

Impact of Supplementation of Various Selenium Forms in Goats on Quality and Composition of Milk, Cheese and Yoghurt

A. PECHOVÁ¹, B. JANŠTOVÁ², L. MIŠUROVÁ¹, M. DRAČKOVÁ², L. VORLOVÁ², L. PAVLATA¹

¹Clinic of Ruminant Diseases, Faculty of Veterinary Medicine,
University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

²Department of Milk Hygiene and Technology, Faculty of Veterinary Hygiene
University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

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Abstract

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The objective of this experiment was to monitor the possibility of influencing the composition of milk and milk products by supplementing the feed of lactating goats with selenium in various forms (natrium selenite, selenium proteinate).

The trial was carried out in 30 goats, divided into 3 groups: Group C (n = 10) was a control group, SeI group (n = 10) received selenium in the form of natrium selenite and SeO group (n = 10) in the form of lactate-protein complex produced by *Lactobacillus acidophilus*. Diverse selenium supplementations were carried out from 3.5–4 months before the date of delivery until the 2nd month of lactation. In the time span of 2 weeks, milk from individual goats was taken six times and cheeses and yoghurts were made from the mixed milk from individual groups. During the period of milking and making milk products, the goats in experimental groups received added 565 µg Se per animal and day in relevant forms.

Long-term selenium supplementation in the form of natrium selenite/proteinat had no significant effect on the Se concentration in milk. Se concentration in milk of individual groups was as follows: C: $13.14 \pm 4.21 \mu\text{g}\cdot\text{l}^{-1}$, SeI: $12.50 \pm 5.59 \mu\text{g}\cdot\text{l}^{-1}$, SeO: $12.47 \pm 3.71 \mu\text{g}\cdot\text{l}^{-1}$. Similarly, no significant differences in the selenium concentration were determined in yoghurts (C: $17.13 \pm 5.40 \mu\text{g}\cdot\text{kg}^{-1}$, SeI: $18.12 \pm 5.49 \mu\text{g}\cdot\text{kg}^{-1}$, SeO: $19.69 \pm 6.48 \mu\text{g}\cdot\text{kg}^{-1}$) and cheeses (C: $68.79 \pm 7.14 \mu\text{g}\cdot\text{kg}^{-1}$, SeI: $70.41 \pm 6.22 \mu\text{g}\cdot\text{kg}^{-1}$, SeO: $69.66 \pm 12.68 \mu\text{g}\cdot\text{kg}^{-1}$) made from milk of individual goats groups. Apart from selenium concentration in milk and milk products, we also monitored other quality indicators that might have been influenced by selenium supplementation in the goats' feed. Among qualitative milk indicators, we found significantly lower titration acidity and significantly higher rennetability in SeI group. We also determined a significantly higher fat concentration in milk of SeO group compared to the other two groups. Indicators monitored during production of yoghurts and cheeses were not influenced significantly, only fat concentration in yoghurts was higher in SeO group compared to SeI group.

Supplementation of selenium in the form of natrium selenite and selenium proteinate exceeding the physiological needs of goats had no significant effect on the Se concentration in milk and milk products.

Natrium selenite, selenium proteinate, microelements, metabolism

Selenium is an essential trace element present in numerous enzymes (glutathione peroxidase, iodothyronin deiodinase, thioredoxin reductase, etc.) and selenium proteins. Selenium helps to protect the organism against oxidation stress, participates in the synthesis and metabolism of thyroid hormones, proteosynthesis, it is important for reproduction and its anti-carcinogenic effect plays an important role as well (Schrauzer 2000a). In view of the important functions that selenium plays in the organism, options of increasing the selenium intake are being studied. From human nutrition's standpoint, milk and milk products may be a source of selenium. The recommended daily intake of Se is 20–100 µg·day⁻¹ (Biesalski and Grimm 1999), while selenium concentration in cow milk oscillates, according to various authors, from around

Address for correspondence:

