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Production of selenium-enriched milk and dairy products

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Abstract. Until the middle of the last century, selenium was considered to be toxic, but recently it turned out to be a micronutrient with important physiological effects, whose lack impedes the functioning of several enzymes, while in the case of a prolonged deficiency, disease processes can also occur in the body. Hungary belongs to the selenium-deficient regions in Europe; therefore, our aim was to contribute to the improvement of selenium supply of the population through increasing the selenium content of milk and dairy products. A daily supplementation of 1-6 mg organic selenium to the feed of dairy cows increases the selenium content of milk from the value of 18 μg/kg to 94 μg/kg in 8 weeks, decreasing again to the initial value in 6 weeks after stopping the supplementation.

After producing various products from the control milk (18 μg/kg selenium content) and the selenium-enriched milk (53 μg/kg) obtained from dairy cattle fed on a feed supplemented with 2 mg selenium/day, we concluded that the selenium content of selenium-enriched milk compared to the products produced from the control milk increased from the value of 18.6 to 58.5 μg/kg in the case of yogurt, from 66.0 to 138.1 μg/kg in the case of *telemea*, from 80.8 to 163.7 μg/kg in the case of *orda* (urdă) and from 88.6 to 200.0 μg/kg in the case of semi-hard cheese obtained by mixed-coagulation. The selenium content of whey also increased significantly (from 8.8-9.7 μg/kg to 20.1-25.8 μg/kg), which could also be used as a food for people or feed for animals. According to our calculations, the selenium requirements of the developing organism could be satisfied by the consumption of 2-3 dl selenium-enriched milk until the age of 8 and with 4-6 dl selenium-enriched milk until the age of 20.

1 Introduction

Until recently, selenium was considered to be a toxic heavy metal because the consumption of larger amounts of it leads to the devastation of the living organism. Recently, however, with the improvement in sensitivity of analytical methods, it could be found that it has important physiological effects since the human body itself contains about 15 mg of selenium, which, together with some tocopherols, is involved in metabolic processes. The body's antioxidants play an important role in the defence against harmful free radicals; these natural protective mechanisms are involved in eliminating the harmful

effects of free radicals. Parts of these protective mechanisms, for example the glutathione peroxidase and thioredoxin reductase, are selenium-dependent enzymes, which, in the absence of selenium, are not able to neutralize the harmful and sometimes carcinogenic components. Glutathione peroxidase protects unsaturated lipids and helps preserve the integrity of cell membranes by catalyzing the peroxide degradation reaction. It was discovered in the recent decades that selenium is necessary for normal life activities and helps in treating certain forms of cancer and even in their prevention. Foods produced in Scandinavia and some other European countries are very selenium-deficient. The selenium amount entering into our bodies through our daily meals (0.05-0.10 mg) is not significant.

The relative atomic mass of selenium is 78.96 g. Among its isotopes, the isotope ^{80}Se occurs more frequently. Depending on the environmental influences, it occurs with the following oxidation states: -2, 0, +4, +6, (*Skinner*, 1999). In 1930, selenium was considered to be toxic and in 1943 its carcinogenic properties were also described (*Nelson et al.*, 1943). *Clayton & Baum* (1949) showed that the presence of selenium is essential in the living organism and selenium supplementation reduces the number of cancer diseases. The essential role of selenium was pointed out in 1957 (*Schwarz & Foltz*, 1957), when it was found that selenium added to the diet could prevent liver necrosis. In 1966 (*Shamberger & Rudolph*, 1966), the anticarcinogenic effect of selenium was also published, but at that time the total selenium content of the food was mentioned. In 1973 (*Turnder & Stadtman*, 1973), the catalytic activity of selenium-dependent enzyme proteins was studied, among which the glycine reductase plays a significant role in anaerobic bacteria, while the glutathione peroxidase (*Rotruck et al.*, 1973) plays a significant role in mammals. In 1976 (*Cone et al.*, 1976), the glycine reductase and the seleno-cysteine, the selenium analogue of cysteine, were discovered.

Researchers found that it is not possible to conclude the selenium supplementation suitability of a given biological system from its total selenium content as the different chemical modifications considerably differ from each other due to their toxicity, absorption and availability in the human body. Therefore, if we try to get information about their availability in the human body, each of the different chemical modifications must be determined (*Thomassen & Nieboer*, 1995). There is a need to consider the oxidation state of selenium, the distribution of metal complexes and the relationship with chelating agents, as these effects can significantly influence availability. It is therefore necessary to examine the distribution, quality and quantity of selenium modifications with the help of speciation analysis.

Foods of plant origin contain only selenomethionine, while those of animal origin contain both selenomethionine and seleno-cysteine. As methionine is an essential amino acid for humans as well as for animals, they cannot produce selenomethionine from inorganic sources, but the selenomethionine entered into the body can be converted into seleno-cysteine (*Beilstein & Whanger*, 1986). In addition to these seleno-amino acids, the derivatives of these, seleno-methyl-seleno-cysteine and γ-glutamil-seleno-methyl-seleno-cysteine, are also present. These two components occur in greater concentrations in the food of vegetable origin if the soil is treated with a significant amount of fertilizer.

