THESES OF DOCTORAL (PHD) DISSERTATION

PRODUCTION OF SELENIUM ENRICHED MILK AND DAIRY PRODUCTS

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1. Introduction and aim of the dissertation

Selenium is an essential element for vital function. Since the discovery of the first selenium-containing enzyme in the human body (LOCHÉ et al., 1973), we have known that selenium exerts its antioxidant effect through these enzymes, thus protecting the body against some viruses, as well as against inflammatory processes and the formation of harmful free radicals (PECORARO et al., 2022; STEINBRENNER & SIES, 2009). Its role is essential in anti-inflammatory processes and in regulating the functioning of the normal hormonal system (KARAG et al., 1998). It can be said that it reduces the risk of developing cardiovascular diseases, joint diseases of rheumatic origin, fatty liver, as well as 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 effects of selenium seem indisputable, STRANGES et al., in their 2007 study, draw attention to the fact that its long-term use, at an amount of 200 μg/day, may promote the development of type 2 diabetes, but STEINBRENNER et al. (2022) pointed out in their latest study that measurable selenium-containing markers are not the root cause, but the consequence of diabetes. DIAS et al. (2021) confirmed that selenium intake has no effect on the development of type 2 diabetes.

The human body contains 10-15 mg of selenium, it accumulates mainly in the pancreas, spleen, liver and kidneys. The RDA is 55 μg/day for children and adults over 14 years of age and 60-70 μg/day for pregnant and nursing mothers (INSTITUTE OF MEDICINE, 2000; NATIONAL INSTITUTES OF HEALTH, 2021). According to a report by a committee set up by the FAO, IAEA and WHO, the daily maximum tolerable limit (UL) is 400 μg Se, above which the symptoms of selenosis should be expected (WHO, 1996). Symptoms of short-term high selenium intake are garlic-smelling or metallic taste in the mouth, hair loss, loss of nails, while nausea, vomiting, diarrhea, skin rashes, tooth discoloration, fatigue and nervous system abnormalities may also occur with chronic selenium poisoning (NATIONAL INSTITUTES OF HEALTH, 2021). However, this UL value cannot be taken into the body with food alone in Hungary.

Research has shown that selenium absorption from the digestive tract is limited, most of it is excreted in the urine, so its utilization is limited (BENDHAL & GAMMELGAARD, 2004). Selenium supplementation is needed in areas deficient in Hungary. It is advisable to do this using organic selenium forms such as selenomethionine and selenocysteine. The population has two options: to make up for the deficiency by using dietary supplements or by consuming foods with an increased selenium content.

Food contains selenium in varying amounts. The highest selenium content is found in Brazil nut ('*Bertholletia excelsa*'), which contains 70-90 μg per piece (CHANG, 1995). Seafood, fish, animal offal, meats and meat products, and dairy products are our selenium-rich foods (NAVARRO-ALARCON & CABRERA-VIQUE, 2008). Dietary supplements have been available to the public since the 1980s to replenish vitamins and minerals. Commercially available selenium tablets and capsules contain inorganic selenite, selenate, and organic seleno-methionine - bound to yeast (HORACSEK et al., 2006). According to many people, it is optimal to supplement selenium with food, because the absorption of organic forms of selenium, seleno-amino acids, is better than that of inorganic forms (SURAI, 2000).

Annual milk consumption in Hungary has shown a slightly increasing trend in the recent years; according to the milk balance of the Central Statistical Office, in 2019 it was 206.4 liters (KSH, 2021). Milk alone covers 6-10% of our daily selenium intake, making it one of the basic sources of intake (CSAPÓ & CSAPÓNÉ, 2002). Selenium added to the feed of dairy cows gives the opportunity to enrich milk with selenium. The supplement can be done with sodium selenite, selenocysteine, selenomethionine or selenium-enriched yeast. When feeding animals, it is worth preferring organic forms, because inorganic selenium forms have a higher toxicity, as well as part of them is excreted with rumen and intestinal gases, urine, and feces (BOKORI et al., 2003).

Our work can be significant both in terms of animal science and food science, since the milk and dairy products consumed daily are considered a basic source of selenium, and optimal nutrition is hard to imagine without them.

2. Materials and methods

2.1. Pre-experiment

We considered it important to conduct the preliminary experiment because we wanted to know whether Selplex-2300, which contains organic selenium, is suitable for enriching cows' milk with selenium by adding it as a premix to the basic feed.

Three Simmental-type cattle were included in the experiment, which were kept in domestic conditions and in confined housing. During the experiment, we measured the Se content of the feed (0.43 mg/kg), then additional selenium supplementation was carried out with selenium yeast (SelPlex-2300, Alltech Hungary Kft.). Selenium-containing yeast was added to the feed at 1 mg/cow/day for two weeks and 2 mg/cow/day for another two weeks.

2.2. Main experiment

We set up our feeding experiment in two dairy farms in the county of Hajdú-Bihar, involving six Holstein-Friesian cattle each. The cows were selected so that their milk production represented the average of the herd, therefore we did not look for exceptionally high or low performance individuals. The animals calved within nine days at the end of October 2018, so they were in the same lactation period, their ages and the number of calvings at the start of the experiment are shown in Table 1.

1. Table: ***Individuals participating in the experiment***

Farm	Bödönhát						Hajdúböszörmény					
Individual	**2618**	**4372**	**4730**	**4784**	**4828**	**5396**	**5850**	**6075**	**6438**	**6722**	**6781**	**7197**
Age (year)	7	3	3	3	3	2	3	3	3	3	3	3
Nr. of calving (pc.)	5	2	2	2	2	1	2	2	2	2	2	2

The animals were isolated for the duration of the experiment, which lasted from October 29, 2018, to April 8, 2019.

