



EFFECT OF SELENIUM ON ITS CONTENT IN MILK AND PERFORMANCE OF DAIRY COWS IN ECOLOGICAL FARMING

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ABSTRACT

Currently, the ecological farming is increasingly spread in the European Union. The aim of this relatively young farming method is a friendly approach to agricultural production with an emphasis to deliver healthy raw materials and food to final consumer. Selenium is included in an essential trace micronutrients which are necessary for the proper process of physiological reactions. It is a part of glutathione peroxidase, which is a powerful antioxidant. At present, selenium-deficiency can occur in feed and food in central Europe. Selenium deficiency is one cause of the higher occurrence of cardiovascular diseases. The aim of the experiment was to study whether the addition of selenium to the diet of dairy cows in ecological farming can increase its concentration in milk and affect quantitative (milk yield) and quality (content of protein, fat, lactose, somatic cells and urea) milk indicators. The experiment included twenty cows of Holstein breed. The first experimental group of cows ($n = 10$) was fed with selenium in an amount of $0.3 \text{ mg} \cdot \text{kg}^{-1}$ (as selenomethionine) in the feed dose. The control group ($n = 10$) was not fed with the increased selenium in the feed dose. The basic feed dose contained 0.17 mg of Se/kg in the diet. For dairy cows, daily intake was of 20.5 kg of dry matter feed. The duration of the experiment was set at 45 days. The selenium concentration in milk was measured from 0.13 to $0.15 \mu\text{g} \cdot \text{mL}^{-1}$ in the experimental group of cows during the evaluation. The control group of cows without the addition of selenium to the diet showed a selenium concentration below the detection limit. During the experiment, milk yield, lactose, fat and protein were not affected. A significant decrease ($p < 0.05$) of somatic cells by 58% occurred in milk in the experimental group. The amount of urea was significantly lower in both groups in the experimental (by 52%; $p < 0.05$) and control (50%; $p < 0.05$). These results show that the addition of selenium may increase the selenium concentration in milk (the production of functional food). The addition of selenium reduces the amount of somatic cells, which are the indicators of inflammatory diseases of the mammary gland.

Keywords: selenium; milk; cow; ecological farming

INTRODUCTION

Ecological farming has recently developed in the European Union. The food from this production is increasingly popular for final consumer (Gambelli et al., 2014; Horký, 2014). Selenium is important as the essential microelement in animal and human areas. The receiving adequate level of selenium in the diet is essential for the maintaining of good health and reproduction parameters. Selenium is a part of the enzyme glutathione peroxidase (GPx) transforming hydrogen peroxide to water and molecular oxygen (Horký et al., 2012a; Horký et al., 2013). The food is low on selenium content and the total amount of antioxidants, which are associated with civilization diseases in many cases (Hadas et al., 2015; Navia et al., 2014). The amount of selenium varies significantly in the soil by the region. Selenium proves the ability to activate cells of the defense system of the organism and thus prevents serious diseases e.g. cancerous tumors (Mehdi et al., 2013). The application of selenium in feed doses is one of the ways how to increase selenium in animal food and products (Horký, 2014; Van Metre and Callan, 2001). The addition of minerals (especially selenium) to the diet of ruminants can also improve the

reproductive performance and animal health (Horký et al., 2012b; Nevřkla et al., 2014; Pechova et al., 2012). Selenoproteins support the metabolism of hormones because of improving activity of the thyroid gland (Horký and Cerkal, 2014; Wichtel, 1998).

The aim of the experiment was to study the effect of addition of organically bound selenium on the quality of the produced milk in terms of functional food into the diet of dairy cows in an ecological farming.

MATERIAL AND METHODOLOGY

The experiment was carried out on an ecological farm (the farm was registered in accordance with the Czech Republic's Act No. 242/2000 Coll. under registration number 42318335) keeping dairy cattle in Lesoňovice, the Czech Republic. The experiment included 20 Holstein dairy cows divided into two equal groups by weight (the experimental group had an average weight of $622 \pm 15 \text{ kg}$; the average weight of the control group was $630 \pm 11 \text{ kg}$) and at the same stage of lactation. Dairy cows in the experimental group had completed an average of 3.4 ± 0.1 lactations prior to the experiment while cows in the control group had completed an average of 3.6 ± 0.1 lactations.