Selenium is present in the human diet in the form of selenite and selenomethionine. After entering into the human organism, it reacts with thiols, in a reaction which is catalyzed by the enzyme glutathione. In the reduction process, selenate converts into selenite, and then hydrogen-selenide is formed. During the process of trans-sulphurization, seleno-cysteine will be formed from selenomethionine through seleno-cystationine. Due to the effect of a β-lyase enzyme, the seleno-cysteine will be broken down into hydrogen-selenide. In another pathway, it will be metabolized through trans-amination and decarboxylation, and about 90% of it gets incorporated through non-specific means into the organism (*Mitchell et al.*, 1978). During the reaction with S-adenosyl-methionine, methyl-selenol is formed from methyl hydrogen, which then converts into dimethyl selenide first, and then into trimethyl-selenonium ion (*Janghorbani et al.*, 1999). When treating plants with large amounts of selenium, γ-glutamyl-methyl-seleno-cysteine is formed primarily, whose availability is similar to that of methyl-seleno-cysteine. Due to the effect of β-lyase enzyme, the methyl-seleno-cysteine becomes converted into methyl selenol (*Dong et al.*, 2001). Assuming an average selenium intake, the excess selenium will be excreted through the urine in the form of amino sugars, but, if it enters into the organism in large amounts, it can also be excreted via the lung in the form of dimethyl-selenide, while through the urine in the form of trimethyl-selenonium ion (*Suzuki & Ogra*, 2002; *Kobayashi et al.*, 2002; *Bendhal & Gammelgaard*, 2004).

Selenium acts as an antioxidant through the binding to various enzymes. Skeletal muscle proteins integrate even the selenomethionine and seleno-cysteine from external sources (these are selenium-containing proteins), but those seleno-proteins which play an active role in metabolism can use only the seleno-cysteine formed in the body (*Levander & Burk*, 1996). These seleno-proteins are able to operate solely in the presence of selenium and their amount significantly decreases in the case of a selenium-deficient diet. The role of seleno-proteins in biochemical processes became well known already in the

last century; this way, seleno-protein P, iodotironin-deiodinase, thioredoxin reductase and seleno-phosphate synthase have also been identified (*Allan et al.*, 1999).

In 1975, *Awashti et al.* identified the antioxidant glutathione peroxidase, and it was found that it is present in all of the human tissues and that its function is primarily determined by the amount of reduced glutathione and the selenium supply of the body (*Meister & Anderson*, 1983). It was found about glutathione peroxidase that it protects the structure of the membranes, it inhibits DNA damage, reduces the formation of carcinogenic substances in the body through the removal of hydrogen peroxide as well as lipid and phospholipid hydroperoxides.

We know today that selenium integrates into the enzyme as seleno-cysteine, occupying the location of sulphur, and – as it gets reduced more easily than sulphur – it plays a very important role in the defence system of the body against oxidation (*Cser et al.*, 1998). In the last century, four selenium-containing glutathione peroxidases have been identified, of which all have antioxidant properties (*Holben & Smith*, 1999). The administration of selenium increases the activity of these enzymes, which, measured in platelets, serves as a good indicator of the body's supply of selenium. The selenium-containing iodotirozin-deiodinase enzyme plays an important role in the synthesis and activation of tri-iodide-iodothyronine and thyroxine hormone; therefore, the selenium is strictly necessary for normal development and growth (*Wilson et al.*, 1998; *Holben & Smith*, 1999). The thioredoxin reductase is involved in the regeneration of the organisms' antioxidant system, it converts dehydro-ascorbic acid back into ascorbic acid and – with the reduction of thioredoxin – plays an important role in the regulation of cell growth (*Holben & Smith*, 1999; *Mustacich & Powis*, 2000). Seleno-protein C has likely a role in the transmembrane transport processes and, due to its antioxidant effect, it protects the endothelial cells against the chemical attack of peroxy-nitrites.

From the literature data, it appears, therefore, that the availability and utilization by the body of metallic and inorganic state of selenium is very low, firstly because of limited absorption from the gastrointestinal tract and, secondly, because much of the absorbed selenium-containing compounds will be excreted in the urine, while the forms not excreted in the urine are of limited availability. The selenomethionine form of selenium, which is formed in plants due to the selenium content of the soil and which animals convert into seleno-cysteine, can be well utilized. In addition to these two forms of selenium, there exist some other selenium-containing compounds too, but, according to the literature, their use as selenium supplementation is very low. Due to the

above-mentioned facts, during the selenium supplementation of the Hungarian population, it is advisable to use cystine and methionine selenium analogues.

There are two possibilities to supplement the body with selenium: firstly, with food of plant and animal origin, and secondly with the consumption of foods with increased selenium content. Despite the fact that our foods contain selenium in very various amounts, in general, it can be stated that our most commonly consumed foods have very low selenium content. The richest source of selenium is considered to be animal organs (liver, kidney), marine fish, crustaceans and finally meat. There are certain foods that are distinguished by their high selenium content. Thus, Brazil nuts may contain even 100 μg of selenium (*Chang*, 1995, a, b). Since plants do not require selenium for the normal functioning of their organism, the selenium content of these can vary significantly.

The selenium content of foods of vegetable origin are affected by the selenium content of the soil and its oxidation forms, the pH of the soil, which affects even the different selenium forms, the organic compounds of the soil, the iron and aluminium content and rate of the soil, which can bind selenium, the content of sulphur-containing compounds, which, if present in large amounts, can inhibit the absorption of selenium from the soil, precipitation, which can wash out the selenium from the soil, and microorganisms which can convert insoluble selenium compounds into soluble forms.

