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Increasing selenium concentration in Finnish organic milk using participatory action approach

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Selenium (Se) is an essential trace element for animals. In Finland, Se supplementary feeding and Se-enriched macronutrient fertilisers are used in conventional farming because the Se availability from soil is extremely low. The objective of this study was to improve Se concentration in organic milk by replacing sodium selenite (SS) in mineral -vitamin mixture with selenium yeast (SY) either completely or partly (50/50). The feeding experiment was conducted on 15 certified organic dairy farms in Eastern Finland during spring 2008. The cross-over design consisted of three treatments (SS treatment, SY treatment, and SS/SY treatment), three experimental periods (28 days each), and three farm groups (different order of treatments). Replacing SS with the same amount (0.2 mg Se kg^{-1} feed dry matter) of SY more than doubled Se concentration in organic milk and their mixture resulted in slightly lower Se concentrations. In conclusion, SY supplementary feeding is needed to improve Se concentration in organic milk and Se supply to cows, their calves, and consumers.

Key words: sodium selenite, selenium yeast, organic dairy farming, participatory action research

Introduction

Selenium (Se) is the most controversial trace element, which has a narrow margin between deficiency, essentiality, and toxicity (Hartikainen 2005). In addition, it is very unevenly distributed in the soils of the earth's surface. In animal production, utilisation of the Se can vary widely between farms and diets due to Se form, composition of total diet, level of concentrates as well as contents of other minerals and vitamin E in diets (Gerloff 1992, Ortman 1999). Globally, milk Se concentration has been reported to vary in the range of 2–60 µg l^{-1} (Knowles et al. 2006). Aspila (1991) suggested that a desirable Se concentration in milk would be around 20 µg l^{-1}. According to Wichtel et al. (2004) a milk Se concentration of less than 9.6 µg l^{-1} represent observable Se deficiency for cows, and a milk Se concentration of greater than 21.8 µg l^{-1} represent adequacy, but also clearly higher concentrations have been suggested to improve human Se supply. Milk Se concentrations up to 300 µg l^{-1} (Walker et al. 2010) and even close to 600 µg l^{-1} (Sun et al. 2021) have been reported with supranutritional experimental Se dosages. How high should Se concentration in organic milk be: above minimum requirements for calves, like in conventional farming, or able to provide Se enriched milk for consumers in future?

Dietary Se recommendation for bovines vary worldwide between 0.1 and 0.3 mg Se kg^{-1} feed dry matter (DM). However, the bioavailability of Se strongly depends on Se form in the diet. In most Se-deficient areas, inorganic sodium selenite (SS) is added to mineral supplements to provide adequate Se intake for animals, although organic Se increases the Se concentration in milk more efficiently compared to SS (Conrad and Moxon 1979, Ortman and Pehrson 1999, Heard et al. 2007, Ceballos et al. 2009). At the end of year 2006, the European Union permitted the use of selenium yeast (SY) products in feeding of all animal species (EC Regulation 1750/2006), but during 2014–2015 the use of SY products was temporally prohibited for ruminants in organic farming (Implementing Regulations 505/2012, 2016/673).

Selenium yeast is produced by cultivating common yeast *(Saccharomyces cerevisiae*) in the presence of SS, which makes the yeast (similarly to plants) synthesise inorganic Se into organic forms, mainly into selenomethionine (SeMet) (Malbe et al. 1995, Ortman 1999). It is absorbed in the small intestine via methionine pathway while SS is absorbed mainly by passive diffusion, and it must undergo a metabolic transformation before it can be assimilated (Weiss 2005, Arshad et al. 2021). Furthermore, SY has been reported to result in higher SeMet concentration in milk compared with SS (Juniper et al. 2006) and with increasing SY dosages to higher proportion of SeMet in total milk Se (Sun et al. 2021). Recently, other novel SeMet containing feed additives have been introduced for conventional dairy farming and the potential of nano-Se has been discussed (Arshad et al. 2021).