Doc. MVDr. Alena Pechová, CSc.
Clinic of Ruminants Diseases, Faculty of Veterinary Medicine,
University of Veterinary and Pharmaceutical Sciences Brno,
Palackého 1-3, 612 42 Brno
Czech Republic

Phone: +420 54156 2408
E-mail: pechovaa@vf.u.cz
<http://www.vfu.cz/acta-vet/actavet.htm>

2 $\mu\text{g}\cdot\text{l}^{-1}$ in Finland (Varo et al. 1980) up to 1270 $\mu\text{g}\cdot\text{l}^{-1}$ in the USA in areas with high selenium concentration in soil (Levander 1987). In the CR, selenium concentrations in milk were measured around $28.6 \pm 7.1 \mu\text{g}\cdot\text{l}^{-1}$ (Pechová et al. 2007).

Apart from natural selenium content in ruminant feeds, numerous trace elements are currently supplemented in the form of mineral feed additives. Supplementation of selenium in ruminant feed is traditionally monitored in relation to animal health, reproduction and production. In recent years, attention has also been diverted to selenium supplementation with the aim to increase the nutrition value of food. These on-farm methods represent an important path to increased nutrition value of food. Most trials with the supplementation of selenium were carried out in cattle (Pavlatá et al. 2003, 2004), while less attention has been paid to this issue in other ruminants, probably because of their less serious health problems due to deficiency diseases. Goat milk is known to have numerous nutritional properties, and this is why we decided to study possible increase in the nutritional value of goat milk and subsequently selected milk products (fresh cheeses, yoghurts) by supplementing various Se forms.

The goal of the experiment was to monitor the effect of selenium supplementation in various forms (natrium selenite, selenium proteinate) in the feed of lactating goats on the composition of milk and milk products.

Materials and Methods

The experiment was carried out in 30 goats divided into 3 groups: C - control group ($n = 10$) without increased selenium supplementation (natural Se content in granulated feed $0.14 \text{ mg}\cdot\text{kg}^{-1}$); Sel - experimental group ($n = 10$) receiving granulated feed containing selenium $0.94 \text{ mg}\cdot\text{kg}^{-1}$ in the form of natrium selenite; SeO - experimental group ($n = 10$) receiving granulated feed containing selenium $0.94 \text{ mg}\cdot\text{kg}^{-1}$ in the form of lactate-protein complex (Selene chelate, AGROBAC Karel Gebauer, Czech Republic), which contains 0.8% of Se. This form of Se complex was produced by cultivation of *Lactobacillus acidophilus* on the substrate containing natrium selenite. Producers assume that this complex contains Se-methionine and Se-cystein, but there are no particular data on the amounts of this Se containing amino acids. Different selenium supplementation was carried out from 3.5–4 months before delivery until the end of 2nd month of lactation. Goats received granulated feed in the amount of 0.35 kg per animal and day for the entire pregnancy period, and in the amount of 0.6 kg per animal and day divided into three parts during lactation. During the milking period and production of milk products, goats in the experimental groups received supplemented 565 μg Se per animal and day in the relevant form. The feed also included meadow hay *ad libitum* and 0.9 kg of barley.

At the end of 2nd month of lactation, individual goats were milked six times in two weeks during morning milking and cheese and yoghurt were made from the mixed milk in the workshop of the Department of Milk Hygiene and Technology. These cheeses are described by Czech legislation, Directive 77/2003 Coll. (2003), amended by 124/2004 Coll. (2004), as natural, non-maturing cheeses, whole-fat to reduced-fat (over 25% fat in milk solids and over 45% fat in milk solids, inclusive). Se concentration was established in the group samples of milk and subsequently made products. Analyses of physical and chemical variables were carried out for individual milk products as well.

Selenium was measured in individual milk and milk products using the HG-AAS method and the AAS Solaar M6 (UNICAM, Great Britain) device, after microwave mineralization of samples in the Milestone Ethos TC (MILESTONE, Italy) unit using the method according to Pechová et al. (2005).

