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The role of selenium in nutrition and the manufacturing of selenium-enriched milk

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Abstract. The role of selenium has increased after the discovery of the first seleno-enzyme in the human body. Selenium supports growth, the immune system, the reproductive organs, thyroid and muscle function, has an antioxidant effect, and protects against free radicals. The recommended daily intake of selenium for adults is 55 μg/day, which cannot be covered with food alone in selenium-deficient areas. That is the reason why we chose as our research topic the production of functional food – in this research: milk – in which the selenium level has been elevated naturally.

In our work, we supplemented six Holstein-Friesian cattle feeds with a daily 1, 2, 4, and 6 mg/individual organic selenium, and then we measured the selenium content by ICP-MS. With a selenium enrichment of 1 mg/day, a 60% increase in selenium content was achieved, as the selenium content of milk increased from 32.93 μg/kg to 52.79 μg/kg. With the 2, 4, and 6 mg supplementation, the milk contained 97.2, 182.69, and 231.31 μg/kg selenium respectively. The latter is seven times more than the selenium content of the control sample. We have developed a recommendation for the amount of naturally selenized milk that should be consumed for different age groups. In our opinion, milk with increased selenium content could contribute to improving the selenium status of the population.

Keywords and phrases: functional food, dairy, Se-milk, selenium-enriched yeast feeding, health preservation

1. Introduction

Selenium is an essential element for the normal function of the human body. It has been known since the first selenium-containing enzyme (glutathion peroxidase) was discovered in humans (*Flohé et al.*, 1973) to exert its antioxidant activity through selenium-containing enzymes, thus improving the immune function, protecting the organism against certain viruses (*Pecoraro et al.*, 2022), decreasing inflammatory processes, and reducing the formation of harmful free radicals (*Steinbrenner & Sies*, 2009). Its role is essential in the regulation of anti-inflammatory processes and the functioning of the normal hormonal system (*Karag et al.*, 1998). Without wishing to be exhaustive, it reduces the risk of developing cardiovascular diseases, rheumatoid arthritis, fatty liver, and insulin resistance and dyslipidemia associated with polycystic ovary syndrome (PCOS) (*Hajizadeh-Sharafabad et al.*, 2019; *Kanafchian et al.*, 2018; *Shidfar et al.*, 2018; *Weeks et al.*, 2012). Although the positive physiological effect of selenium appears to be indisputable, *Stranges et al.* (2007) pointed out in their study that the long-term use of high doses (200 µg/day) of selenium might promote the development of type 2 diabetes (T2D). However, *Steinbrenner et al.* (2022) stressed that the measurable selenium-containing markers may not be the cause but the consequence of diabetes. *Dias et al.* (2021) also clarified that selenium intake has no effect on the development of T2D.

In the human body, selenium is found in amounts of 10–15 mg; it accumulates mainly in the pancreas, spleen, liver, and the kidneys. The recommended daily intake is 55 µg for children and adults over 15 years and 60-70 µg for pregnant and lactating mothers (*Institute of Medicine*, 2000; *National Institutes of Health*, 2021). According to a report by a committee set up by the FAO, the IAEA, and the WHO, the maximum daily tolerable limit, or tolerable upper limit intake level (UL), is 400 µg selenium, above which symptoms of selenosis are to be expected (*WHO*, 1996). Symptoms of short-term high selenium intake include garlic smelling or metallic palate, hair and/or nail loss, while chronic selenium poisoning may include nausea, vomiting, diarrhoea, skin rashes, tooth discolouration, fatigue, and nervous system abnormalities (*National Institutes of Health*, 2021). However, this UL dose cannot be taken in Hungary with food alone.

Research has shown that the absorption of selenium from the digestive tract is limited, most of it being excreted in the urine, so its utilization is low (*Bendhal & Gammelgaard*, 2004). Selenium supplementation is needed in areas with insufficient selenium supply, e.g. in Hungary. This is achieved using organic forms of selenium such as selenomethionine and selenocysteine. The population has two options: make up for the deficiency by using food supplements or consume foods with increased selenium content.