For the feeding experiment, we prepared a premix containing cornmeal (Nagyhegyesi Takarmány Kft.) and selenized yeast. For dosing, we made a dosing spoon, which could fit a supplement containing 1 mg of selenium (43.5 g). The premix was added to the daily feed ration once a day, according to the given dose per animal. The basic feed at the beginning of the experiment, the summer basic feed in Bödönhát contained 0.7 mg/kg, then from 01.12.2018 the winter basic feed contained 0.6 mg/kg of selenium, while in Hajdúböszörmény it contained 0.6 mg/kg, and after the feed change (2018.12.03.), 0.5 mg /kg was the selenium content of the feed.

The quantity of the selenium premix was increased every 28 days (Table 2), its dosage was 1; 2; 4; 6 Se/individual/day. Control samples were taken before the start of the experiment, the selenium feeding was continuous until 25.02.2019, and then, stopping the supplement, we carried out an emptying test for 6 weeks.

2. Table: ***Selenium intake during the experiment***

Week of experiment	**Selenium content of basic feed**	**Selenium supplementation *(mg/individual/day)***
1-2	0,7/0,6 mg	0 mg
3-6	0,7/0,6 mg	1 mg
7-10	0,6/0,5 mg	2 mg
11-14	0,6/0,5 mg	4 mg
15-18	0,6/0,5 mg	6 mg
19-24	0,6/0,5 mg	0 mg

The selenium content was determined exclusively from milk, so the animals were not slaughtered at the end of the experiment.

The milk samples were taken individually at the once a week (7^{th}; 14^{th}; 21^{st}; 28^{th} day of the dose) with the automatic sampling unit connected to the milking machine and stored below -20 °C until further processing.

2.3. Production of dairy products

When producing dairy products, we took into account the current provisions of the Codex Alimentarius Hungaricus. According to the MÉ 2-51 directive, we made yogurt, kefir, cottage cheese, semi-hard cheese, unsalted and salted lump cheese ("gomolya") and whey cheese ("orda"). We measured the selenium content of the raw milk and finished dairy products, as well as examined the selenium content of whey and permeate left over during production.

2.4. Determination of selenium content by ICP-MS measurement

2.4.1. Sample preparation

To ensure the quality and the original conditions of the samples we performed digestion. After complete thawing, the frozen samples were homogenized and then measured in a special glass tube. The sample preparation was carried out by wet digestion with concentrated acid and heat in a block digestor. After complete cooling, the digested samples were diluted to 50 cm^3 and then filtered (KOVÁCS et al., 1996).

2.4.2. ICP measurement

To determine the selenium content, a Thermo Scientific X-1 Series 2 type inductively coupled plasma emission mass spectrometer with a plasma temperature of 6000 K was used. Before the measurements, we prepared a dilution series using a selenium reference stock solution, and then recorded an 11-point calibration curve. The test was carried out from the destroyed samples with three repetitions each. The detection limit was 0.06 μg/kg.

2.5. Statistical analysis

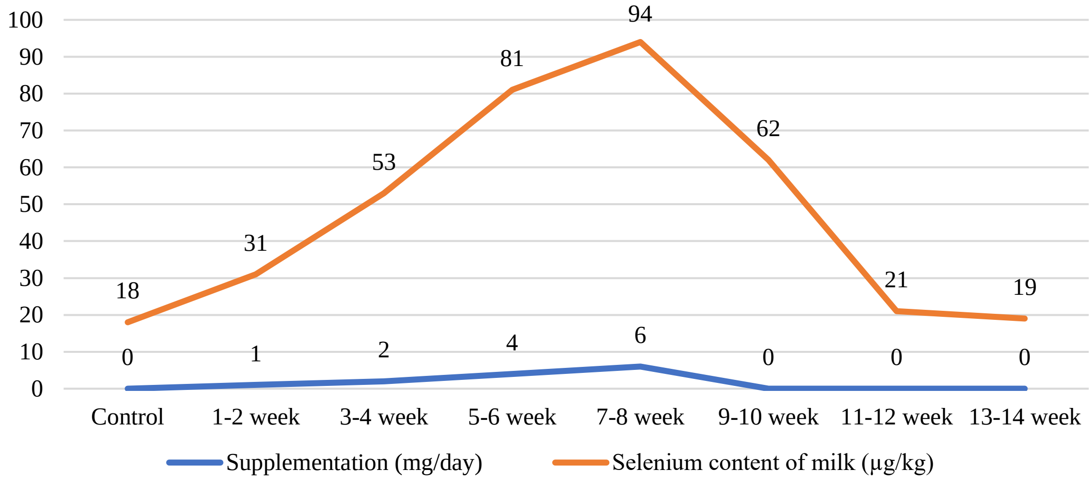
Microsoft Excel was used to evaluate the results and IBM SPSS Statistic 26 to perform statistical analyses. A Oneway ANOVA analysis was performed to investigate the effect of different treatments (1; 2; 4; 6 mg/day). When the analysis showed a significant difference, we analyzed the relationship of the variables (treatments) to each other using a Post Hoc test. If the number of variables was three, then an LSD (Least Significant Difference) Post Hoc test was used. If the number of variables was more than three, then the widely used and less controversial Tukey HSD Post Hoc test was used (SZÉL & JÓNÁS, 2016; NÉMETH, 2018; SAJTOS & MITEV, 2007). To investigate the relationship between microelements and selenium content, Pearson's correlation was calculated, the combined significance of the correlation was examined separately.

3. Results

3.1. Results of pre-experiment

With the preliminary experiment, we wanted to make sure that the selenium content of the milk is increased as a result of the selenium supplementation added to the feed. The animals were sampled at the end of every other week and the samples were analyzed, the results of which are shown in Figure 1.