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In Finland, the soil Se availability is extremely low because soils are young and weakly weathered, acid, and high in adsorptive oxides (Hartikainen 2005). Therefore, besides SS feed additives, sodium selenate has been added to multiple macronutrient fertilisers since 1984. Consequently, the average Se concentration in conventional Finnish milk has increased from 4 to 28 µg l^{-1} (Eurola et al. 2003, Eurola et al. 2016). However, annual Se-enriched fertilisation is necessary, as fertilisation has not increased the amount of soluble Se in soils, although only 8% from given Se is approximately utilised by crops (Yli-Halla 2005). Consequently, at Finnish organic farms the Se concentration in home-grown feeds and milk is extremely low (Eurola et al. 2003). In the European Union, organic farming is regulated according to the strict rules of organic regulations (EC 2022). The use of mineral fertilisers is restricted, but micronutrients are permitted in some cases. Recently, it has been suggested that Se fertilisation should be permitted. However, in Finland Se-alone fertilisation of each field on a yearly or even each harvest basis would be an inefficient and unsustainable practice.

Organic products are regarded as healthy and safe products (Ditlevsen et al. 2019), but their low Se concentration may affect their demand adversely. According to a recent meta-analysis of 170 studies, organic bovine milk has a more desirable fatty acid composition than conventional milk and higher α-tocopherol and iron but lower iodine and Se concentrations (Średnicka-Tober et al. 2016). Organic farming relies on the utilisation of nutrient recycling and biological nitrogen fixation (legumes), the use of home-grown feeds, and the feeding of calves with bovine milk (EC 2022). High calcium concentrations in the forage typical to legume-rich diets are known to interfere with the absorption of Se (Gerloff 1992). Maternal nutrition affects the performance and health of the progeny, as all Se required by the developing offspring is transferred from dam via the placenta, the colostrum, and the milk (Pappas et al. 2008). Suckling calves may be at risk of Se deficiency if their dams are fed with Se-deficient fodder or even provided with SS containing feed supplement (Pehrson et al. 1999). Besides cow's and calf's welfare, Se supply may impact on milk hygienic quality (somatic cell count and total bacterial count) (Malbe et al. 1995).

In our preliminary study, the average Se concentration in organic milk was 10 µg l^{-1} and 1/3 lower than that in milk from farms certified only to organic field farming (Kuusela and Okker 2007). It was concluded that Finnish organic milk relying on SS containing feed supplements could not achieve the recommended Se concentration, underlining the need for organic Se supplements. Organic Se could be derived from crops grown in Se-rich soils (Haug et al. 2007, Heard et al. 2007). However, transporting large amounts of feed from long distances would not be a sustainable or cost-effective solution. The use of SY was suggested, although it was not known how it would work in mixtures fed with typical organic farming diets, calcium-rich legume-grass silage or with SS.

The aim of this study was to improve the Se concentration in Finnish organic milk by replacing supplementary SS totally or partly (50/50) with SY. To our knowledge, the effect of SS/SY mixture on milk Se concentration has rarely been studied. The hypothesis was that the utilisation of SY will be a powerful tool to improve the Se concentration in organic milk.

Material and methods

The feeding experiment was conducted with the permission of Finnish Animal Experiment Board on 15 Finnish organic dairy farms which had participated in the preliminary study. The farms were situated in the North Karelia and Kainuu regions between N 62° 35′ 54′′ and 64° 31′ 14′′ and E 26° 24′ 55′′ and 30° 0′ 18, respectively. The experiment started on 2 February and ended on 29 April 2008.

The experiment was conducted following a cross-over study design, consisting of three treatments and three experimental periods. Furthermore, the treatments were implemented in three farm groups of the same size, which makes the experiment design similar to 3×3 Latin square design. The treatments were SS treatment (SSt), SY treatment (SYt), and (50/50) mixture of SS/SY treatment (SS/SYt). The 15 participating farms were allocated to three similar groups (five farms in each) according to herd size, annual milk yield, proportion of forage in the diet, and housing system (Table 1). The length of each experimental period was 28 days. The concentration of Se in milk is known to reach a plateau value within one week after supplementation (Ortman and Pehrson 1999), but the longer adapting time was chosen to minimise the carry-over effect. The cow breeds were Finnish Ayrshire or Holstein Friesian dairy cows which are the main dairy cow breeds in Finland. On three farms there were also a few cows of Eastern Finncattle.