Foods contain varying amounts of selenium – Brazil nuts (*Bertholletia excelsa*) have the highest selenium content, which means 70–90 µg per piece (*Chang*, 1995). Our richest foods in selenium are seafood, fish, animal offal, meat and meat products, and dairy products (*Navarro-Alarcon & Cabrera-Vique*, 2008).

Dietary supplements have been available to the general consumers since the 1980s to complement vitamins and minerals with tablets and capsules. Commercially available selenium products contain inorganic selenite, selenate, and organic (yeast-bound) selenium (Se-methionine) (*Horacsek et al.*, 2006).

According to many, it is optimal to replace selenium with food because the absorption of organic forms of selenium and selenino-amino acids is better than that of inorganic ones (*Surai*, 2000).

Annual milk consumption has shown a slight upward trend in the recent years. According to the Milk Balance of the Hungarian Central Statistical Office, in 2019, this was 206.4 litres (*KSH*, 2021). Milk alone provides 6–10% of our daily selenium intake, making it one of the main sources (*Csapó & Csapóné*, 2002). Selenium added to the feed of dairy cows allows the milk to be fortified with selenium. Supplementation could be done with sodium selenite, selenocysteine, selenomethionine, or even selenium-enriched yeast (*Cobo-Angel et al.*, 2014). For animal feed, the organic forms should also be preferred because of the higher toxicity of inorganic selenium forms and the fact that some of them are excreted in the rumen and intestinal gases, urine, and faeces (*Bokori et al.*, 2003).

Many research groups investigated the effect of selenium added to livestock feed. A Spanish work group, *Azorín et al.* (2020) added the same dose of inorganic and mixed (inorganic/organic) selenium supplementation and found that they could reach higher selenium milk level with the mixed feed additive. *Stockdale et al.* (2011) made an experiment on two groups of Holstein-Friesian cows. They tested if pasture feeding and TMR feeding influences the selenium level of milk when the selenium supplement is added as pellets within the high range of daily 14.5–36 mg. The researchers found that the way of feeding (grazing or not) has no effect on the selenium level of milk, which is a better indicator of feed-based selenium intake than the selenium content of blood serum.

2. Materials and methods

Our feeding experiment was set up in a dairy farm on the northern edge of Hajdú-Bihar County (Hungary) with six Holstein-Friesian cattle. The cows were selected so that their milk production represented the average of the herd; thus, we were not looking for individuals with exceptionally high or low performance. The cows were isolated in the "calving section" for the duration of the experiment, which ran from 29 October 2018 to 8 April 2019. The animals were calved at the end of October 2018, and their ages at the start of the experiment are shown in *Table 1*.

Table 1. Age of dairy cattle individuals in the experiment

Cattle	**1**	**2**	**3**	**4**	**5**	**6**
Age (year)	7	3	3	3	3	2
Nr. of calving	5	2	2	2	2	1

A premix was prepared for the feeding experiment, which contained cornmeal (Nagyhegyesi Takarmány Kft.) and selenized yeast (SelPlex-2300, Alltech Hungary Kft.). A dosing spoon containing 1 mg of selenium supplement (43.5 g) was prepared for dosing. The premix was thus administered to the daily ration once a day per animal according to the given dose (*Table 2*). The basic feed contained 0.7 mg (summer) of selenium at the beginning of the experiment and 0.6 mg (winter) of selenium from 1.12.2018.

Table 2. Daily selenium intake during the experiment

Week of experiment	**Selenium content of basic feed (mg)**	**Daily supplementation (mg)**	**Total selenium daily intake (mg)**
1–2	0.7	0	0.7
3–6	0.7	1	1.7
7–10	0.6	2	2.6
11–14	0.6	4	4.6
15–18	0.6	6	6.6
19–24	0.6	0	0.6

The amount of selenium premix was increased every 28 days from 1 to 2, 4, and then 6 mg Se/cow/day. Control samples were taken before the start of the experiment; selenium feeding was continuous until 25.2.2019, and then, ending the supplementation, a clearance study was continued for 6 weeks.

The selenium content was determined exclusively from milk, so animals were not slaughtered at the end of the experiment. Milk samples were taken individually at the time of milking with an automatic sampling unit connected to the milking machine and were also tested on an animal-by-animal basis, and the results were evaluated by dose and sampling time.