1. Figure: **Relationship between the selenium content of milk and selenium supplementation during the pre-experiment**

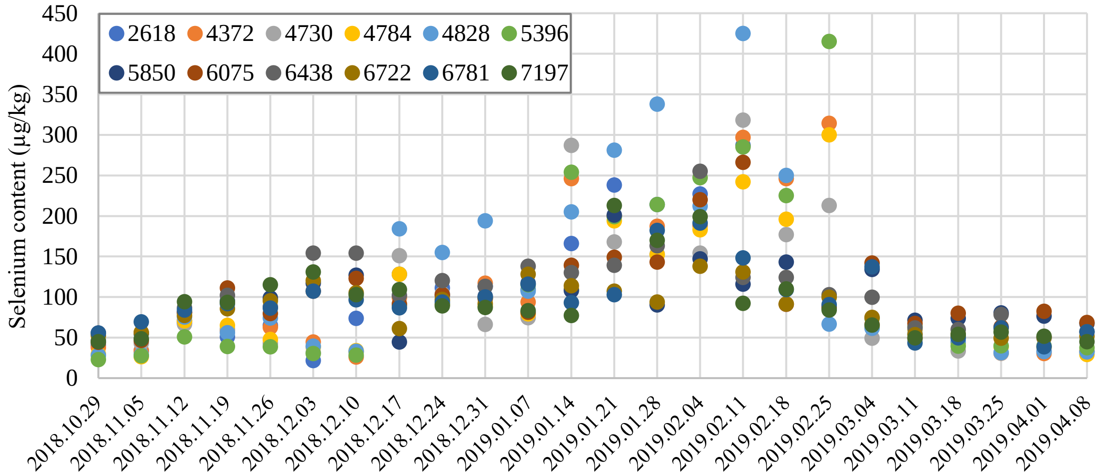


Compared to the control milk, the amount of selenium increased by 73% by the second week of the experiment. As a result of supplementation with 2 mg Se/cow/day, the milk contained almost three times more selenium (53 µg/kg) than the milk of cows consuming only basic feed. Continuing the selenium feeding, the selenium content of the milk already exceeded the value of 81 µg/kg, but by the next week, the selenium seemed to be saturated, so even though we increased the dose to 6 mg/kg per day, the achieved increase in selenium concentration was only 16%. Six weeks after the end of selenium feeding, the selenium content of milk returned to the initial value of 18 µg/kg. Overall, it can be said that with the dosage of 6 mg/day of selenium, we managed to increase the selenium content of milk from the initial 18 µg/kg to more than five times that amount, to 94 µg/kg.

3.2. Results of main experiment

In our research, we conducted a feeding experiment with dairy cattle raised in intensive housing conditions at two locations. We managed to collect a total of 280 milk samples (Figure 2) from the two farms, process and measure them. We produced dairy products on three dates, during which we produced 11 types of dairy products each time, i.e. we managed to produce 33 samples.

2. Figure: ***Summary diagram of the milk samples examined by individuals***



3.2.1. Analysis of milk samples

The samples taken during the experiment were processed at the Faculty of Agriculture, Food Science and Environmental Management of the University of Debrecen, and the measurements were carried out with an ICP-MS device after digestion in the laboratories of the Institute of Food Science.

3. Figure: ***The average selenium content of milk according to the average of treatments***

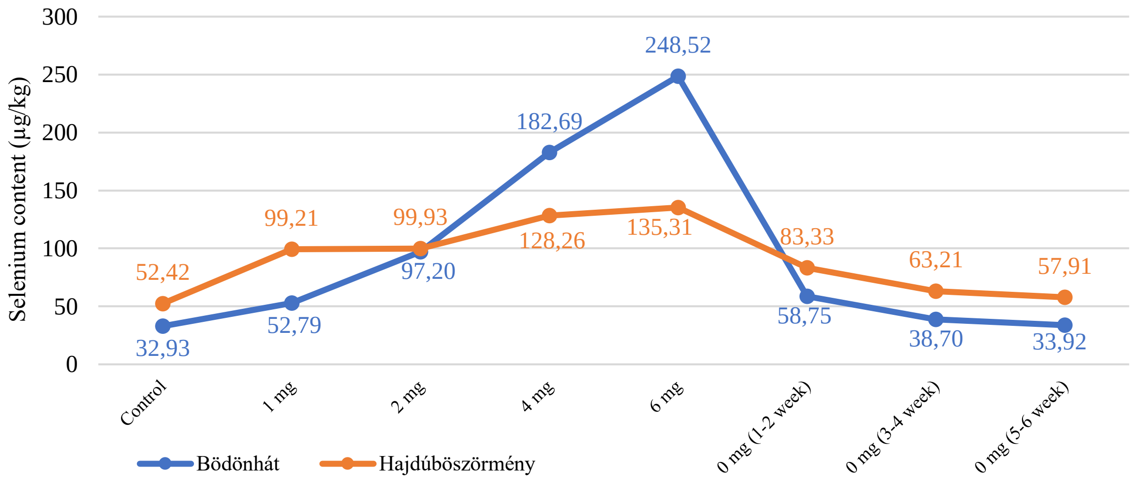
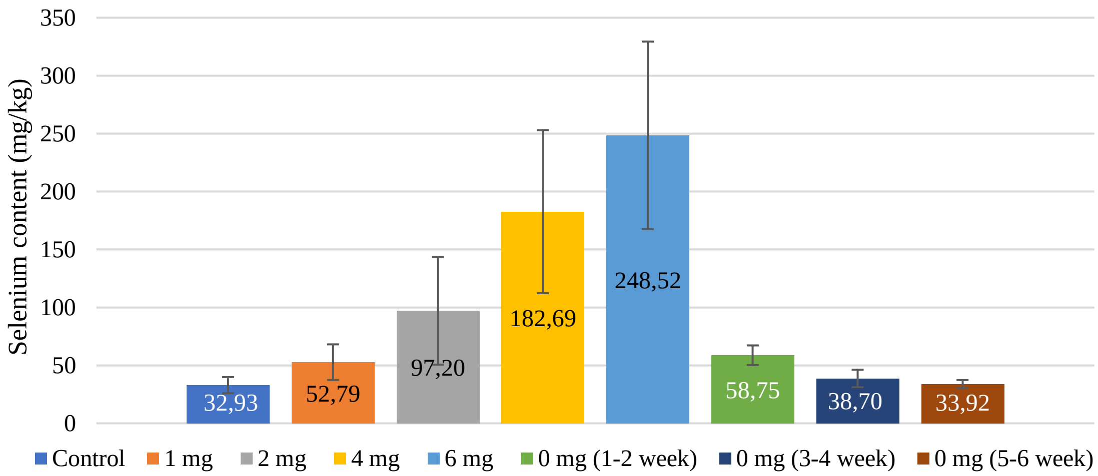