Table 1. Description of farm groups prior to the experiment and order of selenium feeding treatments in each group

	Group A	Group B	Group C	SEM[1]	Sig.[2]
Number of farms in each group	5	5	5		
Average number of cows per farm	28.8	31.4	28.4	7.31	*ns*
Average annual milk yield, l	7774	7636	7600	499	*ns*
Average proportion of forage in the diet, %	71.6	67.5	70.0	3.70	*ns*
Housing system:					
Loose-house dairy barn	2	3	2		
Tie stalls dairy barn	3	2	3		
Feeding order of treatments[3]	SYt SS/SYt SSt	SS/SYt SSt SYt	SSt SYt SS/SYt		

[1] SEM = standard error of mean; [2] Significance: *ns* = not statistically significant (p>0.05); [3] SYt = selenium yeast, SY/SSt = (50/50) mixture of selenium yeast and sodium selenite, SSt = sodium selenite

Experimental farm-ready mineral-vitamin mixture and feeding

The amount of Se was similar in each experimental feed (20 mg kg^{-1}). Selenium was included in mineral-vitamin feed which contained Ca (24 g kg^{-1}), P (28 g kg^{-1}), Mg (68 g kg^{-1}), Na (120 g kg^{-1}), Cu (450 mg kg^{-1}), Mn (590 mg kg^{-1}), Zn (1600 mg kg^{-1}), Co (35 mg kg^{-1}) and I (60 mg kg^{-1}). The vitamin content was vitamin A (200 000 IU kg^{-1}), vitamin D3 (40 000 IU kg^{-1}) and vitamin E (600 mg kg^{-1}). Customised mineral content of the experimental feed was first designed based on silage mineral content monitoring of ProAgria organic farming project (Luomu Itää 2006) and further checked before the first feeding period started based on the experimental farms' silage analysis. On two farms there was less calcium in the silage than predicted and farmers were advised to give extra calcium until switching to legume-rich silage.

The experimental feed was provided and prepared by Raisio Nutrition Ltd. The SS used was Sel-Plex™ (Alltech Inc.) which is a specific strain of *Saccharomyces cerevisiae* (CNCM I-3060). After measuring each component, the compound was mixed, molasses was incorporated, and the feed was granulated. The feed sacks were coded clearly and delivered to local dairy stores nearest each farm. Farmers picked up the feeds either all at once or for one feeding period at a time. The dosage (0.2 mg Se kg^{-1} feed DM) of the experimental feed was adjusted according to expected daily milk yields (Table 2) which are easier to record on farms than DM intakes.

Farmers received a measure cup and were advised to give the experimental feed according to a given table. Most of the farms (n = 11) gave the experimental feed individually to each cow. Those farms (n = 4) using mixer feeding systems (total mixed ration or concentrate mixer) were advised to include the milk yield-based average amount of supplementary feed into the mixture. For mixer feeding farms, the actual intake determined the daily Se doses. The daily dosage of mineral feed was planned so that mixed feed intake-based dosages resulted in approximately similar Se intakes as milk yield-based ones.

Table 2. Daily dosages of experimental feed and selenium (mg d^{-1}) adjusted according to expected daily milk yield (l d^{-1})

Daily milk yield, l d^{-1}	10	20	30	40	50
Experimental feed, g d^{-1}	100	150	200	250	300
Selenium, mg d^{-1}	2	3	4	5	6

Sampling

Samples (1 kg) from silage and home-grown grain mixture intended for feeding during the experiment were taken before the experiment and when the silos were changed. Silage and grain samples for Se analysis were sent to MTT Agrifood Research Finland and silage samples for other mineral analysis to the Valio Lapinlahti laboratory. Milk samples were collected for analysis before the experiment started and at the end of each experimental period, on the last two days of each period synchronised according to the visit of the bulk milk tanker that took standard samples (30 ml) for the milk basic composition analysis and delivered them to the Valio Lapinlahti laboratory. Experimental samples were taken before the car collected the milk. Thus, most of the milk was from three or four milkings.

When taking the samples, one litre of +4 °C milk was taken from the tank after automatic mixing of the milk tank for two minutes into five plastic bottles prior to any analyses, avoiding stratification of the milk fat. Sample bottles were put into cool bags and frozen at –23 °C on the same day after delivery to the Joensuu Campus. For milk Se analysis reported in this paper, 200 ml milk samples were sent to MTT Agrifood Research Finland for Se analyses.