Milk composition (fat, protein, lactose) was measured by FT-NIR method using Nicolet Antaris Near-IR Analyzer Spectrometer (Thermo Electron Corporation, Madison, USA), the content of milk solids and non-fat milk solids by reference method and calculation according to CTS 570530 (1972), titration acidity by Soxhlet-Henkel method (CTS 570530, 1972). We also monitored indicators of the technological processing of milk to fresh cheeses and yoghurt. We monitored rennetability using Černá and Cvak's method (1986), volume of released whey, weight and yield of cheeses and souring period of yoghurts. In cheese samples, we measured the content of fat in milk solids, milk solids, fat and titration acidity (CTS 570107, 1965). Yoghurts were analyzed according to CTS 570530 (1972).

The results were statistically assessed using F-test for the assessment of the variance of individual sets, and using dependent Student's *t*-test for sets with equality/non-equality of variances according to results. The results are presented as a mean value with standard deviation. The assessment was carried out using EXCEL software.

Results

Long-term supplementation of selenium in the form of natrium selenite and in the form of proteinate had no significant impact on the Se concentration in milk. Se concentration

in milk in individual groups was as follows C: $13.14 \pm 4.21 \mu\text{g}\cdot\text{l}^{-1}$, SeI: $12.50 \pm 5.59 \mu\text{g}\cdot\text{l}^{-1}$, SeO: $12.47 \pm 3.71 \mu\text{g}\cdot\text{l}^{-1}$. Similarly, no significant differences in the selenium concentration were determined in yoghurts (C: $17.13 \pm 5.40 \mu\text{g}\cdot\text{kg}^{-1}$, SeI: $18.12 \pm 5.49 \mu\text{g}\cdot\text{kg}^{-1}$, SeO: $19.69 \pm 6.48 \mu\text{g}\cdot\text{kg}^{-1}$) and cheeses (C: $68.79 \pm 7.14 \mu\text{g}\cdot\text{kg}^{-1}$, SeI: $70.41 \pm 6.22 \mu\text{g}\cdot\text{kg}^{-1}$, SeO: $69.66 \pm 12.68 \mu\text{g}\cdot\text{kg}^{-1}$) made from milk of individual groups of goats. Selenium concentrations in milk, whey, yoghurt and cheese are compared in Fig. 1.

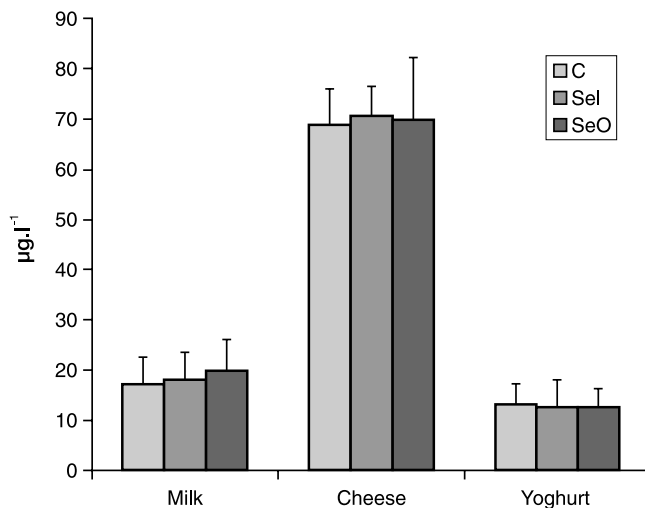


Fig. 1. Selenium concentrations in milk ($\mu\text{g}\cdot\text{l}^{-1}$), cheese and yoghurt ($\mu\text{g}\cdot\text{kg}^{-1}$) in individual groups (C-control, SeI-supplemented with natrium selenite, and SeO-supplemented with selenium proteinate)