Because of the fact that the selenium content of most of the foods is very low, with the increasing of their consumption, the selenium supply of the body cannot be increased, the selenium requirement of the body can be satisfied with dietary supplements, while, on the other hand, with selenium-enriched foods. Dietary supplements spread in the 80s of the past century and the well-controllable manufacturing technology of present day makes highly utilizable products available for the consumers in the form of encapsulated or tablet supplements (*Horacsek et al.*, 2005). These products contain mainly selenite, selenate, selenomethionine or selenium-enriched yeast.

Selenium-enriched foods with particular nutritional uses contain selenium in its natural or close-to-natural form. The production technology of such foods is extremely complicated as the selenium supplementation added to the feed or prepared for plants suffers several modifications and transformations until it reaches its natural form. During the transformation, the oxidation state of selenium may change; thus, it is important to monitor in what form the plant or animal nutrient contains selenium. The following selenium-enriched products are on the market in Hungary: selenium-enriched cereal, egg, margarine, bread, respectively bakery products. There appeared many data in

the literature regarding the selenium-enrichment of foods of animal and plant origin (*Gergely et al.*, 2004; *Shah et al.*, 2004; *Rayman*, 2000, 2002) and the supplementation of chicken meat, egg, broccoli, onion and wheat has also been reported.

According to *Schauzer* (2000), the most widely traded selenium supplementation is selenium-enriched yeast. The enrichment can be carried out with sodium selenite, sodium hydrogen selenite, sodium selenate and selenomethionine. The selenium content of selenium-enriched yeast may reach the value of 3,000 mg/kg, integrated mainly as selenomethionine into the yeast proteins (*Polatajko et al.*, 2004); on the other hand, methyl seleno-cysteine is present only in small amounts in the selenium-enriched yeast (*Kotrebai et al.*, 2000).

The selenium-enriched yeast is the result of the fermentation process of *Saccharomyces cerevisiae* in a medium with high selenium content, where the broth is usually a solution with high sugar content, containing usually sodium-selenite as selenium source. In order to assure an optimal growth of the yeast, the broth is supplemented with vitamins, minerals and nitrogen-containing compounds. After producing the selenium-enriched yeast paste, the yeast cells are killed through a heat treatment procedure and the paste is dried by spray-drying, obtaining this way a product which contains organically bonded selenium in high concentration. After the manufacturing process, the inorganic and organic selenium content has to be controlled as it affects primarily the quality of the end-product. The inorganically bonded selenium is present mainly in the form of selenomethionine, which is able to replace non-specifically the methionine of the organisms' proteins, acting as a selenium reserve for the consumer (*Thomson*, 2004, a, b).

The inorganic selenium content of the selenium-enriched yeast (sodium-selenite) is an excellent substrate for protein formation, but the organism cannot form a selenium stock from it (*Varo et al.*, 1988). Some countries approved to add selenium-enriched yeast even to the feed, where the aim is to produce foods of animal origin rich in selenium due to the different yeast strains used, the different manufacturing techniques applied and the different quality traits of the selenium forms used for enrichment. *Fox et al.* (2004) found that the selenium absorption may be of different quality. They also concluded that the selenium content of the selenium-enriched yeast is better utilizable than the inorganic forms and it remains longer in the organism after the selenium supplementation. This can be explained by the integration of selenomethionine into the tissue proteins, which then, after escaping from its bond due to protein degradation, adequately supplies the organism with selenium.

Levander et al. (1997) compared the utilization of inorganic selenium and organic selenium derived from selenium-enriched yeast and wheat, and they found that the supplementation with selenium-enriched yeast and/or wheat is similar in terms of uptake and retention, but it is much higher than that of the inorganic selenium. While studying the utilization of seleno-methionin by lactating and non-lactating mothers, *Mcguire et al.* (1993) found that the selenium content of blood plasma of lactating mothers who did not receive selenium supplementation was significantly lower than that of the non-lactating mothers. In the case of selenomethionine supplementation, the selenium content of blood plasma increased in both types, while selenium-enriched yeast increased the plasma selenium content only in the case of non-lactating mothers. Due to the supplementation with selenomethionine, the selenium content of breast milk increased significantly compared to those who consumed selenium-enriched yeast as supplement.

According to the data reported in the literature, it can be concluded that the seleno-enzyme activity increases due to selenium yeast supplementation and the selenomethionine content of yeast – being integrated into the organisms' proteins – allows the selenium to become only slowly eliminated from the organism, ensuring this way the selenium requirement of the organism. The utilization of selenium-enriched yeast is lower than that of selenomethionine; this can be explained by the fact that the selenium-containing yeast has to be broken down by the organism in order to make the selenomethionine become available. In addition to this, the lower utilization can also be explained by the fact that selenium is not only present in the form of selenomethionine, but a major part of it is in the form of inorganic selenium. According to the analysis of *Schrauzer* (2000), the half life of selenomethionine in our body is 252 days and that of selenite is 102 days, which also explains the better storability of the organic form of selenium. A similar result came out when the LD_{50} value of these two selenium forms was measured in a study made on rats. In the case of selenium-containing yeast, this value was 37.3 mg/kg, while in the case of sodium-selenite the value was 12.7 mg/kg, which proves that, in order to obtain toxicity levels, a higher amount is needed from the selenium-containing yeast than from inorganic selenium. However, there arises the problem that the long-term consumption of organic selenium-containing compounds can lead to selenosis as the body is capable to accumulate it. Therefore, it is very important to monitor the selenium accumulation of the body and to know what kind of selenium form the selenium-supplemented food or the selenium-enriched food contains.