Analysis

Milk and feed Se concentrations were analysed at MTT Agrifood Research Finland using the Electrothermal Atomic Absorption Spectrometry method (Kumpulainen et al. 1983). Analyses for fat, protein, and lactose were carried out in the Valio Lapinlahti laboratory using an infrared milk analyser (MilcoScan FT 6000, Foss Electric, Hillerød, Denmark), somatic cell counts by flow cytometry (BactoScan FC), and bacteria using a Fossomatic 5000. Mineral contents of silage samples were analysed in the Valio Lapinlahti laboratory using Perkin Elmer Elan 6100 ICP-MS.

Participant farmer consultation

Instructions for experimental feeding were explained orally and given on paper during a farm visit before the experiment started. The farmers were also advised on phone or email if they had any questions. When milk samples were collected at the end of each period, the feed for the next period was checked during the farm visit. Farmers received all their feed and milk results during the experiment, and they were interviewed on how they experienced this study.

Statistical analysis

The relationships between milk Se concentration and measured milk composition were studied by Pearson correlation coefficients. Prior to more advanced modelling, the effect of Se treatment was studied using One-way ANOVA. The experiment used a crossover design with three treatments (SSt, SY/SSt, SYt), where treatment order was altered between three farm groups (Table 1). It is important that the carry-over effect either does not exist or if it exists, it needs to be independent with respect to the previous factor (Lawson 2015). The statistical analysis was conducted using a linear mixed-effects modelling (LME).

The model which was used in the analyses is

$$y_{ijkl} = \mu + \lambda_{prev,i} + s_j + \pi_k + \tau_l + \delta_i + \epsilon_{ijkl},$$

where y_{ijkl} is Se level for farm *j* from group *i*, receiving treatment *l* on period *k*. So, μ is intercept, $\lambda_{prev,i}$ is carry-over effect, π_k is period effect, τ_l is treatment effect and δ_i is farm group effect. We assume that random intercept (farm effect) s_j follows normal distribution with zero mean and variance σ_s^2, and error term ϵ_{ijkl} follows normal distribution with zero mean and variance σ_ϵ^2.

Statistical analyses were conducted using R version 4.2.1 (R Core Team 2022), R-packages lme4 (Bates et al. 2015), and lmerTest (Kuznetsova et al. 2017). Post hoc -tests were conducted as general linear hypothesis testing using multcomp-package (Hothorn et al. 2008).

Results

Farmers were highly motivated and their responses to the experiment were positive, and they did not find feeding practices too stressing. The Se concentrations in home-grown grain mixtures and silage used during the experiment were extremely low (≤ 0.01 mg kg^{-1} DM). Red clover *(Trifolium pratense)* was the predominant legume in silages and the average mineral contents were typical of clover-grass silages (Ca 8.9 g kg^{-1} DM, P 2.9 g kg^{-1} DM, K 22.4 g kg^{-1} DM, Mg 3.5 g kg^{-1} DM).

The average Se concentration in milk prior to experiment was 15.3 µg l^{-1} with 95% confidence interval of (11.3, 19.4), and varied from 4 to 32 µg l^{-1}. During the experiment milk Se concentration varied from 8 to 41 µg l^{-1}. In the initial analysis, the effect of Se treatment was significant ($F_{2,41}$54.2, p< 0.001), but experimental period ($F_{2,41}$0.41, p = 0.666) or farm group ($F_{2,41}$= 0.335, p= 0.717) had no impact on milk Se concentration.

From the model presented, neither the farm group effect, (Chisq= 0.53, df= 2, p= 0.768), nor the period effect was found statistically significant (Chisq= 4.92, df= 2, p= 0.085). Also, the carry-over effect was not significant (Chisq= 1.56, df= 1, p= 0.211). Thus, the only statistically significant fixed effect was treatment effect. Another significant effect was a farm effect being a random intercept term. The random effect variance of LME model was 5.46 µg l^{-2} yielding farm effects to vary in range of (–2.9, 3.5) µg l^{-1}. The treatment level estimates, and their 95% confidence intervals were (µg l^{-1}); SYt: 26.9 (22.4, 31.0), SY/SSt: 21.6 (15.1, 27.0), SSt: 10.5 (4.5, 15.6). Thus, replacing SS with the same amount of SY more than doubled the organic milk average Se concentration, and 50/50 mixture of SS and SY resulted in 19.7% lower Se concentration than SY. According to pairwise post hoc comparison, each treatment estimate is statistically significantly different from one another (estimates = 11.1, 16.4, 5.3, standard errors = 1.2, 1.4, 1.5, p-values: <0.001, <0.001 and 0.002). The 95% confidence intervals based on LME model and based on data using independent observations and normality assumption with group-specific variances are compared with each other in Figure 1. LME model assumes homogeneity of variance. Levene's test supports homogeneity of variance ($F_{2,41}$= 2.12, p= 0.133).