Table 1. Physical and chemical indicators of raw goat milk from the control (C, n = 6) and individual experimental groups supplemented with selenium in the inorganic form (SeI, n = 6) and in the form of lactate-protein (SeO, n = 6)

		Fat (%)	Protein (%)	Lactose (%)	Fat in milk solids (%)	Titration acidity (SH)	Milk solids (%)	Rennetability (s)
C	mean	2.49 ^c	2.98	4.24	8.18	6.13 ^A	12.98	81.00 ^a
	SD	0.16	0.40	0.87	0.46	0.50	2.15	6.00
SeI	mean	2.55 ^b	2.94	4.77	8.01	4.80 ^{A,b}	11.93	97.83 ^a
	SD	0.26	0.20	0.24	0.22	0.52	0.64	14.44
SeO	mean	2.96 ^{b,c}	2.71	4.87	8.11	5.70 ^b	11.63	81.50
	SD	0.30	0.17	0.70	0.38	0.53	0.53	10.86

The same indexes in columns mean statistical significance of differences in average values between the groups:

^A $p < 0.01$

^{a,b,c} $p < 0.05$

Apart from selenium concentration in milk and milk products, we also monitored other quality indicators that might have been influenced by selenium supplementation in the goats' feed. Milk composition from control and individual experimental groups is shown in Table 1. Among qualitative milk indicators, we found significantly lower titration acidity and significantly higher rennetability in SeI group. We also determined a significantly higher fat concentration in milk of the SeO group compared to the other two groups. Physical and chemical indicators monitored during production of yoghurts and cheeses were not

Table 2. Physical and chemical indicators of cheese from the control (C, n = 6) and individual experimental groups supplemented with selenium in the inorganic form (SeI, n = 6) and in the form of lactate-protein (SeO, n = 6)

		Fat (%)	Milk solids (%)	Fat in milk solids (%)	Titration acidity (SH)	pH	Rennetability (s)
C	mean	17.08	35.99	46.81	95.92	4.82	48.33
	SD	2.40	2.18	10.23	9.61	0.24	22.19
SeI	mean	14.78	36.27	40.68	96.17	4.92	42.50
	SD	1.94	1.27	4.49	17.45	0.40	2.50
SeO	mean	18.46	36.85	50.07	95.17	4.84	42.50
	SD	3.24	1.19	8.34	9.82	0.26	2.50

Table 3. Technological indicators of cheese production from milk from goats of the control (C, n = 6) and individual experimental groups supplemented with selenium in the inorganic form (SeI, n = 6) and in the form of lactate-protein (SeO, n = 6)

		Whey (ml)	Cheese weight (g)	Yield (%)
C	mean	1701.00	281.96	14.09
	SD	113.51	25.63	1.28
SeI	mean	1686.00	278.10	13.91
	SD	56.78	34.27	1.71
SeO	mean	1594.40	305.30	15.00
	SD	128.52	46.89	1.82

influenced significantly (Tables 2 and 3). The differences in the fat content in cheese and fat in milk solids were the only ones on the border of statistical significance - the lowest fat content was found in the group receiving inorganic selenium. Composition of fresh yoghurts was not significantly influenced either. From the monitored indicators, only fat concentration in yoghurts was higher in SeO group compared to SeI group (Table 4).

Table 4. Physical and chemical indicators of yoghurt from the control (C, n = 6) and individual experimental groups supplemented with selenium in the inorganic form (SeI, n = 6) and in the form of lactate-protein (SeO, n = 6).