Table 1 Composition of the feed ration and dairy cows' average daily intake per head (Dry matter/kg).

Feedstuff	Control group	Experimental group
Maize silage	6.38	6.38
Clover–grass haylage (first cutting)	5.95	5.95
Grass haylage (first cutting)	4.00	4.00
Winter wheat	2.64	2.64
Spring barley	1.76	1.76
Detamin GA Spezial ^b	0.13	0.13
Total intake	20.86	21.86

Note: ^bDetamin GA Spezial is a mineral supplement for ruminants intended to be used in organic farming systems (H. Wilhelm Schaumann GmbH, Pinneberg, Germany).

The experimental and control group of cows received up to 20.5 kg of dry matter of feed /piece/day. The basic diet contained 0.17 mg of selenium/kg. The animals had ad-libitum access to water. The first group of cows (n = 10) was added the selenium in the diet at a dose of 0.3 mg.kg⁻¹ (as selenomethionine). The second group of cows (n = 10) served as a control group without the addition of selenium (this group of animals received only selenium from native sources). The premix of selenium was mixed to the basic ration (TMR) and fed in the morning dose. The average milk yield of the animals was 7,600 ±50 kg/lactation. All animals were fed a basic feed ration in the form of a total mixed ration (Table 1 and Table 2) and were allowed *ad libitum* access to water. The quantity of feed provided was recorded by the mixer-wagon (Luclar, the Czech Republic). Uneaten amounts were estimated and not analysed in any way.

In the control group of cows, the average duration of lactation lasted for 103 days at the beginning of the experiment (stage of lactation varied from 86 to 110 days). The experiment began on average 107th day of lactation (lactation phase ranged from 85 to 114 days) in the experimental group of cows. The cows were in free housing. The feeding was carried out twice a day (morning and evening). The duration of the experiment was set at 45 days.

Before starting the experiment and then at 15 day intervals (0, 15, 30 and 45th day), milk samples were taken for selenium determination. Milk samples for the determination of yield and milk components (protein, fat, lactose, somatic cells, urea) were taken at the beginning and end of the experiment, ie. 0th and 45th day. The samples were taken before feeding in each morning and

subsequently subjected to an appropriate analysis.

Determination of Selenium Concentration in the Silage

The determination of selenium concentration in the silage was carried out using the atomic absorption spectrometry.

Determination of Selenium Concentration in Milk

Selenium has been set on the 290Z Agilent device using method of atomic absorption spectrophotometry (Agilent, USA) with the electrochemical atomization. The ultrasensitive lamp with hollow cathode (Agilent) was used for selenium. The lamp of power of 10 mA was utilized as a radiation source. The spectrometer operated at 196 nm with a spectral bandwidth of 1.0 nm. The volume of sample was of 20 mL, which was injected into the graphite tube. The flow rate of argon as the inert gas was of 300 mL/min. The repetition was applied in the field strength of 0.8 Tesla. Selenium was determined in the presence of palladium as a chemical modifier. The samples were always measured in two repetitions.

Microwave Decomposition for the AAS determination

A 40 mL of milk was pipetted into tubes, in which took place the decomposition. Nitric acid (65%) and hydrogen peroxide (30%) were used for the decomposition of the samples. Overall, a 500 ml of folding mixture was used. The ratio between the nitric acid and hydrogen peroxide was 7:3. The samples were determined using a 3000 microwave (Anton Paar GmbH, Austria), MG - 65 rotor. The program is repeated at regular ten minute intervals with power ranging from 50 W to 0 W (cooling). The microwave power was 100 W in the main portion of the program (duration 30 minutes) at 140 °C.

Assessment of milk components

Table 2 Composition of Detamin GA Spezial mineral supplement for dairy cows (the content is presented in 1 kg of premix).