The measurements were performed with ICP-MS (Thermo Scientific X-1 Series 2) at the Institute of Food Science of the University of Debrecen after concentrated acid digestion. Samples were prepared by two-step digestion for selenium analysis. A sample of 2.00 ± 0.01 g each was weighed into the disruptor tubes. For pre-digestion, 10 cm^3 of concentrated HNO_3 was added to the samples, which were then allowed to stand overnight and then held in a block digester at 60°C for a duration of 30 minutes.

For the main digestion, 3 cm^3 of 30% H_2O_2 was added, and the samples were repeatedly placed in the block digester and kept at 120°C for 90 minutes. The samples were cooled to room temperature, made up to 50 cm^3 with deionized water, and filtered through filter paper (Filtrak 388).

MS Excel (Microsoft) was used for data processing and graphical representations, while SPSS 20.0 (IBM SPSS Statistics) was used to perform the statistical analysis of the experimental results.

3. Results and discussion

The results are shown in *Figure 1*, which shows the selenium content of milk by treatment average.

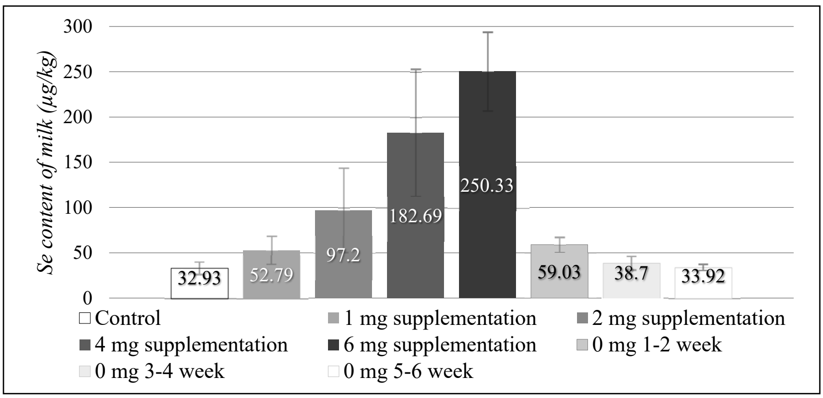

Figure 1. Changes in the selenium content of milk per treatment

The columns with the black numbers show the data when the cows did not receive selenium supplementation. The white-coloured column with black frame shows the result of the control sample. The sample taken at the beginning of the experiment contained 32.93 µg/kg selenium. As a result of the additions (3 grey bars with white numbers), the selenium content increased significantly in all cases due to the doses of 1, 2, 4, and 6 mg/cow/day, and treatment averages increased from 52.79 µg/kg to 97.20 µg/kg, to 182.69 µg/kg, and then to 250.33 µg/kg. In the 19th week of the experiment, no more extra selenium was added, following which the selenium content of the milk decreased to the level of the control samples during the six-week monitoring period.

For consumers aiming to cover their personal daily selenium needs from milk alone, the graph included in *Figure 2* could be informative. Based on experimental results, the curve shows the recommended amount of selenium-containing milk with different selenium concentrations.

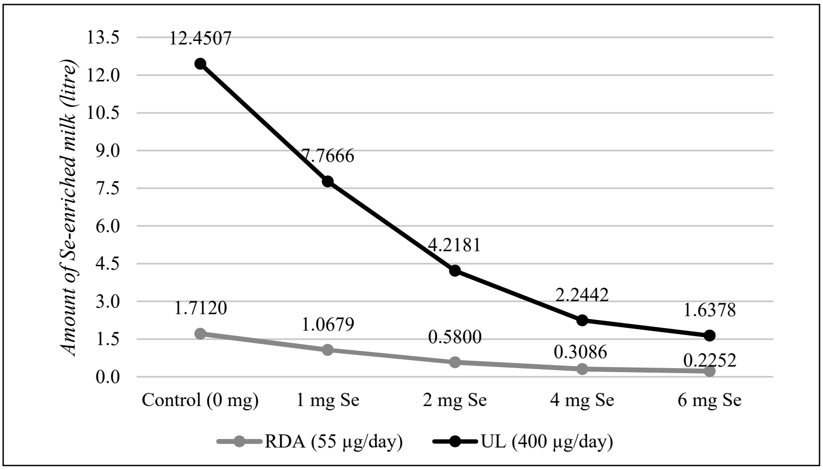

Figure 2. Selenized milk consumption recommendations for adults

The recommended daily intake for adults (according to WHO) is shown in grey, and the upper limit is indicated in black. The average milk consumption of the population cannot be modelled, and selenium is also absorbed from other sources, wherefore we recommend the marketing of milk obtained with a 2 or 4 mg supplement (Se_2-milk and Se_4-milk) for health protection purposes.

