

Effect of supplementation of various selenium forms and doses on selected parameters of ruminal fluid and blood in sheep

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ABSTRACT: Effect of various doses and forms of selenium (organically bound vs. inorganic) on selected parameters of ruminal fluid and blood in sheep was evaluated. The trial was performed with 15 sheep divided into two groups. Animals from group A ($n = 8$) received a feeding mixture with selenomethionine, while sheep from group B ($n = 7$) were fed a mixture with sodium selenite. During the first 14 days, animals from both groups were fed a mixture with optimum dose of selenium (1 mg Se/kg dry matter). For another 28 days, all experimental animals received a Se-deficient mixture (0.1 mg Se/kg dry matter), whereas in the last 21 days of the experiment, animals were fed a high-Se diet (5.0 mg Se/kg dry matter). Throughout the trial, 4 samples of blood and ruminal fluid were taken from each animal. The samples of ruminal fluid were analyzed to determine the concentration of Se and identify other parameters of ruminal fermentations. Selenium levels were also determined in ruminal biomass. In blood, Se concentration, glutathione peroxidase (GSH-Px) activity, and other selected biochemical parameters were measured. The results of the present study demonstrate that the actual intake of both organic and inorganic Se is reflected in Se concentration in ruminal fluid and ruminal biomass and, similarly, in Se content and GSH-Px activity in blood. The form of supplemented selenium did not have a significant effect on ruminal fermentation parameters in dependence on different doses of Se contained in feeding rations (except for the negative effect of a sudden start of feeding high levels of organically bound Se on infusoria count, which was accompanied by the increase of GMT, LDH, and AST enzymes activity in ruminal fluid). The results also suggest possible negative interaction between the intake of organically bound selenium and the concentration of copper in blood of sheep.

Keywords: selenomethionine; organic selenium; inorganic selenium; copper; glutathione peroxidase; zinc; interactions

The importance of a sufficient supply of microelements is based on the fact that they take part in many biological processes and have many structural, catalytic, and regulatory functions in the organism. As such, they influence the organism's health state (Underwood and Suttle, 1999). Microelement supply in ruminants is significantly affected by the place where they are reared, as they are routinely fed on plant feedstuffs produced locally. Feed grains usually contain higher concentrations of microelements than forage. The form of supplementation of microelements

is generally considered as an important factor. Organic forms are usually reported to be more efficient and having higher biological effect (Rabianski et al., 1998; Pavlata et al., 2001a, 2011a; Kuricová et al., 2003; Davis et al., 2008; Pechova et al., 2008; Skřivan et al., 2010; Sevcikova et al., 2011; Wang et al., 2011). However, other studies did not prove this finding and, on the contrary, their authors observed equivalent or even better efficiency of inorganic compounds (Leeson et al., 2008; Heindl et al., 2010; Pavlata et al., 2011b, 2012a).

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Selenium, together with vitamin E, participates in antioxidant protection of the organism, as it is contained in glutathione peroxidase enzyme (GSH-Px). Selenium also plays an important role in the immune system and the metabolism of thyroid hormones. It is also crucial for reproduction (McKenzie et al., 1998; Ruz et al., 1999; Birringer et al., 2002).

Since the Czech Republic is situated in the area with insufficient content of Se in soil, there is no risk of selenium intoxication here. On the contrary, animals living in this region are rather susceptible to Se-deficiency diseases (Pavlata et al., 2002, 2005a; Ludvíková et al., 2005). In order to ensure adequate supply of Se, the animals must be supplemented with this element in an appropriate form. It should be noted that Se added in the feeding ration is not fully utilized by the animal's organism, as its significant part is absorbed and metabolized by ruminal bacteria (Serra et al., 1994).