		Fat (%)	Milk solids (%)	Non-fat milk solids (%)	Titration acidity (SH)	Souring time (hours)	pH
C	mean	2.52	12.13	9.61	53.53	4.50	4.15
	SD	0.32	0.38	0.32	7.01	0.00	0.15
SeI	mean	2.21 ^a	12.10	9.90	54.60	4.50	4.16
	SD	0.39	0.43	0.21	4.99	0.00	0.11
SeO	mean	2.91 ^a	12.77	9.86	53.93	4.50	4.23
	SD	0.37	0.86	1.03	7.45	0.00	0.24

The same indexes in columns mean statistical significance of differences in average values between the groups:

^a $p < 0.05$

Discussion

An important factor that affects Se concentration in milk is the geographic area and natural content of Se in soil. Selenium is introduced into the food chain by plants, which absorb inorganic selenium salts from the soil and convert them into organic forms of the element (mainly as selenium methionine), which are then incorporated into proteins. The concentration of selenium in plants varies widely and depends on the selenium content and characteristics of the soil.

The Se concentrations we measured in goat milk are similar to the values mentioned by Benemariya et al. (1993) $23.1 \pm 4.5 \mu\text{g}\cdot\text{l}^{-1}$, Rodriguez et al. (2002) $9.2 - 31.2 \mu\text{g}\cdot\text{l}^{-1}$ and other authors. Although we supplemented a rather high dose of selenium, we did not succeed in increasing the selenium concentration in milk. It was probably because the basic feed ration saturated the needs of the organism sufficiently. This was probably the

reason why the supplementation of selenium exceeding the physiological needs of goats was not reflected in the increased concentration of selenium in milk. The organic form of selenium in the form of lactate-protein complex we used behaved similarly to the inorganic form and had no effect on the selenium concentration in milk. We obtained similar results as in another experiment (Pechová et al. 2008). It is very likely that if there was a lack of selenium in the basic feed, the selenium supplementation would be reflected in the increased concentration of selenium in milk. Serra et al. (1996) were interested in the effect of selenium in soluble grass bolus on the selenium content of milk and blood of goats. These authors found that five months after Se administration, the does in the treated group had a higher ($p < 0.01$) Se content in their blood (62.2 vs. $25.7 \mu\text{g}\cdot\text{l}^{-1}$) and milk (5.1 vs. $2.5 \mu\text{g}\cdot\text{l}^{-1}$) than does in the control group.

In available literature, we did not find any information about selenium excretion supplemented in the form of proteinate produced by cultivation of *Lactobacillus acidophilus* in milk. Most papers compared supplementation of the inorganic form of Se with the organic form of selenium yeast produced by *Saccharomyces cerevisiae*, where approximately 63% of Se is bound in the form of selenium methionine. The option to increase selenium concentration in milk by supplementing Se in the form of yeast was established in cows (Ortman and Pehrson 1999; Knowles et al. 1999; Givens et al. 2004; Heard et al. 2004; Juniper et al. 2006). The improved bioavailability of yeast Se relative to other forms probably occurs at the intestinal level. Ingested Se-met is absorbed in the small intestine via Na^+ -dependent neutral amino acid transport system (Schrauzer 2000b), which is very effective. The organic form of selenium lactate-protein produced by *Lactobacillus acidophilus* that we used was probably absorbed passively as inorganic sodium selenite, which may be due to the lower content of Se containing amino acids or other to this time unknown factors.

Increased concentration of selenium in milk due to supplementation of selenium methionine is often linked to the fact that selenium methionine replaces methionine in milk proteins. This hypothesis was corroborated by Muñiz-Naveiro et al. (2007), who studied the selenium content in milk using speciation analysis (liquid chromatography coupled with hydride generation-atomic fluorescence spectrometry). The milk samples obtained after organic supplementation of the feed as selenised yeasts contained three species of selenium, SeCyst2, Se (IV) and SeMet, while only SeCyst2 and Se (IV) were present in milk samples obtained after inorganic supplementation of feed. Interesting results were also published by Aspila (1991), who investigated the metabolism of inorganic and organic Se sources at different dietary Se levels in lactating goats. The true absorption of Se was 63% and 65% and excretion of Se in milk 4% and 7% in the goats dosed intraruminally with sodium selenite and orally with grass sprayed with sodium selenite one week before cutting. The effect of dietary Se content was non-significant. In goats dosed with sodium selenite and selenium methionine intravenously, 3.6% and 33% of Se was excreted in milk, respectively. Increased concentration of selenium in milk after parenteral application of inorganic forms of selenium was found in cows by Grace et al. (1997) and in sheep by Cuesta et al. (1995) and Gabryszuk et al. (2005). Rodríguez et al. (2002) investigated 78 samples of goat milk from the Canary Islands and determined concentrations of Se from 9.2 to $31.2 \mu\text{g}\cdot\text{l}^{-1}$ (mean value $19.98 \mu\text{g}\cdot\text{l}^{-1}$). These authors found that the milk of goats milked once per day had significantly higher concentrations of Se than the milk of goats milked twice per day. The concentration of Se in goat milk decreased significantly from March to June.