Parameter	Unit	Quantity
Zinc (as zinc oxide)	mg	8000
Manganese (as manganese oxide)	mg	6000
Copper (as copper sulfate pentahydrate)	mg	1200
Iodine (as calcium iodate)	mg	100
Cobalt (as carbonate cobaltous hydroxide)	mg	18

The milk was preserved using 2-bromo-2-nitropropane-1,3-diol and cooled to 4 – 6 °C until the analysis, which was carried out within hours of the sampling. The milk components were analysed in a commercial laboratory using a MilkoScan FT2 (Foss Electric, Hillerød, Denmark). Fat was acidobutyrometrically established according to standard CSN ISO 2446. Crude protein was established by the Kjeldahl method according to CSN 57 0530. The urea in milk was enzymatically determined using a commercially available urea/ammonia assay kit (Megazyme, Wicklow, Ireland) according to CSN 57 0533. Somatic cells were analyzed using FTIR technology (Fourier Transform InfraRed) - (MilkoScan, FT 6000) according to ČSN EN ISO 13366-2.

Statistica

The data were processed statistically using STATISTICA.CZ, version 10.0 (the Czech Republic). The results were expressed as mean ± standard deviation (SD).

RESULTS

In the experiment, the effect of selenium on selenium concentration in milk and performance of dairy cows was

studied. During cow feeding of selenium in an ecological farming, the selenium content was monitored in the milk of animals before the beginning of the experiment under the detection limit. In the experimental group, a detectable amount of selenium was monitored after 15 days of the application of selenium to the diet, which was in the constant level throughout the duration of the experiment. The experimental group with selenium level ranged from 15th to 45th day in the interval from 0.13 to 0.15 g.mL⁻¹. In the control group, the amount of selenium was under the detection limit for the entire duration of the experiment (Table 3).

Within the experimental observation, the qualitative and quantitative parameters of the produced milk were also evaluated. Milk yield was similar in both groups with no significant changes. At the end of the experiment, an increase of 22 resp. 18 % was observed in the experimental and control groups. For lactose, no differences were also observed. The lactose value were within the physiological range (from 4.6 to 4.8%). The values of milk yield and lactose are shown in Figure 1A and Figure 1B.

Table 3 Effect of selenium feeding on its content in milk and antioxidant potential.

Index	Experimental group				Control group			
	Day of experiment				Day of experiment			
	0	15	30	45	0	15	30	45
Selenium content (µg/ml)	UDL	0.15 ±0.01	0.13 ±0.05	0.14 ±0.03	UDL	UDL	UDL	UDL