Sodium selenite and sodium selenate administered at optimum dose show similar bioavailability. Also, they are distributed in rumen in a similar way and at a similar rate. Selenium is very quickly embedded in bacteria – in fact, 30% of Se is present in bacteria 1 h after the administration (Serra et al., 1994). It has been reported that supplementation of sodium selenite together with vitamin E affects ruminal activity in terms of increased production of fatty acids and growing number of protozoa. At the same time, average daily gain increases (Naziroglu et al., 1997). On the other hand, certain authors did not observe any significant changes after *in vitro* application of selenium (Kim et al., 1997; Feasenhiser, 2005). Mihalíková et al. (2005) reported that selenomethionine has protective effect on the growth of some protozoa species. It has been also published that higher Se concentrations can harm ruminal microflora or even ruminal mucosa. Ruminal bacteria and mucosa in the rumen contain enzymes whose concentration in ruminal fluid increases

after the cells are damaged. Monitoring of activity of selected enzymes in ruminal fluid can therefore provide information about possible toxic action of microelements in the rumen (Weekes, 1972; Cheng et al., 1976).

The present study evaluates the effect of the dose and form of supplemented selenium on selected parameters of ruminal fluid and blood in sheep. The aim is to find out whether the bioavailability of organic Se compounds is higher as compared with inorganic compounds and whether higher concentration of Se in one or other form affects ruminal environment, metabolic function of rumen, or metabolism of other microelements.

MATERIAL AND METHODS

The experiment was performed with 15 wethers which had been fed and housed in the same way for 4 months before the trial. The animals were divided in two groups (A and B) and housed in two independent open-air pens with *ad libitum* access to water. During the trial, all animals were fed the same feeding ration. The only exception was the form and concentrations of selenium added in the supplementary feed mixture (manufactured by Bio-kron s.r.o., Blučina, Czech Republic). Daily feeding ration of one animal included 2.5 kg meadow hay and 0.3 kg supplementary grain feeding mixture containing additional selenium. The ration was divided into two meals (one fed in the morning, the other in the evening – see Tables 1 and 2). Animals from group A ($n = 8$) received feeding mixture containing organic selenium (Sel-Plex 2000; Alltech, Nicholasville, USA). Wethers from group B ($n = 7$) were fed a mixture enriched with inorganic Se (sodium selenite, Na_2SeO_3). From day 1 to day 14, the animals received 1.0 mg of Se per 1 kg feeding mixture. In the second stage of the experiment (days 15–42), only a small amount

Table 1. Intake of selenium in feeding ration and hay per animal and day throughout the experiment (daily ration divided to two meals)

Dose	Duration of feeding (weeks)	Feeding mixture		Hay		Intake of Se (mg/day)
		intake of feeding mixture (kg/day)	Se content (mg/kg dry matter)	intake of hay (kg/day)	Se content (mg/kg dry matter)	
Adaptation	2	0.3	1.0	2.5	0.03	0.32
Deficient	4	0.3	0.1	2.5	0.03	0.09
Increased	3	0.3	5.0	2.5	0.03	1.34

Table 2. Nutritional composition of supplementary feeding mixture

Dry matter (g/kg)	894.6
Crude protein (g/kg)	158.4
Fat (g/kg)	23.1
Fiber (g/kg)	56.3
Nitrogen-free extract (g/kg)	682.8
Organic matter (g/kg)	920.6
Ash (g/kg)	79.4
Ca (g/kg)	9.7
P (g/kg)	9.6
Mg (g/kg)	4.0
Cu (mg/kg)	45.8
Zn (mg/kg)	182.1

of Se was administered (0.1 mg/kg), whereas in the last three weeks (days 43–63), the wethers received high dose of selenium (5.0 mg/kg) in the supplementary grain feeding mixture (Table 1).

Throughout the trial, four samples of blood and ruminal fluid were taken from each animal. Ruminal fluid (300 ml) was collected by a ruminal probe and a suction pump 2 h after morning feeding. At the same time, blood was taken from *vena jugularis* to two test tubes, one without anticoagulant to obtain serum sample, the other with heparin to obtain whole blood sample. The first sampling was done after 14 days of feeding the mixture containing 1 mg/kg Se (day 14), the second sample was taken after three weeks of feeding low dose of Se (day 35), the third sample was collected after the first feeding the mixture with higher concentration of Se (day 43), and the last sampling was performed after 3 weeks of feeding a diet high in Se (day 63).