Monitoring selenium concentration in milk products is assessed by most authors only as food screening in a selected area, but in the available research we could not find the monitoring of the influence of supplementation of various selenium forms on the contents in milk products. Therefore, comparison with other authors in this regard is difficult. Smrkolj et al. (2005) monitored the selenium content in food including milk products

in Slovenia. In cow milk, they found Se concentration of 11.6–13.4 $\mu\text{g}\cdot\text{l}^{-1}$, in yoghurts 11.9–12.8 $\mu\text{g}\cdot\text{kg}^{-1}$ and in cheeses 20.5–26.7 $\mu\text{g}\cdot\text{kg}^{-1}$. These values are quite low compared to our values, especially in cheeses, but other authors mention higher values, similar to ours. For example Barclay et al. (1995) found Se concentration of 16–140 $\mu\text{g}\cdot\text{kg}^{-1}$ in cheeses and 14 $\mu\text{g}\cdot\text{kg}^{-1}$ in yoghurts in the UK. These values attest to great variability of selenium concentration in milk products. Composition of goat cheese was monitored by Pappa et al. (2006) in northern Greece. They found selenium content in goat cheese varying from 27.0 to 59.5 $\mu\text{g}\cdot\text{kg}^{-1}$ ($43.3 \pm 16.3 \mu\text{g}\cdot\text{kg}^{-1}$). These values are a little lower than what we found in goats supplied with selenium for a long time. Feta, a typical Greek cheese made from sheep or goat milk, had Se content between 24.1 and 66.0 $\mu\text{g}\cdot\text{kg}^{-1}$ ($51.8 \pm 5.4 \mu\text{g}\cdot\text{kg}^{-1}$) (Pappa et al. 2006). Higher selenium concentration, although statistically non-significant, was found by these authors in low-fat yoghurts, compared to whole products.

Apart from selenium concentration in milk products, we also monitored physical and chemical characteristics. Significant differences were found only in rennetability and titration acidity of milk. Milk rennetability was higher by approximately 16 s ($p < 0.05$) in the group receiving inorganic form of Se, compared to the control and the other experimental group. From the practical standpoint, the difference is non-significant, as the limit defined in the method we used is 420 s. Similarly, slightly worse variables were established in titration acidity, which was the lowest in the group that received the inorganic form of selenium. We could not find any paper in the available literature dealing with this issue. We also found a higher fat content in the milk of goats supplemented in the long-term with Se in the form of proteinate. Since the main precursors of milk fat originate in the rumen, the increased milk fat content may have been influenced by rumen fermentation. The effect of Se supplementation in the form of yeast on the rumen fermentation was studied by Faixová et al. (2007). They found significantly higher activity of alkaline phosphatase and glutamate dehydrogenase in ruminal fluid in Se-supplemented group. They explained it by the supportive effect of Se on rumen microbial population, increasing their resistance and activity. Similarly Mihalíková et al. (2005) documented a protective effect of Se feed supplementation on the development of some rumen ciliate species in young sheep. The higher milk fat content also reflected in the products, with a higher fat content in cheese and milk solids, as well as higher fat concentration in yoghurts from the group that received Se-proteinate.

