKT 41). Colostrum density was measured by densimeter (colostrometer). Immunoglobulins were determined by means of zinc sulfate turbidity reaction (Slanina et al., 1976) after removal of casein by rennet coagulation (Dolezalova, 1978).

### Assessment of health disorders

An assessment of the occurrence of clinical forms of diseases was done in the animals of different groups. Cases which required treatment during

the first 30 days of the postpartum period were included in the assessment.

### Statistical methods

Basic statistical parameters of results (means, standard deviations) in individual groups, and a comparison between results of groups (using the Student's *t*-test after *F*-test for equality of variations) were computed using Microsoft Excel 97.

## RESULTS AND DISCUSSION

In experimental groups (E1 and E2) higher selenium concentrations were found in comparison to control group (C) on the day of parturition (Table 1). Mean selenium concentrations in whole blood in all groups were far below the recommended value of 100 µg/l (Van Saun, 1990; Pavlata et al., 2000). In cows of group E2 the difference was significant ( $P < 0.05$ ). Table 1 furthermore shows that a significantly higher vitamin E concentration was found in cows in group E2 compared to both control group and group E1 ( $P < 0.05$ ). Mean vitamin E concentration in serum in all groups were around the minimum recommended value of 8.12 µmol/l (Jagos et al., 1981). The results showed that repeated administration of Selevit inj. a.u.v. to pregnant cows was beneficial, although the deficiency in blood was not completely compensated. 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

Table 1. Effect of parenteral administration of selenium and vitamin E on blood parameters of cows (C – control group, E1 and E2 – treatment groups) at parturition

		Se – whole blood (µg/l)	Vit. E – serum (µmol/l)	T3 – serum (nmol/l)	T4 – serum (nmol/l)
C	Mean ± SD	41.13 ± 11.08 <sup>a</sup>	7.13 ± 1.59 <sup>a</sup>	1.88 ± 0.71 <sup>a</sup>	48.64 ± 15.98
E1	Mean ± SD	51.64 ± 16.42	8.22 ± 1.91 <sup>b</sup>	1.81 ± 0.30 <sup>b</sup>	43.21 ± 8.58
E2	Mean ± SD	61.63 ± 8.23 <sup>a</sup>	10.50 ± 1.86 <sup>ab</sup>	3.05 ± 0.42 <sup>ab</sup>	56.10 ± 13.18

<sup>a,a</sup>; <sup>b,b</sup>  $P < 0.05$ )

occurrence of clinical forms of mastitis, immunity disorders, frequent occurrence of respiratory and gastrointestinal infections in calves, disorders of thermoregulation in calves born in cold weather, etc. (Brzezinska-Slebodzinska et al., 1994; Cartens, 1994; McDowell et al., 1996; Smith, 1996; Smith et al., 1997; Weiss, 1998; Underwood and Suttle, 1999; Allison and Laven, 2000).

The examination of thyroid hormones revealed that in cows injected with selenium and vitamin E 4 and 8 weeks prior to parturition had higher T3 concentrations at parturition ( $P < 0.05$ ) than cows injected 4 weeks prior to parturition or control cows (Table 1). The difference can be probably explained by an increased activity of iodothyronine deiodinase, which participates in the conversion of T4 into active T3 and which activity is influenced by selenium (Arthur et al., 1990; Larsen and Berry, 1995; Kohrle, 2000). Similar results confirming a positive effect of selenium on T3 concentrations were presented for instance by Wichtel et al. (1994) and Awadeh et al. (1998), who observed increased T3 concentrations in cows and calves after higher selenium intake. The ratio of T3 : T4 calculated in our work (0.039–0.055) is close to the results of the previously mentioned authors (0.023–0.040). It can be therefore concluded from our observations that after a repeated administration of a product containing the combination of selenium and vitamin E during the period prior to parturition a positive effect of increased selenium intake on higher T3 concentrations and higher T3 : T4 ratio was confirmed.

No conclusive relation was found between the activity of enzymes detecting muscular damage

and the intake of selenium and vitamin E. Mean activities of the enzymes in blood serum in different groups of cows were slightly increased (AST 1.40–1.56  $\mu\text{kat/l}$ ; CK 3.17–5.59  $\mu\text{kat/l}$ , and LD 28.00 to 38.34  $\mu\text{kat/l}$ ) over the reference limits for healthy animals (AST  $< 1.41$ ; CK  $< 0.60$ ; LD  $< 29.05$   $\mu\text{kat/l}$ ) (Vrzgula and Sokol, 1990; Pechova, 1992). However, there were no significant differences among the groups. Higher increase in CK activity compared to the values of AST and LD may be attributed to the fact that samples were collected on the day of parturition when the cows were stressed to a different extent and certain muscular damage may occur. Obviously a low intake of selenium and vitamin E may predispose to muscular damage (Pavlata et al., 2001b).