The following parameters of ruminal fluid were examined: pH, total acidity, infusoria count, lactic acid concentration, ammonia, volatile fatty acids, percentage of individual acids (acetic, propionic, *n*-butyric, and *n*-valeric), Se concentration, and ALP, ALT, AST, GMT, LDH, and amylase activity. Concentration of Se in ruminal biomass was determined as well. The rumen fluid samples were preserved by toluene for determining volatile fatty acids, by formaldehyde for determining ciliate protozoa, and by mercuric chloride for determining the other indicators (pH, total acidity, and ammonium). Total acidity was established by titration method. The pH level was measured

by the pH Meter Lab 850 (SCHOTT Instruments GmbH, Mainz, Germany). Lactic acid was measured with the use of enzymes (Noll, 1988) and with the Quantum (STANGEST, Valls, Spain) and L-, D-lactate dehydrogenase test kits (Megazyme International Ireland Ltd., Wicklow, Ireland). Ammonia was determined by the modified Berthelot reaction according to Chaney and Marbach (1962). Volatile fatty acids (VFA) were determined by gas chromatography (Agilent 6820 Gas Chromatograph System; Agilent Technologies, Santa Clara, USA). The ruminal enzymes were analyzed with the use of AMS Liasys Clinical Chemistry System (AMS Diagnostics, Summerville, USA), the individual enzymes were analyzed using individual test kits produced by Lachema (Brno, Czech Republic): LDH (L LDH cat. No.12352), ALP (L ALP cat. No. 10061), AST (L AST cat. No.10351), ALT (L ALT cat. No. 10451). Individual test kits (BioVendor – Laboratorní medicína a.s., Brno, Czech Republic) were as follows: GMT (GMT KIN 100 cat. No. 1003208) and amylase (AMS L 100 cat. No. 10003302). Infusoria count was done with the use of a microscope in a Fuchs-Rosenthal Counting Chamber (Hausser Scientific Co., Horsham, USA) by dilution 1 : 20 and staining with 0.1% methylen blue. Conservation was done by the use of 10% formaldehyde solution.

The ruminal biomass separation was done according to the Czerkawski method (Czerkawski, 1976) which was slightly modified. The method begins with filtering of the ruminal fluid through several layers of cotton gauze. After this the filtrated fluid is passed through a 0.315 mm sieve. Centrifugation by 15 000 rpm for 15 min is the following step. The formed sediment is diluted with 0.9% NaCl and centrifuged again. The created sediment is first dried with heat and later it is placed in an exsiccator with silica gel. The biomass prepared in this manner was analyzed in the university's laboratory. The following blood parameters were analyzed: concentrations of total protein, albumin, bilirubin, urea, and selenium and activity of AST, GMT, and GSH-Px. The glutathione peroxidase activity was assessed in whole heparinized blood according to the method described by Paglia and Valentine (1967) with the use of the Ransel set (Randox Laboratories Ltd., Crumlin, UK) and Roche Cobas Mira Automated Chemistry Analyzer (Roche, Basel, Switzerland). Zinc and copper concentration in blood plasma were determined by flame atomic absorption spectrophotometry

using Solaar M6 (Unicam, Leeds, UK) following the previous deproteinization of the sample by adding trichloroacetic acid in the 1 : 1 ratio. The minerals were determined in the supernatant after centrifugation. The catalytic activity of the GMT, AST and the concentration of TP, albumin, and bilirubin were determined with Cobas Mira Automated Chemistry Analyzer by standardized photometric methods using the tests supplied by Lachema, BioVendor – Laboratorní medicína a.s. (Total protein cat. No. 12751), Human (Wiesbaden, Germany) (ALBUMIN liquicolor cat. No. 10560), and JK – Trading s.r.o. (Prague, Czech Republic) (Total bilirubin cat. No. 10007762).