UDL - under the detection limit

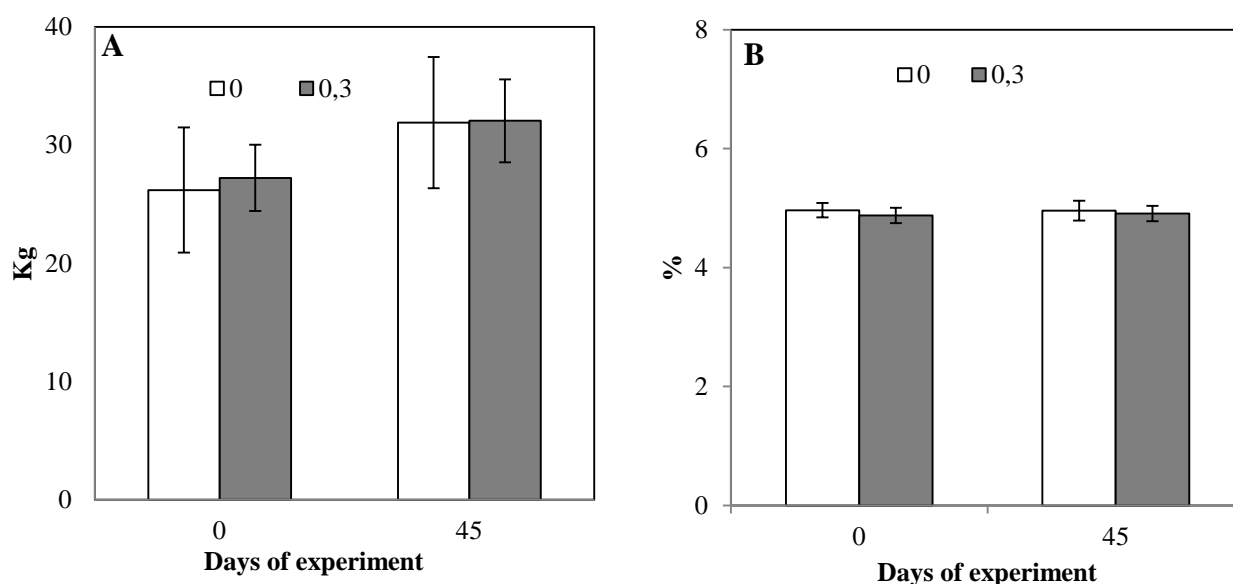


Figure 1 Effect of selenium feeding on milk yield (A) and lactose content (B) in dairy cows in ecological farming.