Figure 3 shows the selenium content of the milk in the two farms according to the average of the treatments. In blue we indicated the results of Bödönhát, while in orange we indicated the results of Hajdúböszörmény farm. At Bödönhát, the selenium content of milk increased significantly as a result of the supplementation, from the control value

(32.93 µg/kg) to 7.5 times the maximum administered dose of 6 mg/individual/day (247,83 µg/kg). With the end of the supplement, the selenium content of the milk decreased sharply and returned to the control value after six weeks.

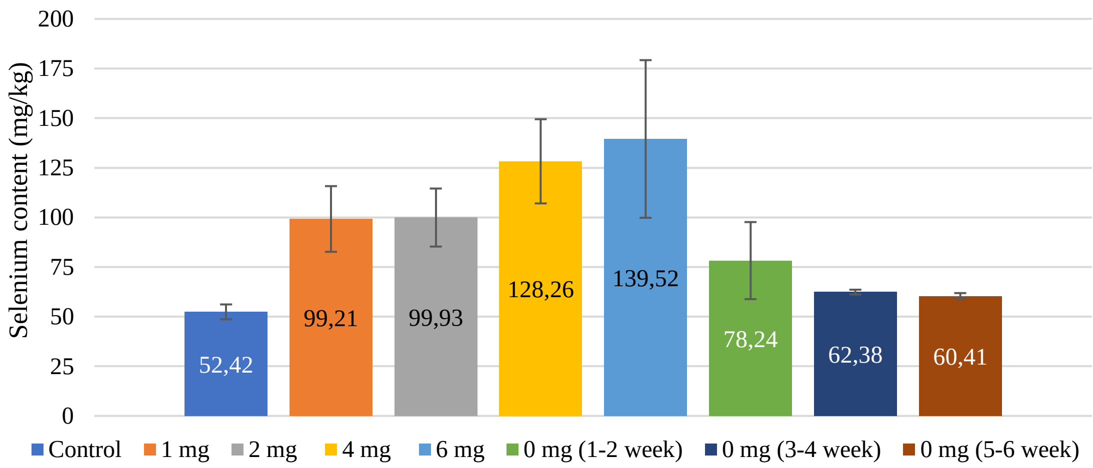
The results of the Hajdúböszörmény plant are more balanced, they do not show such a big jump. Although there is a significant difference between the highest dose supplementation and the control samples, the average of the two treatments is almost the same because of the feed change between 1 and 2 mg/individual/day supplementation. For this reason, we did not make dairy products from milk taken with 2 mg supplementation, we only used milk from the control, 4 and 6 mg supplementation for this purpose.

4. Figure: **Average selenium content of milk (Bödönhát)**



Based on the results shown in Figure 4, it can be established that the selenium content of the control sample taken at the beginning of the Bödönhát experiment was 32.93 µg/kg (± 6.96 µg/kg). As a result of selenium supplementation in different doses, the selenium content in milk is constantly increased. With selenium supplementation of 1 mg/day, the average selenium content of milk increased to 52.79 µg/kg (± 15.38 µg/kg). With selenium supplementation of 2 mg/day, the selenium content of milk is 97.20 µg/kg (± 46.54 µg/kg), in the case of 4 mg/day supplementation it is 182.65 µg/kg (± 70.34 µg/kg). With the highest selenium supplementation of 6 mg/day, the selenium content in the milk reached a peak, at which time the milk of the cattle tested contained an average of 248.52 µg/kg (± 80.88 µg/kg) selenium. At week 17 of the experiment, selenium dosing was ended, so from this time on, the selenium content of milk decreased continuously and rapidly, by week 22 to almost the control value.

5. *Figure:* **Average selenium content of milk (Hajdúböszörmény)**



Based on the results, it can be concluded that the mean selenium content of the control sample taken at the beginning of the experiment was 52.42 μg/kg (± 7.02 μg/kg). Due to the constantly increasing doses, the selenium content of the milk also increased during the experimental period, although the increase is not as spectacular as in the case of the Bödönhát farm. Looking at the results, it can also be seen that although the 1 mg/day and 2 mg/day supplementation differ from the control, the difference between the two doses is hardly noticeable in the graph.

Based on the averages per treatment, the selenium content of the control sample taken at the beginning of the experiment was 52.42 μg/kg (± 7.02 μg/kg). With the addition of 1 mg/day of selenium supplementation, the average selenium content of milk increased to 99.21 μg/kg (± 18.47 μg/kg), which is 46.80 μg/kg higher, almost double the control value. In the case of 2 mg/day selenium supplementation, the average selenium content of milk is 99.93 μg/kg (± 21.03 μg/kg), which is 47.52 μg/kg higher than the control value. The difference between the control and the 2 mg/day treatment is significant, but the selenium content measured after 1 mg/day and 2 mg/day selenium supplementation is almost the same, the difference between them is not significant, which can be explained by the winter feed change. In the case of the 4 mg/day dose, there is a significant increase in the average selenium content of milk, compared to both the control and the 1 mg/day and 2 mg/day selenium supplementations. The average selenium content in the milk of cows receiving selenium supplementation 4 mg/day is 128.26 μg/kg (± 37.32 μg/kg), which is 75.84 μg/kg higher than the control value. It is not surprising that milk achieved the highest selenium content during the period of selenium supplementation of 6 mg/day.