1.1 Selenium enrichment possibilities of foods of plant origin

Plants are able to convert the inorganic selenium from the soil or that sprayed onto their leaves into organically bonded selenium compounds. This method presents a safety selenium supply for people compared to the initial selenite, as the risk to overdose the body with selenium by consuming selenium-containing food of plant origin is low (*Terry et al.*, 2000). Most plants contain up to 1-2 mg/kg selenium at 100% dry matter, and, even if the soil is rich in selenium, the selenium content does still not exceed the value of 10 mg/kg. These plants do not accumulate selenium, but there are some which are able to store large amounts of selenium in their organs if grown in selenium-rich soil.

Among the plants that accumulate selenium, the plants of the families of *papilionaceae* and *cruciferous* are considered to be primarily accumulating plants and are able to accumulate 1000 mg/kg selenium, while the plants considered to be secondary accumulating plants are able to take up only smaller amounts of selenium from the soil, and as a consequence their tissues contain only a few hundred mg/kg of selenium (*Bell et al.*, 1992; *Ellis & Salt*, 2003). The capability to take up high amounts of selenium can be explained by the fact that these plants synthesize seleno-amino acids primarily (90-95% methyl-selenocysteine), which the plant is capable to store for a prolonged period (*Brown & Shrift*, 2001). The plants called primarily accumulating plants do not integrate the seleno-amino acids into their proteins; therefore, they can be used for the detoxification of soils containing toxic levels of selenium (*Banuelos et al.*, 1996). In the case of plants grown on soils containing normal levels of selenium, the selenium gets integrated into the plant proteins and enters the food chain mainly in the form of seleno-cysteine and selenomethionine.

The main selenium-containing compound of plants growing on soils with higher selenium content is methyl-seleno-cysteine; selenium is stored in this form by e.g. broccoli and other bulb vegetables. The last ones contain sulphur compounds in high amount and, due to the fact that selenium is synergistic with sulphur, it displaces the sulphur from the amino acids; therefore, bulb vegetables are frequently chosen as host vegetables for the production of selenium-containing foods. While studying the supplementation of bulb vegetables with selenium (*Kotrebai et al.*, 2000), it was concluded that in the case of a low selenium concentration of the soil selenium is stored as γ-glutamyl-methyl-selenocysteine in garlic, whereas in the case when the selenium concentration of the soil becomes increased the main selenium-storing compound will be methyl selenocysteine, but selenomethionine also occurs in small amounts in the plants.

In an experiment where garlic was enriched with 3 mg/kg selenium, *Ip et al.* (1992, 1998) found that this amount could reduce the development of breast cancer from 83% to 33% in the case of rats. Experimenting with broccoli, *Finley et al.* (2001) concluded the same. *Dong et al.* (2001) concluded that selenium-enriched garlic was more effective in the reduction of breast cancer development than selenium-enriched yeast, which was explained with the fact that selenium is present in the form of selenomethionine in the case of yeast, while in the case of garlic it is present as methyl-seleno-cysteine or the glutamyl derivative of it. These latter two derivatives are assumed to provide a very effective protection against breast cancer.

In addition to the previously mentioned plants, selenium accumulation was also studied in the case of wheat, corn, rise and soy (*Olson et al.*, 1970; *Beilstein et al.*, 1991), whereby they found that selenium is present mainly in the form of selenomethionine in these plants. In contrast, the findings of the previous author, *Ip et al.* (2000), *Kápolna & Fodor* (2006) and *Cai et al.* (1995) found that the main selenium form in the garlic, green onion, chive and broccoli enriched with selenium is the form of methyl-seleno-cysteine.

After having examined the literature data, it becomes clear that the greatest threat to people is the consumption of selenium in inorganic form as the acute toxicity can develop in this case quickly and easily. It is less dangerous if the selenium is introduced into the body in its organic forms such as selenomethionine; however, in this case, the accumulation of selenium poses a risk as selenomethionine can be stored for a longer period by the body. It seems to be a better solution if selenium is consumed through selenium-enriched natural foods, but the solution to introduce the necessary amount of selenium into the body through products of animal origin enriched with selenium seems to be an even better solution as the animal executes such a conversion after which the food of animal origin does not pose any danger to the organism of the consumer-assuming normal eating habits.

The selenium absorbed from the soil occurs in some plants in the form of methyl-seleno-cysteine of glutamyl methyl selenocysteine; in the other parts of plants, the main selenium-containing organic compound is selenomethionine, while in foods of animal origin, the organic compound of selenium is present almost exclusively in the form of selenomethionine. Some plants are able to accumulate high amounts of selenium, while others contain selenium in an amount adequate for human consumption even when grown on soils with high selenium content. In the case of plants, their selenium content could be increased by various selenium-containing fertilizers; in the case of animals, inorganic selenite and selenate may be suitable for increasing the selenium

content of the animal tissues, but it is better if the animal feed also contains organically bonded selenium.