Colostrum examination revealed a trend towards higher selenium concentrations in cows after the administration of Selevit inj. a.u.v. (Figure 1). Mean selenium concentration found in group E2 (45.43  $\pm$  10.56  $\mu\text{g/l}$ ) is significantly ( $P < 0.05$ ) higher than in control group (29.29  $\pm$  8.42  $\mu\text{g/l}$ ). Figure 2 shows that after the administration of Selevit inj. a.u.v. the confirmed higher concentration of vitamin E in blood of cows did not lead to a corresponding increase in colostrum. On the contrary, in cows of group E2, where the concentration of vitamin E was the highest, lowest levels of this vitamin were found in colostrum. No relation between the concentration of vitamin E in serum of the dam and its concentration in colostrum was determined. Sufficient intake of vitamin E through colostrum is extremely important for newborn ruminants, because fat-soluble vitamins are transported only in a limited degree

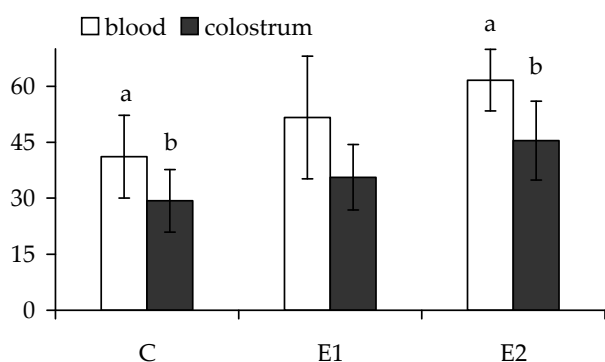


Figure 1. Concentration of selenium ( $\mu\text{g/l}$ ) in whole blood and colostrum of cows (C – control group, E1 and E2 – treatment groups) at parturition

a:a; b:b  $P < 0.05$

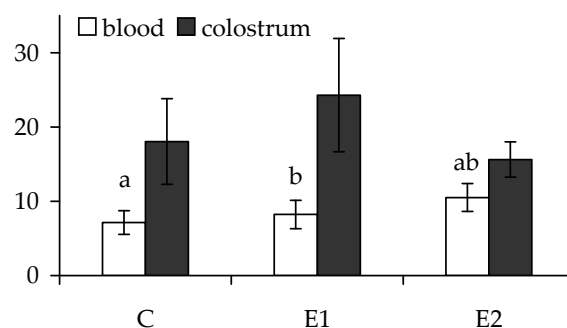


Figure 2. Concentration of vitamin E ( $\mu\text{mol/l}$ ) in serum and colostrum of cows (C – control group, E1 and E2 – treatment groups) at parturition

a:a; b:b  $P < 0.05$

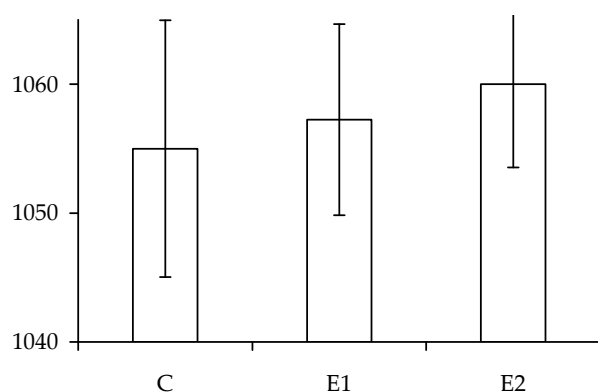


Figure 3. Colostrum density ( $\text{kg}/\text{m}^3$ ) of cows (C – control group, E1 and E2 – treatment groups) at parturition