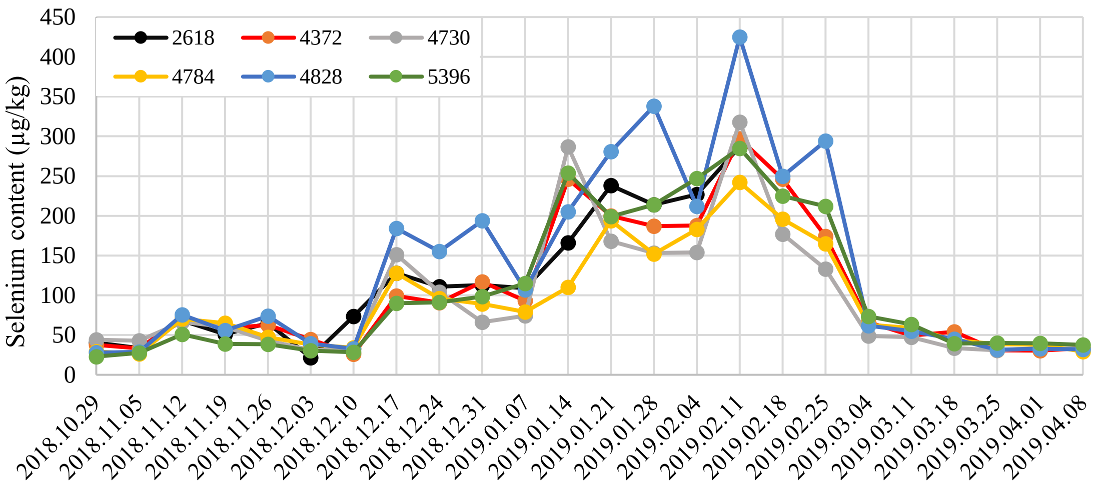
The average selenium content for the 6 mg/day treatment is 139.52 µg/kg (± 52.45 µg/kg), which is 87.10 µg/kg higher than the control value.

The average selenium content measured in the 1-2 weeks after the end of selenium feeding was 78.24 µg/kg (± 15.85 µg/kg), in the 3-4 weeks 62.38 µg/kg (± 10.96 µg/kg), and in 5-6 weeks it was 60.41 µg/kg (± 11.72 µg/kg). This is 7.99 µg/kg higher than the control value, but the difference compared to the control is not significant (SE= 12.09 pTukey= 0.998).

3.2.2. Measurement of selenium content by individuals

Figure 6 shows the change in the selenium content of milk at the Bödönhát farm during the examined period for each individual.

6. Figure: **Changes in selenium content per individual (Bödönhát)**



The figure shows that after the control period (first two measurement times), the selenium content of the milk increased slightly due to the 1 mg/day and 2 mg/day selenium supplementation. All tested individuals experienced a significant increase at the measurement on 17.12.2018.

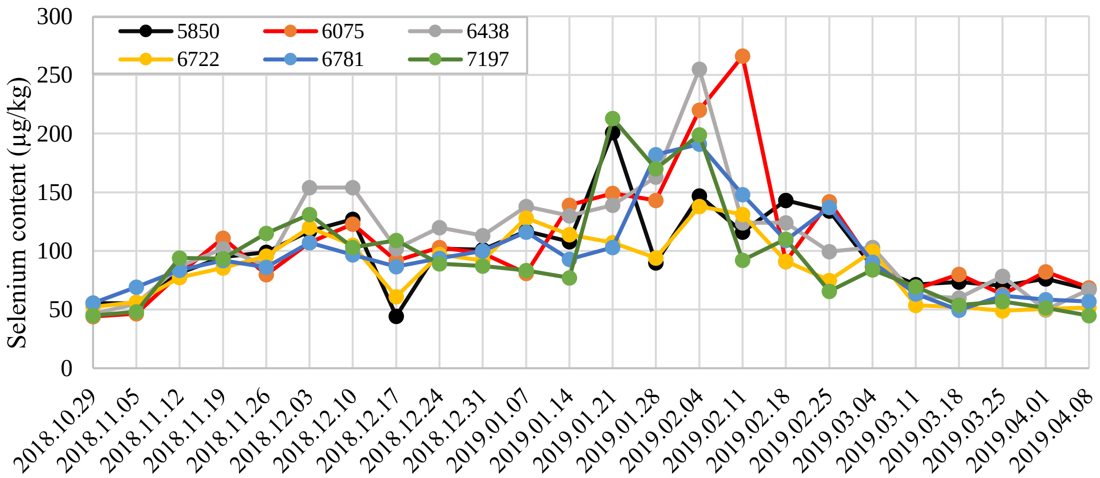
Treatment at doses of 1 mg/day and 2 mg/day are separated as shown. Another significant increase can be seen from the beginning of 2019 for all examined individuals, because this is when the dosing of the 4 mg/day dose began. The doses of 4 mg/day and 6 mg/day are separated based on the individuals, especially for cows with ear numbers 4784 and 4828. Since March 2019, we have not fed selenium supplements, the process of selenium depletion was visible for all individuals.

In the case of the six examined cows of the Hajdúböszörmény farm, the change in the selenium content of the milk during the examined period is shown in Figure 7 for each individual.

The figure shows that after the control period (first two measurement times), the selenium content of the milk increased for all examined individuals. The 1 mg/day and 2 mg/day treatments do not differ sharply. From the beginning of 2019, another significant increase can be seen for all examined individuals. The doses of 4 mg/day and 6 mg/day can be separated in the case of individuals with ear numbers 6075 and 6438, while in the case of the other individuals these two periods are merged.