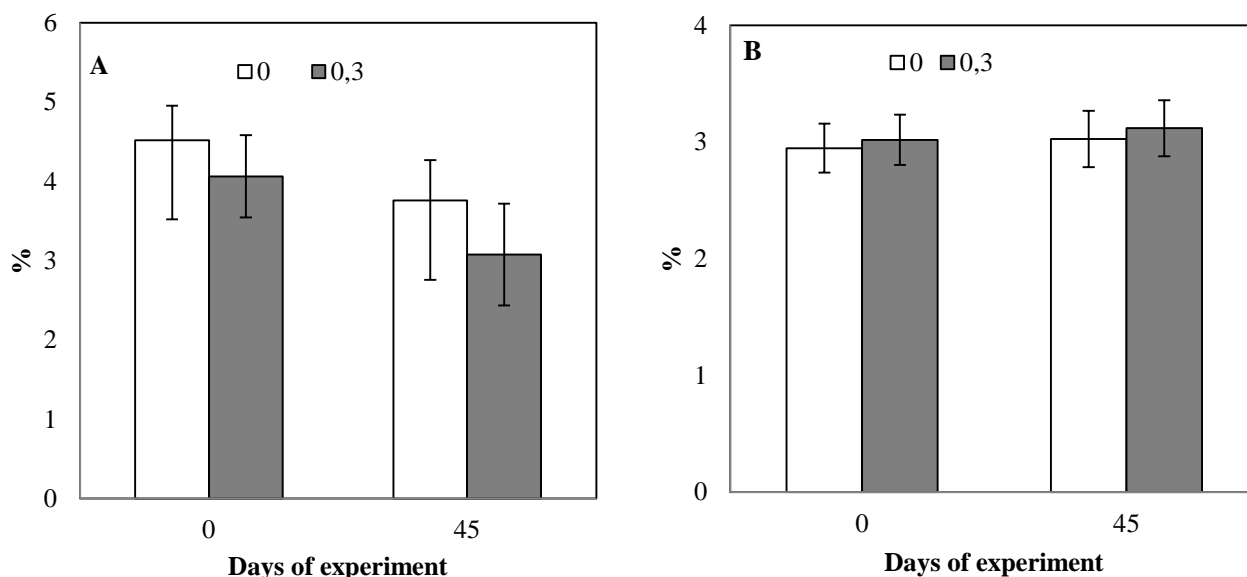


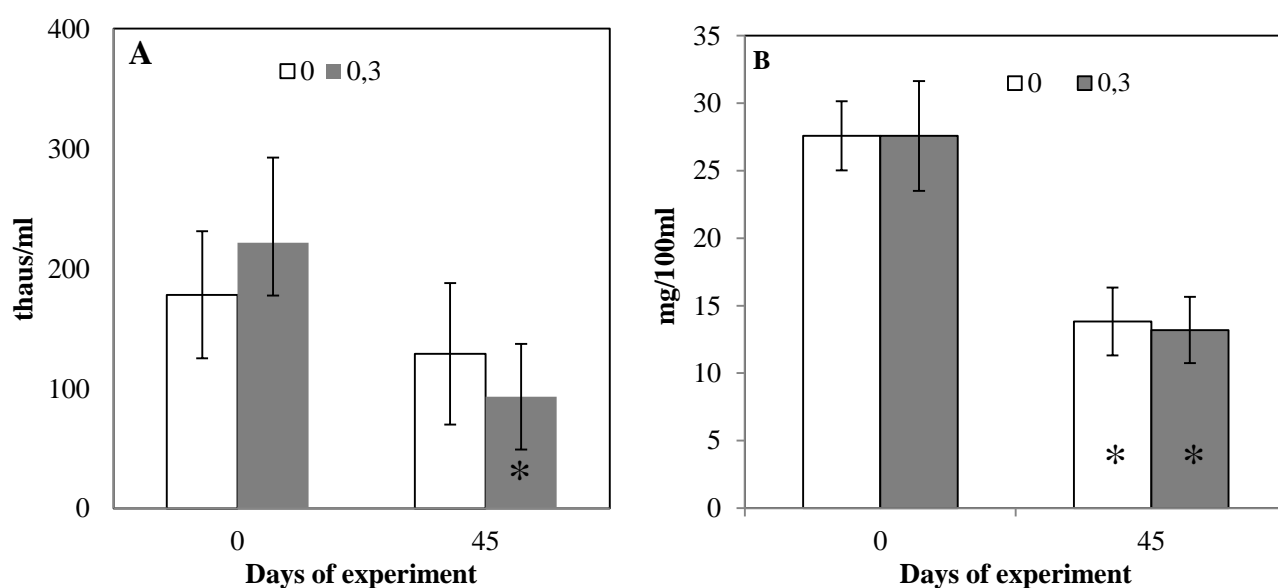
Figure 2 Effect of selenium feeding on the content of fat (A), protein (B) in dairy cows in ecological farming.

The fat decrease occurred in both groups in the experimental (by 17%) and control (by 24%) during the observation. The protein in the milk of the experimental and control groups of cows was practically in the identical level throughout the experiment. The fat and protein values in the milk of dairy cows can be seen from the Figures 2A, 2B.

The amount of somatic cells was significantly reduced in the test group of dairy cows by 58% ($p < 0.05$). The decrease of the number of somatic cells was also observed in the control group (by 28%). The urea concentration was significantly decreased in the experimental and control groups by 52% ($p < 0.05$) respectively 50% ($p < 0.05$). The values of somatic cells and urea in milk are shown in Figures 3A, 3B.

DISCUSSION

The experiment was focused on the feeding of organic selenium ($0.3 \text{ mg} \cdot \text{kg}^{-1}$ of diet) in the ecological farming of dairy cows in order to increase the element in cow milk (the production of functional food with higher selenium level). We also studied the quantitative and qualitative indicators of the produced milk. In the experiment, in which the selenium of the same amount ($0.3 \text{ mg} \cdot \text{kg}^{-1}$ of diet) was fed by dairy cows similarly to our observation, was higher selenium level of the serum (Hall et al., 2014). The dose of $0.278 \text{ mg Se} \cdot \text{kg}^{-1}$ of diet affected neither the performance of dairy cows nor selenium concentration in milk. No significant differences were observed in the content of lactose and protein. Conversely, the increased milk fat, the reduction of somatic cells in the milk and the



* = statistical significance $p \leq 0.05$ between Day 0 and Day 45 of the experiment

Figure 3: Effect of selenium feeding on the amount of somatic cells (A), urea (B) in dairy cows in ecological farming.