1.2 Possibilities of selenium enrichment of foods of animal origin

It is possible to produce selenium-enriched meat in the case of products of animal origin, but selenium-enriched poultry eggs and cattle milk could also be used as a selenium supplementation for the population with a very high efficiency. Since the selenium content of most of the foods is extremely low, by an increased consumption of these, the selenium intake cannot be increased. The selenium requirement of the organism can be satisfied with dietary supplements, on the one hand, and with selenium-enriched foods, on the other hand.

The preparation of selenium-enriched foods requires an extremely complicated technology because selenium is supplemented to the plants or to animals as a nutritional supplement, and subsequently the natural form of selenium will be reached through several transformations. During these transformations, the oxidation state of selenium can also change; therefore, it is important to monitor the selenium form contained in the foods of animal or of plant origin.

Through the supplementation of swine feed with inorganic (selenite, selenate) or organic (selenium-enriched yeast) selenium, it is possible to produce high selenium-containing pork. The similar supplementation of the feed of laying hens results in organic eggs with high selenium content, while the supplementation of cattle feed increases the selenium content in milk.

From the data reported in the literature, it can be concluded that the activity of seleno-enzymes increase due to selenium supplementation, and the selenomethionine – integrating into the organisms' proteins – allows the selenium to be slowly excreted from the body, ensuring this way the continuous selenium requirement of the organism. In the case of pigs and piglets, the consumption of a selenium-containing diet (sodium selenite and feed with selenium-enriched yeast) significantly increases the selenium content of organs and precious muscle parts.

Despite a decline in milk consumption, milk, as a basic food, has to be counted as a selenium source for humans; therefore, its selenium content should not be indifferent. It was also suggested that the selenium content of milk could be a suitable indicator to determine the selenium state of the herd. Knowing the concentration of selenium in milk, we could indicate the selenium supply

of the animal organism and the health state of the udder. By supplementing dairy cattle feed with selenium, it is possible to produce selenium-enriched milk.

1.3 The aims of the experiments

It is well known that Hungary belongs to the selenium-deficient regions within Europe. The selenium-deficient supply of the population may lead to various diseases which could seriously threaten the health of the population. To solve the selenium supplementation, a number of attempts were born – in which the authors were also involved (bread and egg with selenium content) – and even currently some research is being conducted (the analysis of selenium content in wheat, flour and bread, the possibilities of selenium enrichment of flour, the analysis of breast milk selenium content, the selenium content of breast milk and the selenium content of the mothers' diet as well as the link between these).

The investigation of dairy feed supplementation methods by which the selenium content of milk and dairy products could be increased and the analysis of the facts how the selenium content of milk passes into dairy products made from this milk fits well into the researches listed above. It would also be useful to examine the effect of inorganic and organic selenium added to raw milk on the growth of lactic acid bacteria and the kefir yeast applied in the dairy industry, as well as to measure how the inorganic selenium content converts into organic form during the production of dairy products.

The selenium supply of the population was also explored in Hungary, but relatively few have dealt with the selenium content of cow's milk and with the possibilities to increase it through the feed. Due to the above ones, we aimed at developing feeding methods which allow us to increase the selenium content of milk to the health protection level. We have studied and continuously examined the effect of various, commercially available selenium supplements and selenium supplements developed by us (selenite, selenate, selenium-enriched yeast and other organic forms of selenium) on the selenium content of milk. We have produced kefir and yogurt, cheese produced with different technologies, butter and butter creams from milk with high selenium content, and examined how the selenium passes from the milk to the various dairy products. The ultimate aim is to develop a technology through which it is possible to produce milk and dairy products with high selenium content in order to better supply the population with healthy selenium sources.

Since the selenium-containing compounds are not directly added to the food,

selenium overconsumption or poisoning can be safely avoided. In the present research, we supplemented the dairy cattle feed with selenium-enriched yeast, hoping that the selenium content increases significantly in the milk, and we may produce dairy products with high selenium content from this milk. We want to report in this paper about the results obtained due to the organic selenium supplementation, the increased selenium content of the milk and about the production of high selenium-containing dairy products.

2 Materials and methods

2.1 Experimental animals and selenium supplementation

We included in our experiments three Simmental dairy cows, which were four or five months pregnant. The milk production based on corn silage and hay meadows feed systems was of 4,000-5,000 litres of milk during their lactation period. The selenium supplementation was carried out by adding selenium-enriched yeast (Selplex-2300), which contained 2,300 mg of selenium in the form of selenomethionine and seleno-cysteine. We prepared a premix using ground corn grain in a way that 10 g of the premix contained 1 mg selenium in order to ease the administration. This premix was added to the daily feed of dairy cattle. The selenium content of the feed consumed by the animals was of 0.42 mg daily. Before beginning the experiment, we took milk samples from each cow (control group). In addition to the previous feed ratio (control group), the animals got 1 mg of selenium supplementation in the first two weeks, 2 mg in the 3^{rd} and 4^{th} week, 4 mg in the 5^{th} and 6^{th} week and 6 mg in the 7^{th} and 8^{th} week. After the 8^{th} week, samples were taken three more times every two week and the selenium content of the milk was measured. *Table 1* shows the selenium supplementation of dairy cows in the form of selenium-enriched yeast.