The last period (from March 2019 without supplementation) can be characterized by decreasing selenium concentrations; the process of selenium depletion can be seen in all individuals.

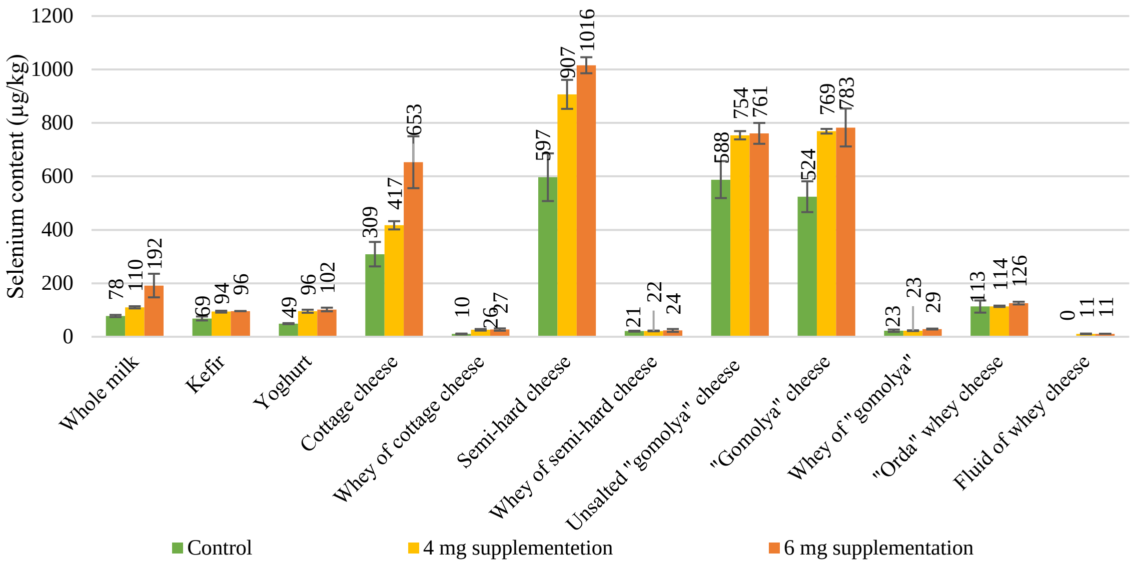
7. Figure: **Changes in selenium content per individual (Hajdúböszörmény)**



3.3. Selenium content of the produced dairy products

Figure 8 contains the results of the examination of the 11 dairy products produced from whole milk taken during the control period and when 4 mg/day and 6 mg/day selenium doses were administered.

8. Figure: **Selenium content of dairy products**



In the whole milk, which is the basis of the dairy products, the selenium content of the control sample was 77.7 µg/kg (±4.11 µg/kg), which was increased to 110.2 µg/kg (± 4.04 µg/kg) after the 4 mg/day treatment, and to 195.6 µg/kg (± 3.33 µg/kg) after the 6 mg/day treatment.

In case of kefir, the selenium content in the control sample is 68.6 µg/kg (± 7.44 µg/kg), which after the 4 mg/day treatment is 93.9 µg/kg (± 3.49 µg/kg), and it increased to 96.0 µg/kg (± 0.17 µg/kg) after treatment with 6 mg/day. There is a significant difference between the different samples based on the selenium content.

In the case of yogurt, the selenium content in the control sample was 49.1 µg/kg (± 1.90 µg/kg), which increased to 95.5 µg/kg (± 5.75 µg/kg) after the 4 mg/day treatment. and increased to 102 µg/kg (± 6.95 µg/kg) as a result of the 6 mg/day treatment.

In the case of the cottage cheese, the selenium content in the control sample was 309.3 µg/kg (± 45.76 µg/kg), which increased to 417.3 µg/kg (± 15.50 µg/kg) because of the 4 mg/day treatment and increased to 653.0 µg/kg (± 96.44 µg/kg) after the 6 mg/day treatment.

The selenium content in the control sample of the whey of cottage cheese was 10.4 µg/kg (± 1.54 µg/kg), which was increased to 25.9 µg/kg (± 4.32 µg/kg) after the 4 mg/day treatment. It increased to 26.5 µg/kg (± 2.91) after treatment with 6 mg/day.

In the control sample of the semi-hard cheese, the selenium content was 597 µg/kg (± 89.27 µg/kg), which increased to 906.7 µg/kg (± 54.22 µg/kg) after the 4 mg/day treatment. As a result of 6 mg/day treatment, it increased to 1015.7 µg/kg (± 30.09 µg/kg).

In the case of the semi-hard cheese whey, the selenium content in the control sample was 20.9 μg/kg (± 1.67 μg/kg), which was increased to 21.8 μg/kg (± 1.35 μg/day) because of the 4 mg/day treatment. kg), and it increased to 23.7 μg/kg (± 5.31 μg/kg) as a result of the 6 mg/day treatment. There is no significant difference in selenium content between different whey of semi-hard cheese.

In the control sample of the unsalted lump cheese ("gomolya"), the selenium content was 587.7 μg/kg (± 69.10 μg/kg), which increased to 754.0 μg/kg (± 15.52 μg/kg) after the 4 mg/day treatment, and it increased to 761.3 μg/kg (± 38.28 μg/kg) after treatment with 6 mg/day.

In the control sample of the salted lump cheese ("gomolya"), the selenium content was 524.0 μg/kg (± 57.66 μg/kg), which, because of the 4 mg/day treatment, increased to 769.0 μg/kg (± 71.55 μg/kg) and increased to 782.7 μg/kg (± 8.74 μg/kg) after treatment with 6 mg/day.

In case of the lump cheese whey, the selenium content in the control sample was 22.6 μg/kg (± 4.36 μg/kg), which was increased to 22.8 μg/kg (± 1.35 μg/kg) because of the 4 mg/day treatment and increased to 28.9 μg/kg (± 1.69 μg/kg) after the 6 mg/day treatment. There is no significant difference in the selenium content between the different whey samples.