occurrence of mastitis were occurred (Oltramari et al., 2014). In our observation, no significant increase was detected in milk fat content. However, the decrease of somatic cells in cow group treated with selenium was found out as in the previous study. It may suggest that selenium may prove the anti-inflammatory effect from this point of view associated with a lower occurrence of mastitis. The concentration of selenium was effected in this case contrary to the results according to Oltramari et al. (2014). Conversely, the milk yield and protein content are in accordance with the above mentioned authors. Selenium with vitamin E was fed to other groups of cows. A higher synergistic effect was expected because of shared action. Selenium and vitamin E were applied in the injection (three weeks before the birth). In terms of selenium concentration in the animal blood, no difference was found out between oral and injection application (Kafilzadeh et al., 2014). From this viewpoint, it is possible to take the selenium injection as a possible alternative to its application. In another experiment, the selenium was given in a single injection (48.4 mg of selenium for 21 days before birth). After the birth, the decrease of mastitis (by 13%) and somatic cell count in milk was observed in the experimental group (Zigo et al., 2014). As stated in other studies, selenium-enriched milk can be an alternative source of selenium in human nutrition (as a functional food that has a positive effect on the consumer health). We tested two forms of selenium (inorganic and organic). The use of inorganic form may cause an increase in milk content from 13.3 to 17.6 to 19.7 mg.L⁻¹. and the application of organic selenium (selenomethionine) was increased from 13.9 to 24.6 to 54.8 mg.L⁻¹. In both cases, the dose was of 0.4 mg selenium /kg per diet (Meyer et al., 2014). In our case, selenium increase was observed from 13 to 15 µg.L⁻¹ after the feeding of organic form. Because of taking lower selenium dose by 0.1 mg than Mayer et al (2014) did, the selenium increase was not so significant. The control group of cows without the selenium addition showed the levels below the limit detection. It is not identified whether the selenium addition in the diet of cows can increase the milk production and quality. In the experimental observation lasted for 60 days (in this case for 30 days), the dose of 0.3 mg selenium /kg of diet was given to dairy cows in the organic or inorganic form. The performance of the animals was not affected. During evaluation of dairy ingredients (percentage of fat, protein and lactose) no significant differences were also observed. In the assessment, the antioxidant parameters in blood of dairy cows were observed in a higher activity of antioxidant enzymes (catalase and GPx) in a group of cows feeding an organic source of selenium. Selenium was also significantly higher in milk during using of selenomethionine (Gong et al., 2014). In our experiment, we have managed to confirm the results. On the other hand, it must be said that our aim was not to compare the two forms of selenium (organic or inorganic). Our objective was to focus on selenomethionu applications. During the use of organic selenium, neither the milk production nor affecting of milk components were observed. The antioxidant capacity of milk was not measured because of low quantitation of the antioxidant

enzymes. Selenium is still rare element in cattle feed doses. Its deficiency may cause not only a reduction of antioxidant potential but mainly getting lower the reproductive performance. This trend is widespread throughout central Europe (Balicka-Ramisz and Jastrzębski, 2014). The study (Pilarczyk et al., 2011) was devoted to the monitoring of the concentration of selenium in dairy cows from the ecological and conventional farming. In both farming methods, high variability was found out. In ecological farming, the average level of selenium was up to 0.016 µg.mL⁻¹ in milk. In conventional farming, the average level of selenium was at the level of 0.005 µg.mL⁻¹ in milk. As the authors stated, the deciding factor of selenium content is the nutrition in cow milk. Our study only monitors the different methods of cow farming.

CONCLUSION

In an experimental monitoring, the effect of organic selenium addition on its concentration, quantitative and qualitative parameters of milk in the diet of dairy cows in an ecological farming was studied. The selenium addition at a dose of change to 0.3 mg.kg⁻¹ of the diet increased the concentration of selenium in the milk and reduced the number of somatic cells ($p < 0.05$). Other components of milk (protein, fat, lactose, and urea) and milk yield were not affected. From these results, it is obvious that the selenium addition to the diet of cows increases the concentration of essential micronutrients in milk (production of functional food) and reduces the production of somatic cells, which are indicators for the inflammation of the mammary gland.

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