2.2 The production of different dairy products

According to the data from the literature, a 6 mg/kg selenium supplementation is considered to be fully safe; however, we consider to use a 2 mg selenium supplementation daily practice because of safety reasons, and we produce dairy products from the milk of the control group and that of cows obtaining a daily dose of 2 mg selenium supplementation, and examine what kind of selenium-containing dairy product can be produced from the milk of the control group and that containing selenium.

Table 1: Selenium supplementation of dairy cows in the form of selenium-enriched yeast

Number of weeks	Selenium supplementation (mg) cow/day
1-2	1
3-4	2
5-6	4
7-8	6
9-10	0
11-12	0
13-14	0

The selenium content of the basic forage is 0.42 mg/cow/day.

We produced from both the control milk (18 μg/kg selenium content) and the selenium-enriched milk (53 μg/kg selenium content) yogurt, Telemea cheese, curd cheese, Orda cheese and cheese prepared with mixed coagulation. The selenium content of the product as well as that of the whey obtained as a by-product of the production process was measured in each case. The dairy products were produced according to the Romanian standards at the Department of Food Science of the Sapientia Hungarian University of Transylvania, Miercurea Ciuc. The selenium content of the produced dairy products was determined at Kaposvár University, Faculty of Animal Science, Department of Chemistry and Biochemistry and Analytical Laboratory as well as at the Sapientia Hungarian University of Transylvania, Department of Food Science.

Both the control and the selenium-containing milk was pasteurized at 78 °C for 50 seconds, and then, during the yogurt production, the pasteurized milk was inoculated with a pure culture mixture of *Lactobacillus delbrueckii* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* after the milk was cooled down to 27 °C, and then the samples were incubated at 27 °C for 7 hours in a thermostat and frozen at -25 °C.

During the production of the semi-hard cheese with mixed coagulation, its fat content was adjusted to 3.9%, and we treated the milk with a 70 °C heat treatment for 50 seconds in a laboratory pasteurizer, after which it was cooled down to the inoculation temperature of 34-36 °C and inoculated with 0.001% pure culture of *Propioni* bacteria, letting it age until reaching 19 degrees acid. The rennet was dissolved in lukewarm water before using it, and it was added to the cheese milk in order to seed it uniformly. After the addition of the

rennet, the milk was stirred for two minutes and, after stopping the movement, the mixture was left to coagulate for 40 minutes until the curd was separated from the wall of the tub and crushed like porcelain, which meant the end of the coagulation. During the processing of the clot, this was chopped into pea-sized lumps, and it was rotated for 10 minutes in order to precipitate the whey, while we measured the degree of acidity of the whey, which increased by two degrees acid. Then it was poured into shapes and allowed to desiccate at room temperature for 24 hours, rotating it every 30 minutes and storing it in the refrigerator afterwards. During the storing at 8-10 °C with a 95% air humidity, we also rotated it several times a day.

During the production of Telemea cheese, the fat content of the cheese was adjusted to 3.8% and it was pasteurized using a laboratory pasteurizer at 70 °C for 50 seconds. After having cooled down the milk to the inoculation temperature of 35-37 °C, we added 0.0015% pure culture of *Propioni* bacteria, and then allowed to age for 20 minutes, adding to it the above-calculated volume of rennet, and then coagulating it for 35 minutes. During the processing of the clot, we chopped it into walnut-sized lumps, rotated it for 15 minutes in the whey, and then, in order to promote the drying of the curd, we continuously increased the temperature to 38-40 °C while stirring it slowly and continuously. After pouring it into shapes, we allowed it to dry for 2 hours at room temperature, and then put it into a saline solution of a concentration of 12% at 12-14 °C for 24 hours. As a result, the salinity of the Telemea cheese reached 2.5-3.0%. The cheese was allowed to age at 10-12 °C by 95% air humidity and it was repeatedly rotated.

During the production of fresh curd cheese, we heated the raw milk to the temperature of 45-50 °C and skimmed it and adjusted the fat content to 0.9%; after that, it was pasteurized with a laboratory pasteurizer at 70 °C for 50 seconds. Subsequently, the temperature was cooled down to 28-30 °C, after which 1.5% of pure *Propioni* bacteria culture and a 2% pure culture mixture of *Streptococcus termofilus*, *Lactococcus lactis lactis*, *Lactococcus lactis cremoris*, *Lactococcus acidophilus* was added. The milk was allowed to age for 45 minutes. After that, we added 0.001% of rennet in the way described earlier and let it age for 10 minutes. During the processing of the clot, we chopped it into walnut-sized lumps; the whey was removed by filtration, and then the clot was kneaded and packaged.

During the production of Orda (whey cheese), we used the whey left as by-product from the production of the mixed-coagulated semi-hard cheese and the Telemea cheese. The whey was heated to 95-96 °C for 1-2 hours, the precipitated whey proteins were removed and the product was kept in a

refrigerator until the analysis. The whey obtained during the production of Orda practically did not contain any proteins.