In the case of the whey cheese ("orda"), the selenium content in the control sample was 112.6 μg/kg (± 22.67 μg/kg), which was increased to 126.3 μg/kg (± 2.52 μg/kg) as a result of the 4 mg/day treatment. and it increased to 113.7 μg/kg (± 5.13 μg/kg) after the 6 mg/day treatment. There is no significant difference in the selenium content between the different "orda" samples.

Compared to the control sample, an increase can be established for all 11 tested dairy products in both the 4 mg/day and 6 mg/day dose treatments, the biggest difference was measured in the case of semi-hard cheese. Overall, it can be said that there is a significant difference in selenium content between dairy products, apart from whey products.

3.4. Correlations between selenium and microelements

The correlations of microelement content were examined using Pearson's correlation. We searched for the answer to whether there is an actual relationship between the change in selenium content and the amount of other elements in the milk sample, and if so, in what direction and strength.

3. table: **Investigation of the relationship between microelements and selenium (Bödönhát)**

Element	Strength of correlation	Direction of corr.	Significance of corr. coefficient
Copper (Cu)	r=-0,974 (strong)	negative	0,005
Manganese (Mn)	r=0,926 (strong)	positive	0,024
Strontium (Sr)	r=-0,895 (strong)	negative	0,04
Zinc (Zn)	r=-0,755 (stronger than medium)	negative	not significant (n.s.)
Barium (Ba)	r=-0,478 (weaker than med.)	negative	n.s.
Cobalt (Co)	r=-0,309 (weaker than med.)	negative	n.s.
Iron (Fe)	r=-0,262 (weak)	negative	n.s.
Molybdenum (Mo)	r=0,032 (weak)	positive	n.s.

Based on Table 3, it can be concluded that out of the eight microelements examined in Bödönhát, there are three where the relationship between the selenium content and the examined microelement is real and not random with a probability of 95%. These elements are copper, manganese and strontium, for these three elements the correlation relationship is strong (0.81≤|r|<0.99). Selenium shows a strong positive correlation with manganese, while it shows a strong negative correlation with copper and strontium.

4. table: **Investigation of the relationship between microelements and selenium (Hajdúböszörmény)**

Element	Strength of correlation	Direction of corr.	Significance of corr. coefficient
Manganese (Mn)	r=0,891 (strong)	positive	0,043
Molybdenum (Mo)	r=0,742 (stronger than medium)	positive	not significant (n.s.)
Copper (Cu)	r=-0,690 (stronger than med.)	negative	n.s.
Iron (Fe)	r=0,650 (stronger than med.)	positive	n.s.
Zinc (Zn)	r=-0,601 (stronger than med.)	negative	n.s.
Strontium (Sr)	r=-0,329 (weaker than med.)	negative	n.s.
Cobalt (Co)	r=0,140 (weak)	positive	n.s.
Barium (Ba)	r=-0,058 (weak)	negative	n.s.

Table 4 summarizes the results of the correlation test for the Hajdúböszörmény farm. Among the eight tested microelements, there is one where the relationship between the selenium content and the tested microelement has a 95% probability of being real and not due to chance. This element is manganese, which is the only one among the examined elements to show a strong positive correlation (0.81≤|r|<0.99).

In the case of both the Bödönhát and Hajdúböszörmény farms, a significant, strong positive correlation between the selenium and manganese content of the milk was demonstrated.