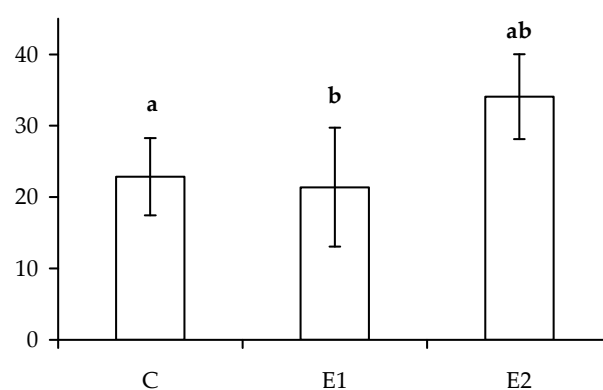


Figure 4. Effect of parenteral administration of selenium and vitamin E on content of immunoglobulins (U ZST) in colostrum of cows (C – control group, E1 and E2 – treatment groups) at parturition

a;a; b;b  $P < 0.05$

through the placenta into the foetus and therefore the stores of the newborn calves are low (Van Saun et al., 1989; Njeru et al., 1994). Analysis of Jagos et al. (1979) show, that the concentrations of  $\beta$ -carotene, as well as of vitamin A and E in the blood plasma of foetuses in the age of 8 to 9 months are much lower than that in the plasma of their cows.

Figures 3 and 4 show the results of additional examinations of colostrum. Despite insignificant differences in colostrum density it appears that the concentration of immunoglobulins in colostrum of cows supplemented with higher doses of selenium and vitamin E was higher. Due to significant ( $P < 0.05$ ) difference between groups E2 ( $34.08 \pm 5.93$  turbidity units according to Snank-Hoagland (U ZST)) versus E1 and C ( $21.38 \pm 8.33$  and  $22.87 \pm 5.41$  U ZST, respectively), a positive effect of selenium and vitamin E on colostrum quality can be considered. Swecker et al. (1995) also confirmed higher concentration of colostral IgG in beef cows with higher selenium intake. On the other hand, Rock et al. (2001) did not demonstrate any positive effect of higher selenium intake on colostral IgG in sheep. Lacetera et al. (1996) likewise did not confirm differences in levels of colostral immunoglobulins in dairy cows but recorded a positive effect of increased selenium and vitamin E intake on the quantity of colostrum and milk produced.

The assessment of occurrence of clinical forms of disease during the first 30 days of the postpartum period revealed 5 treated cases of mastitis in 4 animals and 1 case of endometritis in the cows

of group C. There were 4 treated cases of mastitis in 3 cows of group E1. No cases of disease were detected in the animals of group E2. Although the numbers of animals in separate groups were small, the results concerning the occurrence of disease during the postpartum period suggest a possible positive influence of repeated administration of selenium and vitamin E on the reduction of clinical cases of mastitis. This finding corresponds with the published data on positive influence of selenium and vitamin E administration in cows during the last stage of pregnancy on the reduction of cases of related diseases (mastitis, nutritional myopathy, retained placenta and other reproductive disorders), as well as on improved function and activity of the immune system (Smith et al., 1997; Allison and Laven, 2000).

On the basis of previously mentioned results it is possible to recommend repeated administration of the product Selevit inj. in herds with substantial selenium deficiency. The product should be administered prior to parturition. Higher doses of selenium and vitamin E can be considered in cases when extreme values of selenium deficiency are detected. It has to be noted, however, that injectable products with higher selenium concentration are not available on the market in the Czech Republic. Veterinarians would therefore have to exceed the maximum dose recommended by the manufacturer of the product mentioned above. General recommendations for injectable products usually mention a relatively low dosage of selenium and

in particular of vitamin E. The usual treatment was one injection of approximately 700 IU of vitamin E and about 50 mg of selenium administered about 3 weeks before calving. Positive effects of vitamin E on health status and improved function of the immune system were however described on the basis of considerably higher doses (3 000–5 000 IU of vitamin E) (Politis et al., 1995; Weiss, 1998; Allison and Laven, 2000; Kolb and Seehawer, 2002). If a higher dose of vitamin E is required in practice, it is possible to administer another product containing vitamin E only. We are not of the opinion that long-term low intake of selenium and vitamin E by feed in dairy cows can be effectively compensated by injectable medicinal products. This is a good way just to satisfy increased demands of the organism during pregnancy or during periods of temporary lack of selenium and vitamin E in the ration.

It can be therefore concluded that the administration of the product containing selenium and vitamin E to pregnant dairy cows showed a positive effect on the increase of selenium and vitamin E concentrations in blood, increase of selenium and immunoglobulins concentrations in colostrum, and on the increase of T3 concentration in blood collected from the cows on the day of parturition. In addition, during the first month of the postpartum period a trend of lower occurrence of clinical forms of mastitis was observed.

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