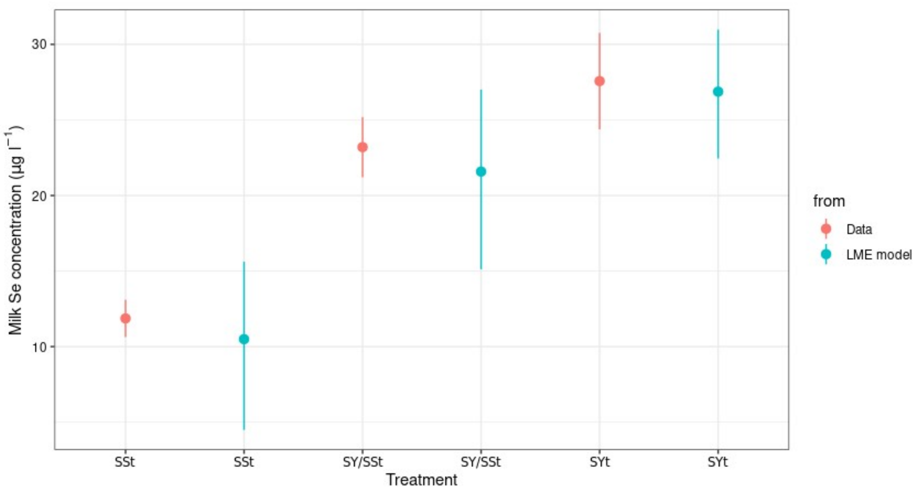

Fig. 1. The effect of selenium treatments (SSt = sodium selenite, SY/SSt = (50/50) mixture of selenium yeast and sodium selenite, SYt = selenium yeast) on milk selenium concentration and the differences between the 95% confidence intervals based on linear mixed-effects model (LME) and based on data using independent observations and normality assumption with group-specific variances.

The average milk fat, protein, lactose, somatic cell counts, and bacterial counts were 41 g kg^{-1}, 32 g kg^{-1}, 46 g kg^{-1}, 166 400 ml^{-1}, 6 800 cfu ml^{-1}, respectively. Milk Se concentration was not associated with the milk basic composition.

Discussion

The current results implicate the adequate Se concentration in milk was achieved without Se fertilisers by including SY into a farm-ready mineral-vitamin mixture. Replacing SS with the same amount of SY resulted in 156% higher Se concentration of milk. This is in line with earlier studies with similar Se-deficient basic feeds, but with different forages used: grass silage (Malbe et. al 1995), and grass pastures (Pehrson et. al. 1999).

Ceballos et al. (2009) summarised in a meta-analysis of 42 studies that Se source (inorganic or organic), Se dose, and their interaction explained 71% of the between-study variance of response to oral Se supplementation. The rest of the variation might be due to geographic factors, diets, animals, state of lactation and other elements that varied widely between studies. When the most of Se in the diet is in organic form (from plants or SY), milk Se concentration is a useful tool to predict animal's and herd's Se supply and status (Ceballos et al. 2009, Walker et al. 2010).