Concentrations of Se in all the examined biological samples were determined by the hydride method of atomic absorption spectrophotometry after mineralization of the samples by microwave digestion technique (Pechová et al., 2005).

Statistical analysis of data in MS Excel 2007 was done using *F* test for the assessment of the variance of individual samples and Student's *t*-test for samples with equality/non-equality of variance to compare group A and B results. Dynamics of changes of individual parameters in each group was assessed by paired *t*-test. The parameters were also subjected to correlation analysis that tested mutual relations of all results obtained throughout the experiment as well as the results of groups A and B independently.

RESULTS AND DISCUSSION

Rumen parameters

Selenium concentrations measured in ruminal fluid and ruminal biomass are stated in Table 3. The results indicate that the vast majority of Se is contained in biomass. Dynamics of absolute Se concentrations in ruminal fluid and biomass roughly correspond with the amount of supplemented selenium: Se concentrations in fluid and biomass were demonstrably lower at the second sampling. However, they significantly increased right after increased dose of Se started to be fed and they remained high until the end of the trial. The form of supplemented selenium did not have significant effect on the absolute content of Se in ruminal biomass. The present results are in accordance with the previously published finding that due to rapid absorption of Se by ruminal

bacteria, the highest selenium levels are found in ruminal biomass, whereas the lowest in ruminal fluid (Serra et al., 1994). Higher concentration of Se in ruminal fluid was observed after the supplementation of sodium selenite. After feeding the mixture with higher content of Se, the concentration of selenium in ruminal fluid of animals fed sodium selenite almost doubled ($4.61 \pm 1.72 \mu\text{g/l}$ – 3rd sampling, $9.68 \pm 5.24 \mu\text{g/l}$ – 4th sampling) as compared with wethers fed organic selenium ($2.60 \pm 1.33 \mu\text{g/l}$ – 3rd sampling, $4.88 \pm 2.16 \mu\text{g/l}$ – 4th sampling). The difference between the groups was statistically significant ($P < 0.05$). Comparison of Se concentration in ruminal biomass and ruminal fluid indicated that biomass and fluid concentrations were highly significantly correlated ($r = 0.78$, $P < 0.001$, $n = 60$). This proves that selenium concentrations in ruminal fluid and ruminal biomass are to a certain degree balanced. Despite the differences in Se content determined in ruminal fluid, it can be said that supplementation of different forms of selenium does not significantly affect the distribution of Se among ruminal fluid and ruminal biomass – particularly when absolute Se concentrations in biomass are taken into account. However, the calculated ratio of Se bound in ruminal biomass vs. ruminal fluid indicates a tendency of Se to bind with ruminal biomass of animals that received organic selenium. Not counting the results obtained at the end of feeding Se-deficient mixture (day 35), average biomass concentrations in organic selenium group were 613, 416, and 487 times higher than fluid concentrations (samplings on days 14, 43, and 63), whereas in animals fed inorganic selenium, biomass concentrations were only 421, 258, and 194 times higher than fluid concentrations. This tendency corresponds with the results reported by Mainville et al. (2009) who observed that inorganic selenium has a lower ruminal microbial uptake than organic selenium sources.