2.3 The determination of selenium content of milk and dairy products

We measured the selenium content of milk after digesting it with nitric acid and perchloric acid by applying fluorometric measurements from the piazselenol form obtained after the derivatization with diamino-naphthalene of the selenite, which was obtained from selenate reduced into selenite with hydrogen chloride. In a series of experiments, the selenium content was measured in the form of hydrogen-selenide both fluorometrically and by atomic absorption spectrophotometer, equipped with hydrogen generator. Since we did not observe significant differences between the two types of measurements, hereinafter we applied the more economical fluorometric method.

3 Results

The changes in the selenium content of the milk obtained as a result of selenium supplementation with Selplex-2300 are shown in *Table 2*.

Table 2: The effect of selenium supplementation on the selenium content of milk

Control/experimental groups	Average daily selenium intake (mg)	Selenium content of milk*** (mg/kg)
Control group (CG)	0.42*	0.018 ± 0.002 ****
CG+1 mg Se**/cow/day 1st and 2nd weeks	1.42	0.031 ± 0.002 (0.022)
CG+2 mg Se**/cow/day 3rd and 4th weeks	2.42	0.053 ± 0.003 (0.022)
CG+4 mg Se**/cow/day 5th and 6th weeks	4.42	0.081 ± 0.005 (0.001)
CG+6 mg Se**/cow/day 7th and 8th weeks	6.42	0.094 ± 0.006 (0.001)
CG+0 mg Se at the end of 10th week	0.42	0.062 ± 0.002 (0.001)
CG+0 mg Se at the end of 12th week	0.42	0.021 ± 0.002 (NS)
CG+0 mg Se at the end of 14th week	0.42	0.019 ± 0.002 (NS)

* Selenium content of the basic materials
** Selenium supplementation in the form of Sel-Plex-2300 (seleno-yeast)
*** Milk sampling at the end of the experimental periods
**** In parentheses, the level of significance is shown compared to the control.

Analysing the data in the table, we found that the selenium content of the milk increased by the increasing amount of the selenium supplementation com-

pared to the value of 0.0145 mg/kg measured in the control group to 0.031 mg/kg in the case of 1 mg/day selenium supplementation, 0.053 mg/kg when supplemented with 2 mg/day, 0.081 mg/kg in the case of 4 mg/day selenium supplementation and 0.094 mg/kg when 6 mg/day of selenium was added. After the completion of selenium supplementation, the selenium content decreased to 0.062 mg/kg after two weeks, 0.021 mg/kg after 4 weeks, 0.019 mg/kg after 6 weeks, which almost coincided with the value of 0.018 mg/kg measured in the control group.

The results of the statistical analysis indicate that the selenium content of milk originating from cows which obtained a varying amount of selenium supplementation was significantly higher at $P<0.001$ level than that of the control group, and the increase of selenium supplementation led to a significant increase in the selenium content of the milk. The greatest increase was measured in the 6^{th} week, when the degree of selenium supplementation was about 4 mg/day; although a further increase of the selenium supplementation to 6 mg/day increased the selenium content of milk significantly again, the degree of this increase was far more less than that of the previous periods. Two weeks after finishing the selenium supplementation, the selenium content of milk was significantly lower than the value measured in the 6^{th} and 8^{th} week, but it statistically exceeded the values measured in the case of animals obtaining only 2 mg/day selenium supplementation. A month after finishing the selenium supplementation (12^{th} week), the selenium content of milk was the same as that of the control group which did not receive any selenium supplementation.

From our experiments, it can be concluded that a daily selenium supplementation of 6 mg in the form of organic selenium increases the selenium content of milk about fivefold. However, the data in *Table 2* show also that in the case of the absence of selenium supplementation the selenium content of the milk decreases to 0.019 mg, which is practically the same value as the one measured at the beginning of the experiment. This draws the attention to the fact that in order to continuously obtain milk with increased selenium content it is necessary to add selenium supplementation to the dairy cattle feed in a continuous manner.

The question arises how the milk with increased selenium content satisfies the selenium requirements of the children and adult population, on the one hand, and, on the other hand, whether the milk with increased selenium content poses a health risk to the public or not. *Table 3* shows the daily selenium requirement of 1–19-year-old people and the milk quantity necessary to satisfy the reported need. If we compare the data shown in *Table 3* with the compo-

sition of the control and the selenium-enriched milk as well as with the milk consumption quantities of the population, then we come to the conclusions reported below.

Table 3: Recommended dietary allowance of selenium and the quantity of milk necessary to satisfy RDA with control and selenium-enriched milk

Age (years)	RDA (μg Se/day)	Quantity of milk (L)	
		Control milk	Selenium-enriched milk
1–3	20	1.00	0.21
4–8	30	1.50	0.32
9–13	40	2.00	0.43
14–18	55	2.75	0.59
19–	55	2.75	0.59

RDA = Recommended Dietary Allowance

The milk and dairy product consumption of Hungarian adult population is 0.26 litre in the case of women and 0.28 litre in the case of men. Comparing these data with the selenium content of milk, it can be concluded that milk contributes to the satisfaction of the selenium requirements in only 7% when it is not supplemented with selenium. If we consider the data of milk with the highest selenium content (0.094 mg/kg), we realize that this milk contributes to the satisfaction of the daily selenium requirement in 34%. As it is well known that a high selenium intake can lead to serious illnesses, it is necessary to examine whether the increased selenium content of milk poses a health risk for the various ages.