The effect of SS/SY mixtures on milk Se concentration have been rarely studied. In Se-deficiency, both SS and SY are known to increase the milk Se concentration and Se status of animals (Malbe et al. 1995). This suggests that combination of the SS and SY might be useful, as SS is less expensive than SY, and when considering the European Union regulation (Implementing Regulation 2019/804) according to which the maximum proportion of organic Se is 0.2 mg of kg^{-1} in complete feed (with a moisture content of 12%) and that of total Se (organic + inorganic) is 0.5 mg kg^{-1}, respectively. However, additional SS to diets already adequate in organic Se (from plants or SY) will not improve milk Se concentration but instead increase the animal Se burden and furthermore the feeding risk of Se toxicity cannot be predicted on the basis of milk Se concentration (Conrad and Moxon 1979, Ortman and Pehrson 1999). Walker et al. (2010) demonstrated that the milk Se averaged 17% of the total amount of Se consumed when cows were supplemented with increasing doses of SY. In this study, 50/50 ratio of SS/SY resulted in 106% higher Se concentration in milk compared to the same amount (0.2 mg Se kg^{-1} DM) of Se fed in the form of SS. Based on this study we cannot conclude if SS increased milk Se over the level obtained with halved dosage of SY. However, according to Walker et al. (2010) it can be estimated that halved dosage of SY would have resulted to the similar milk Se concentration level obtained in this study with SS feeding. In a study with nearly similar Se supplementation level (0.24 mg Se kg^{-1} DM) than in this study, 60/40 ratio of SS/SY resulted in 43% higher milk Se concentration than the same amount of SS alone (Azorín et al. 2020). In another study applying Se supplementation level of 0.4 mg Se kg^{-1} DM, 50/50 ratio of SS/SY was reported to result in 203% higher Se concentration in milk compared to the same amount of SS alone (Ling et al. 2017). The usefulness of SY/SS mixtures should be further studied.

The study design was implemented as 3×3 Latin square design but analysed using a linear mixed effects model. The benefit of this model is that it can take into account potential farm-specific effects and can also be used under the unbalanced design. A better design would have utilised two 3×3 Latin squares, which would have either required more farms or meant only two replications per period and treatment. It would have been very difficult to recruit more eligible farms to participate this experiment. However, as there was evidently no carry-over effect present in the experiment, this does not compromise the experiment results. This is in line with Heard et al. (2007) who reported that after the SY supplementation had ended, the milk Se concentrations decreased rapidly, as milk is produced on daily basis. In their study, after the treatments had ceased, milk Se concentrations of cows receiving SY declined to near pre-experimental concentrations within a week. However, with high SY doses, the elimination of accumulated Se from body tissues might take longer than three weeks. In this study, the average milk Se concentration between periods was similar, indicating no between-period cumulative transfer of Se from body reserves to milk. The average milk Se concentration prior to the study reflected that some farms had already used SY. We assume that the carry-over effect was not found because the adapting time for each treatment was relatively long, and the Se supplementary level was moderate.

This study was carried out in accordance with the participatory action research method and performed on commercial Finnish organic dairy farms, which involved challenges to the experimental design and some inevitable variations in the experiment performance. Variations in milk Se concentration between farms were probably mainly due to variations in dosing. The measured tank milk Se concentration from each farm was a treatment mean of several milkings and from all cows at different lactation phases, breeds, and diets. The present study covered milk Se responses from more than 400 cows. Participating farmers and all the other actors involved could verify that with SY, similar milk Se concentrations compared to conventional milk could easily achieved. Although a farm study is often more demonstrative than a strictly controlled research environment, the results of this study are relevant to practical organic farming situations and can be applied to a great extent to similar low-Se situations. In a Finnish farm survey (with 1067 farms, mostly conventional farms), Se concentration in tank milk samples averaged 25 µg l^{-1} and varied typically between 15–30 µg l^{-1}, but clearly higher and lower concentrations were recorded, reflecting the variation of Se content in basic and content and form in supplementary feeds (Sarjokari 2016, personal communication J. Nousiainen 19 May 2022). During the temporary prohibition of SY products for ruminants in organic farming tank milk Se concentrations (122 organic farms) were in the 86.1% of cases below 15 µg l^{-1} and in the 55.7% of cases below 10 µg l^{-1} (Valio Ltd 2014, personal communication J. Nousiainen 31 May 2022). In a national Se monitoring in 2015, Se concentration in Finnish organic milk was merely half of that of conventional milk, but in the following unpublished Se monitoring in 2019, the levels were on a par with conventional milk (M. Eurola, personal communication 6 May 2022). Recently, the increase in fertiliser prices has led conventional farmers to buy and use lower quantities of mineral fertilisers. Hence the risk of Se deficiency cannot be ignored in any of those Finnish farms, which do not use Se-enriched fertilisers.

Conclusions

Replacing SS with the same amount of SY in complete mineral-vitamin mixture more than doubled the Se concentration in organic milk. The mixture of SS and SY resulted in slightly lower Se concentration than SY. The use of SY is recommended for Finnish organic dairy farming to ensure adequate Se status for cows and their calves, and good Se supply from milk to consumers.

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