The assessment of the effect of different forms and amounts of supplemented Se on other ruminal fermentation parameters indicated no significant influence of selenium. Values of pH, total acidity, concentrations of lactic acid, and volatile fatty acids (acetic, propionic, butyric, valeric) and ammonia remained in physiological range and no significant differences were seen between the groups. The following average values were observed: pH 6.49 ± 0.25 , total acidity 25.67 ± 7.03 arbitrary units, lactic acid 0.45 ± 0.41 mmol/l, VFA 100.0 ± 10.4 mmol/l, acetic acid $69.0 \pm 2.3\%$, propionic

Table 3. Concentration of Se (mean \pm standard deviation) in ruminal fluid and ruminal biomass of sheep during feeding optimum dose of Se (day 14), deficient dose of Se (day 35), 2 h after the first feeding of high-selenium diet (day 43), and during feeding increased dose of Se (day 63)

Sampling day	Group	Selenium in biomass ($\mu\text{g}/\text{kg}$ dry matter)	Selenium in ruminal fluid ($\mu\text{g}/\text{l}$)
14	A	559.7 \pm 195.7 ^{CD}	0.91 \pm 0.47 ^{Cab}
	B	683.5 \pm 189.9 ^{HJ}	1.62 \pm 0.89 ^{GHI}
35	A	197.9 \pm 56.2 ^{CEF}	0.34 \pm 0.24 ^{*DEa}
	B	199.7 \pm 43.3 ^{HKL}	0.10 \pm 0.08 ^{GJK}
43	A	1083.5 \pm 517.8 ^{EG}	2.60 \pm 1.33 ^{DFb}
	B	1192.4 \pm 279.2 ^{IKM}	4.61 \pm 1.72 ^{*HJ}
63	A	2378.9 \pm 974.0 ^{DFG}	4.88 \pm 2.16 ^{CEF}
	B	1881.4 \pm 586.1 ^{ILM}	9.68 \pm 5.24 ^{*IK}

A = sheep fed organic selenium ($n = 8$), B = sheep receiving inorganic selenium ($n = 7$)

C:C to M:M in one column = $P < 0.01$, a:a, b:b in one column = $P < 0.05$

* $P < 0.05$ (significantly higher value as compared with the other group at the same sampling day)

acid $18.4 \pm 1.5\%$, butyric acid $10.6 \pm 1.2\%$, valeric acid $2.0 \pm 0.6\%$, ammonia 7.62 ± 4.03 mmol/l, and infusoria count $194\,000 \pm 97\,000/\text{ml}$. The effect of supplementation of organic selenium (selenomethionine) on concentrations of volatile fatty acids was not seen by Feasenhiser (2005) either. On the contrary, Kim et al. (1997), who studied the effect of selenium on ruminal fermentation *in vitro*, found out that addition of selenomethionine (2 mg/kg) in the ruminal fluid resulted in higher production of certain volatile fatty acids as compared with addition of elementary selenium or sodium selenite, or no selenium supplementation. Differences in production of volatile fatty

acids after supplementation of sodium selenite and vitamin E were observed also by Naziroglu et al. (1997): pre-feeding concentration of VFA in experimental group was lower than in control group, whereas their concentration 6 h post feeding was higher than in control group. Dutt and Chhabra (2008) report that supplementation of 0.5 ppm Se either in inorganic or organic form did not show any significant effect on nutrient intake, rumen microbial population, and protein synthesis in cattle and buffaloes.

The results of the present study however indicate possible negative effect of high doses of Se on ruminal fermentation. This was seen after the

Table 4. Activity of selected enzymes in ruminal fluid of sheep during feeding optimum dose of Se (day 14), deficient dose of Se (day 35), 2 h after the first feeding of high-selenium diet (day 43), and during feeding increased dose of Se (day 63)