As selenium binds to milk protein, the selenium content does not decrease during fat adjustment and lactose removal, so there is no need for selenium replacement during industrial use, and fermented milk products and cheeses can be made from Se-milk. Due to the very high selenium content of Se_6-milk, it is not recommended for industrial use due to the possible accumulation of selenium in dairy products.

Figure 3 shows the recommended intake for milk at 2 mg/day supplementation by age. The recommended amount calculated for Se-milk for infants aged 1 to 3 years is marked in black, which is equivalent to 210 ml, i.e. 1 glass of milk, at the recommended intake of 20 µg/day. Preschool and elementary school children (marked in medium grey) need a daily selenium requirement of about 320 ml of milk, while primary school 5–8th grade students (10–14 years; shown in light grey) can cover their daily selenium needs with 420 ml of milk. For those over 15 years of age (marked with white column on the diagram), the intake value is 55 μg/day, which equals that of the adults and means almost 600 ml of Se_2-milk. In practice, this could be exploited if students in the school milk programme could receive 200 ml of Se-milk or cocoa in order for the beneficial effects of selenium to be felt from an early age.

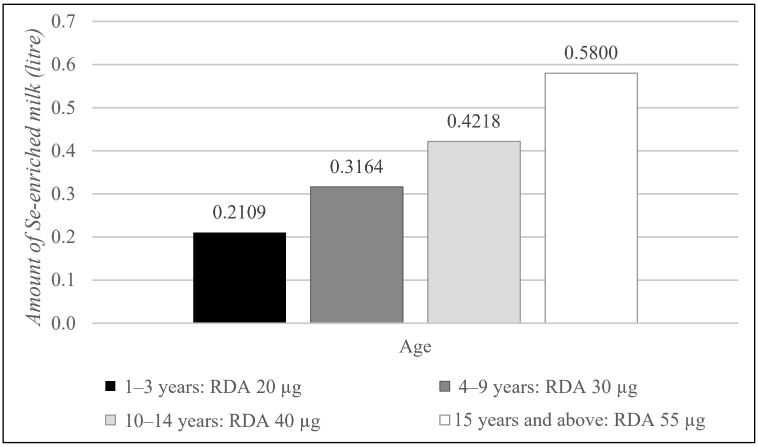

Figure 3. Se_2-milk consumption recommendations for different age groups

4. Conclusions

In our opinion, the experiment was successful because we were able to produce milk with elevated selenium level by feeding dairy cows. With 1 mg of selenium supplementation, the selenium level in milk increased by 60% from 32.93 μg/kg to 52.79 μg/kg. With doses of 2 and 4 mg/cow/day, the milk contained an average of 97.20 μg/kg and 182.69 μg/kg of selenium, which is more than 5.5 times that of the control sample. With a dose of 6 mg/cow/day, milk selenium levels increased to 702% of the original selenium content (to 231.31 μg/kg).

Although the research and clinical trials mentioned in the literature suggest an intake of Se above the RDA in order to maintain health and alleviate certain diseases, in our opinion, the optimal dose for Se supplementation would be 2 to 4 mg/day/cow for direct consumption in the case of healthy adults. In that case, one can take in the daily RDA level of selenium with ~ 3 to 6 dl milk without the risk of overdose [according to the UL, the amount of milk for chronic selenium overdose would be 2.24 litres (4 mg supplementation) and 4.22 litres (2 mg suppl.)]. For dairy production, we suggest using the 2 mg/cow/day selenium dose.

Evaluating the results, we believe that milk with increased selenium content is also important from a food science and health point of view, and as an essential food, it can be one of the cornerstones of adequate selenium intake and healthy eating.

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