Various studies clearly report that the daily selenium intake of adults from food sources of animal origin, but without integrating the amount of dairy products, ranges from 73 to 126 μg selenium, whereas this value is 12 μg in the case of 1–3-year-old children; therefore, the consumption of selenium-enriched milk by the adult population can be evaluated as safe, as the maximal selenium intake by a consumption of one litre milk is 220 μg, while the acceptable daily intake is about 300 μg. In the case of children aged 1–3 years, a daily consumption of 0.5 litre of selenium-enriched milk results together with other foods in a value of 59 μg selenium intake, which is almost the same as the upper limit of selenium intake (60 μg/day). Due to the foregoing, it is recommended that 1–3-year-old children do not consume more than half a litre of selenium-enriched milk. The consumption of selenium-enriched milk should be limited to a maximum of half a litre/day for this age-group.

Table 4 shows the changes in the selenium content of milk and dairy products depending on the selenium content of the raw material. According to the results of the statistical analysis, each dairy product produced from selenium-enriched milk had a significantly higher selenium content at $P < 0.001$ level than the products derived from the control group. The selenium content of the yogurt produced from the control milk was measured to be 18.6 μg/kg, while that made from selenium-enriched milk was 58.5 μg/kg. The minimal growth observable in the value of selenium content of the yogurt produced from the control milk can be explained by the water loss occurred during the heating phases of the production, which increased the selenium content of the resulting fermented product.

Table 4: Selenium content of dairy products as a function of selenium supplementation

The name of the dairy product	Selenium content (μg/kg)	
	Control	Selenium-enriched milk
Whole milk	18.0 ± 1.39	53.0 ± 2.80 (0.001)*
Yogurt	18.6 ± 0.72	58.5 ± 0.40 (0.001)
Telemea	66.0 ± 5.60	138.1 ± 2.01 (0.001)
Telemea whey	9.7 ± 0.60	20.1 ± 0.31 (0.001)
Curd cheese	57.4 ± 0.21	154.8 ± 1.75 (0.001)
Curd cheese whey	8.8 ± 0.20	25.8 ± 0.56 (0.001)
Orda	80.8 ± 1.62	167.2 ± 1.59 (0.001)
Orda whey	4.6 ± 0.10	10.8 ± 0.26 (0.001)
Mixed curding cheese	88.6 ± 1.17	200.0 ± 2.10 (0.001)
Cheese whey	9.2 ± 0.23	21.4 ± 0.85 (0.001)

* In parentheses, the level of significance is shown compared to the control.

The selenium content of Telemea cheese made from the control milk was 66.0 μg/kg and the value of the Telemea cheese made from selenium-enriched milk was 138.1 μg/kg. The whey contained 9.7 μg/kg selenium in the case of the control and 20.1 μg/kg in the case of selenium-enriched milk. Comparing the data obtained for the products produced from the control milk with the data of the products produced from the selenium-enriched milk, it can be stated that the Telemea cheese produced from the selenium-enriched milk contains twice as much selenium as that produced from the control milk. The same is truth in the case of whey as the whey obtained by the manufacturing procedure of Telemea cheese also contains twice as much selenium as the control.

In the case of Orda, the product produced from the control milk contained 80.8 μg/kg selenium; that produced from selenium-enriched milk contained 167.2 μg/kg selenium, which is also double the amount of the control. The selenium content differences among the whey obtained from the production of Orda from control milk (4.6 μg/kg) and that of the whey obtained from Orda produced from selenium-enriched milk (10.8 μg/kg) were even greater. The selenium content of the mixed-coagulated cheese produced from control milk was 88.6 μg/kg, while that of the mixed-coagulated cheese produced from selenium-enriched milk was 200.0 μg/kg.

The selenium content of the whey obtained during the production process from the product produced from the control milk was 9.2 μg/kg, while the value in the case of whey obtained during the production process of the product produced from selenium-enriched milk was 21.4 μg/kg. Compared to the dairy products previously discussed, the increase in selenium content is again a two-fold for the benefit of selenium-enriched milk. The selenium content of the curd produced from the control milk was 57.4 μg/kg and that of the curd produced from the selenium-enriched milk was 154.8 μg/kg. These values are very similar to the values obtained in the case of Telemea cheese. The selenium content of the whey obtained during the production process of curd cheese produced from the control milk was 8.8 μg/kg, while the value in the case of whey obtained during the production process of curd cheese produced from selenium-enriched milk was 25.8 μg/kg.

We concluded, therefore, that the selenium content of Telemea, Orda and mixed-coagulated cheese produced from selenium-enriched milk was each time more than the double of the amount of dairy products produced from control milk. The highest amount of selenium content, 200.0 μg/kg, was measured in the mixed-coagulated cheese, followed by Orda with 167.2 μg/kg selenium content, curd cheese with 154.8 μg/kg selenium content and finally the Telemea with 138.1 μg/kg selenium content. We also concluded that the selenium content of the whey obtained during the manufacturing procedure of the products produced from selenium-enriched milk was more than double the selenium content of the whey obtained from the products produced from control milk. Therefore, it can be concluded that the whey of products produced from selenium-enriched milk can be a valuable source of selenium for people as well as for animals. This is also proved by the high selenium content of 167.2 μg/kg of the Orda cheese obtained from whey.

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