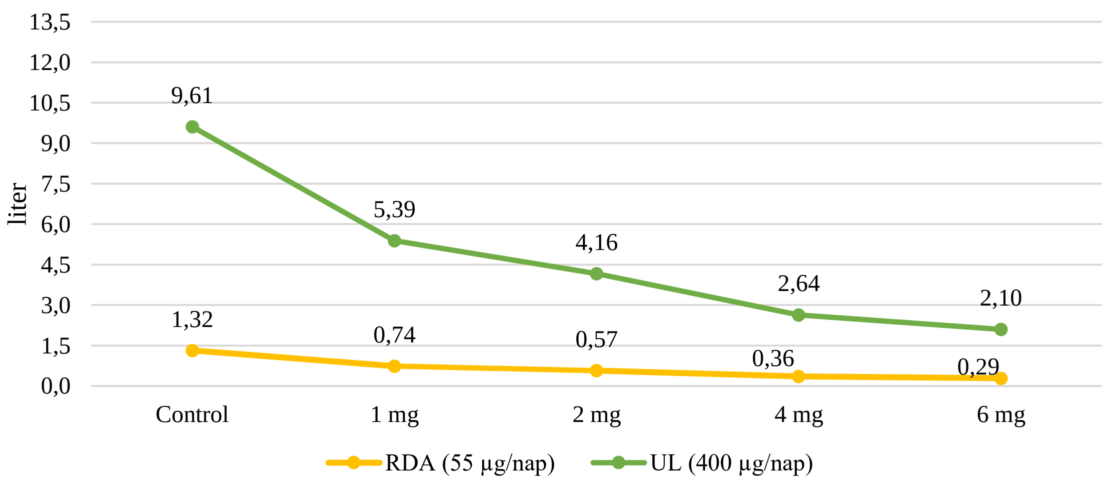
4. New scientific results

1. By mixing different concentrations of organically bound selenium into cattle feed, I proved that the selenium content of milk increases significantly as a function of the dosed selenium concentration as a result of selenium supplementation. I proved that the selenium concentration of the milk increases one week after the start of selenium feeding. By omitting the selenium supplement, a decrease in the selenium content of milk can already be seen in the second week. I found that the selenium content of milk increased significantly because of selenium supplementation of 1 mg/cow per day, and the increase was also significant as a result of supplementation of 2; 4; 6 mg compared to both the control and the cattle that received a smaller supplement.
2. We found that even with the highest (6 mg/day) selenium supplementation, even 1.5 liters of cow's milk per day can be safely consumed, because its selenium content remains below the UL. A milk sample taken at a lower supplementation does not mean any danger to the average consumer.
3. In case of cottage cheese, the selenium content increased from 309 µg/kg to 653 µg/kg, in the case of semi-hard cheese, from 597 µg/kg to 1015 µg/kg, as a result of the 6 mg supplement. The selenium content in the case of the "gomolya" increased from the control level of 524 µg/kg to 783 µg/kg. We could not detect a significant difference between the 4 and 6 mg/day supplementation in all cases. In the case of whey cheese (“orda”), the selenium content varied between 113 and 126 µg/kg, however, there was no significant difference between the control and the 4 mg/day supplement. From the selenium content of cottage cheese, lump cheese (“gomolya”) and semi-hard cheese, it can be concluded that most of the selenium is bound to the casein fraction.
4. I found positive correlation between the selenium and the manganese content, and negative relationship between selenium and copper content of the milk.

1. Practical applicability

In our research, in order to increase the selenium content of milk, we developed a technology to determine the optimal amount of selenium mixed with cows' feed. By supplementing the feed with 1-6 mg/day per individual, the selenium content of cow's milk increased significantly as a result of the treatments; the highest result was obtained when feeding 6 mg. After the end of the feeding, the selenium content of the milk decreased significantly in two weeks, and by the end of the fourth week it was at the level before the selenium supplementation. Due to the previous findings, we came to the conclusion that in order to maintain the increased selenium content of the milk, continuous selenium supplementation is necessary, because the cow is not able to store as much selenium in its body as it would be able to produce milk with increased selenium content after completing the selenium supplementation. In order to continuously produce milk with a high selenium content, we therefore recommend supplementing the feed of dairy cattle with 1-4 mg of selenium per day. During our experiments, the dosage of organic selenium proved to be optimal.

8. Figure: ***Recommended daily intake and maximum tolerated dose for adults from selenium milk (based on farm averages)***



The recommended daily intake value (RDA - Recommended Dietary Allowance) given by the WHO for adults is indicated in yellow, while the maximum tolerable upper intake level (UL - Upper Limit) is indicated in green (Figure 8). Since the population's average milk consumption cannot be modeled, and selenium is also taken from other sources, we recommend marketing the milk obtained with 1-4 mg supplementation for health protection purposes in order to avoid overdose.

Since selenium is bound to milk protein, the selenium content does not decrease during fat content adjustment and lactose removal. During the industrial use of milk produced in the way we recommend fermented milk products and cheeses can also be made from selenium milk. For small, medium and large companies involved in the production of dairy products, in order to improve the population's selenium supply, production of selenium enriched dairy products can be inserted without technology modification.

9. Figure: **Selenium-rich milk consumption recommendation for the different age groups based on the averages of the 1-2 mg of milk taken as a supplement**

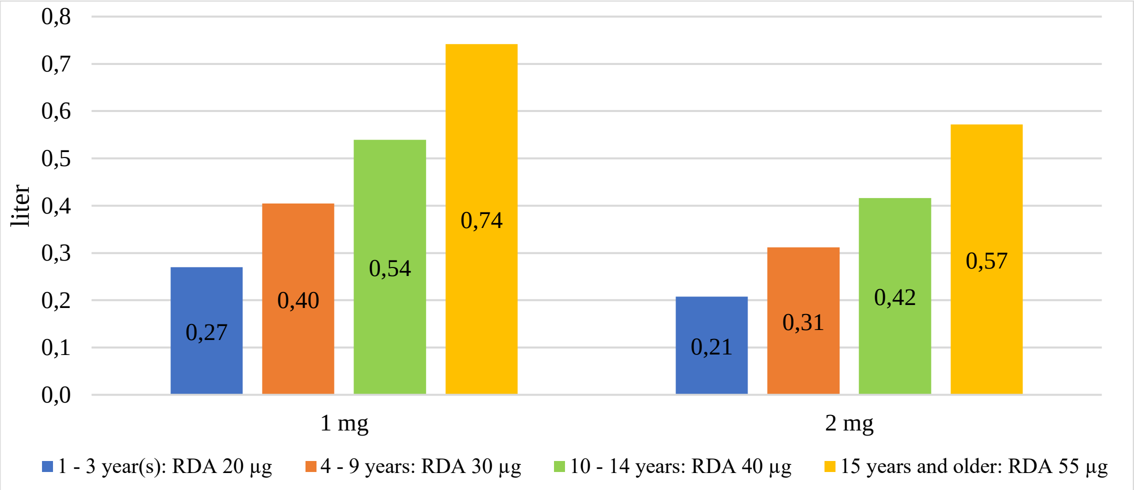


Figure 9 shows how much milk from the 1-2 mg supplement would cover the children's selenium needs. The smallest amount recommended for 1-3-year-old children is marked in blue, which is 210-270 ml, i.e., equivalent to 1 large glass of milk, for the recommended intake value of 20 µg/day. The daily selenium requirement of preschool and elementary school age children (indicated in orange color) can be taken with 320-400 ml of milk, while the age group of primary school seniors (indicated in light green color) can cover their daily selenium requirement with 420-540 ml of milk. For those over 15 marked with lemon yellow, the intake value recommended for adults also applies (55 µg/day), which means approximately 600-740 ml of milk. In practice, this would be feasible if children and students could receive 200 ml of selenium-enriched milk or cocoa in the school milk program or in the canteen, so that the beneficial effects of selenium could be effective from an early age.

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List of publications related to the dissertation

Hungarian scientific articles in Hungarian journals (2)

1. **Juhászné Tóth, R.**, Kiss, D., Zurbó, Z., Csapó, J.: A szelén szerepe a táplálkozásban; szelénnel dúsított tej és tejtermékek előállítása.
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