Our results reveal that a long-term increased selenium supplementation, either in the inorganic (sodium selenite) or organic form (selenium lactate-protein produced by cultivation of *Lactobacillus acidophilus*), had no significant impact on the concentration of selenium in milk and milk products. The monitored physical and chemical milk and milk product indicators revealed a higher milk fat content due to the supplementation of Se lactate-protein, whereas in groups that received sodium selenite lower titration acidity and shorter rennetability period were found.

Vliv suplementace různých forem selenu u koz na kvalitu a složení mléka, sýru a jogurtu

Cílem provedeného pokusu bylo sledování možnosti ovlivnění složení mléka a mléčných výrobků suplementací selenu v různých formách (seleničitan sodný, selen ve formě laktátproteinového komplexu) do krmné dávky laktujících koz.

Pokus byl realizován na 30 kozách, které byly rozděleny do 3 skupin: skupina C kontrolní, skupina SeI dostávala selen ve formě seleničitanu sodného a skupina SeO dostávala selen ve formě laktátproteinového komplexu vyráběného kultivací *Lactobacillus acidophilus* na substrátu s přísadkou seleničitanu sodného. Rozdílná suplementace selenu byla prováděna od 3,5 - 4 měsíců před datem porodu do konce 2. měsíce laktace, kdy bylo v průběhu 2 týdnů šestkrát odebráno mléko od jednotlivých koz a ze směsného mléka jednotlivých

skupin byly vyrobeny sýry a jogurty. V době odběru mléka a výroby mléčných výrobků dostávaly kozy pokusných skupin přísady 565 μg Se na kus a den v odpovídající formě.

Dlouhodobá suplementace selenu ve formě seleničitanu sodného ani ve formě laktátproteinového komplexu neovlivnila významně koncentraci Se v mléce. Koncentrace Se v mléce byla u jednotlivých skupin následující: C: $13,14 \pm 4,21 \mu\text{g}\cdot\text{l}^{-1}$, SeI: $12,50 \pm 5,59 \mu\text{g}\cdot\text{l}^{-1}$, SeO: $12,47 \pm 3,71 \mu\text{g}\cdot\text{l}^{-1}$. Obdobně nebyly zjištěny významné rozdíly v koncentraci selenu ani v jogurtech (C: $17,13 \pm 5,40 \mu\text{g}\cdot\text{kg}^{-1}$, SeI: $18,12 \pm 5,49 \mu\text{g}\cdot\text{kg}^{-1}$, SeO: $19,69 \pm 6,48 \mu\text{g}\cdot\text{kg}^{-1}$) a sýrech (C: $68,79 \pm 7,14 \mu\text{g}\cdot\text{kg}^{-1}$, SeI: $70,41 \pm 6,22 \mu\text{g}\cdot\text{kg}^{-1}$, SeO: $69,66 \pm 12,68 \mu\text{g}\cdot\text{kg}^{-1}$) vyrobených z mléka jednotlivých skupin koz. Vedle koncentrace selenu v mléce a mléčných výrobcích jsme sledovali rovněž další kvalitativní ukazatele, které by mohly být suplementací selenu do krmné dávky koz ovlivněny. Z kvalitativních ukazatelů mléka byla zjištěna u skupiny SeI významně nižší titrační kyselost a naopak významně vyšší syřitelnost. Dále byla zjištěna významně vyšší koncentrace tuku v mléce u skupiny SeO ve srovnání s dalšími dvěma skupinami. Ukazatele sledované při výrobě jogurtů a sýrů nebyly významně ovlivněny, pouze koncentrace tuku v jogurtech byla vyšší ve skupině SeO ve srovnání se skupinou SeI.

Suplementace selenu ve formě seleničitanu sodného ani laktátproteinového komplexu nad fyziologické potřeby koz neovlivnila významně složení mléka a mléčných výrobků.

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