Day	Group	Amylase ($\mu\text{kat}/\text{l}$)	GMT ($\mu\text{kat}/\text{l}$)	LDH ($\mu\text{kat}/\text{l}$)	ALP ($\mu\text{kat}/\text{l}$)	ALT ($\mu\text{kat}/\text{l}$)	AST ($\mu\text{kat}/\text{l}$)
14	A	0.41 \pm 0.46	0.11 \pm 0.03 ^C	0.86 \pm 1.34	0.42 \pm 0.10 ^C	0.15 \pm 0.11 ^{ab}	0.43 \pm 0.42
	B	0.18 \pm 0.05	0.11 \pm 0.01 ^{Fa}	0.19 \pm 0.08 ^{abC}	0.50 \pm 0.10 ^a	0.13 \pm 0.10	0.30 \pm 0.39
35	A	0.17 \pm 0.04 ^a	0.04 \pm 0.02 ^{CDE}	0.29 \pm 0.14	0.58 \pm 0.20	0.31 \pm 0.10 ^{aC}	0.27 \pm 0.11 ^a
	B	0.17 \pm 0.07	0.06 \pm 0.01 ^{FG*}	0.29 \pm 0.04 ^{aD}	0.40 \pm 0.13 ^D	0.25 \pm 0.09 ^d	0.22 \pm 0.09 ^C
43	A	0.15 \pm 0.04	0.10 \pm 0.02 ^D	0.30 \pm 0.19	0.55 \pm 0.06 ^C	0.26 \pm 0.16 ^{bc}	0.42 \pm 0.42 ^a
	B	0.16 \pm 0.10	0.10 \pm 0.01 ^{aG}	0.41 \pm 0.06 ^{CD}	0.50 \pm 0.05 ^b	0.14 \pm 0.08	0.36 \pm 0.31
63	A	0.09 \pm 0.06 ^a	0.11 \pm 0.02 ^E	0.33 \pm 0.13	0.61 \pm 0.33	0.12 \pm 0.04 ^{Cc}	0.13 \pm 0.11
	B	0.14 \pm 0.05	0.08 \pm 0.03	0.55 \pm 0.26 ^b	0.69 \pm 0.14 ^{abD}	0.12 \pm 0.06 ^d	0.11 \pm 0.03 ^C

A = sheep fed organic selenium ($n = 8$), B = sheep receiving inorganic selenium ($n = 7$)

C:C to G:G in one column $P < 0.01$, a:a to d:d in one column $P < 0.05$

* $P < 0.05$ (significantly higher value as compared with the other group at the same sampling day)

first administration of a feeding mixture rich in organic selenium. In this group (A), significantly lower ($P < 0.05$) infusoria count in ruminal fluid ($98\,000 \pm 50\,000$ infusoria per 1 ml ruminal fluid) was observed than in group B ($171\,000 \pm 66\,000$). The decrease of infusoria count in group A was statistically significant ($P < 0.01$) also as compared with previous sampling on day 35 when $191\,000 \pm 68\,000$ infusoria/ml ruminal fluid were determined. This decrease of infusoria count after the administration of higher dose of organic selenium could be explained by toxic activity of Se induced by a sudden rise of its levels without gradual transition. Nevertheless, infusoria count significantly increased ($P < 0.01$) to $215\,000 \pm 67\,000$ /ml after three weeks, which demonstrates that continuous intake of feed high in Se does not have persistent negative effect on infusoria count. In order to assess potential negative effect of high doses of Se, activity of selected enzymes in ruminal fluid was monitored. The measured values are stated in Table 4. Although enzymatic activity was relatively low throughout the experiment, it is obvious that the lowest values in ruminal fluid were determined on day 35. After high doses of Se started to be fed, significant increase of GMT activity was seen in groups A and B, of LDH in group B, and of AST in group A. Significantly higher GMT and ALP activities were observed in group A also on day 63. Considering the obvious tendency of growing enzymatic activities in ruminal fluid on day 43, it

can be assumed that higher activity of enzymes could indicate possible toxic effect of high Se doses. This hypothesis is supported also by the reduction of infusoria count seen in animals from group A. Higher enzymatic activities in ruminal fluid can be associated with disintegration of microorganisms in ruminal ecosystem, or possible damage of ruminal cells caused by supplementation of high doses of Se (Cheng et al., 1976; Moharrery and Das, 2001). Mihaliková et al. (2005) did not see different infusoria counts in ruminal fluid of sheep that received organic or inorganic selenium as compared with control sheep.

Blood parameters

In the present study, concentration of selenium in a feeding ration affected also biochemical parameters of blood (Table 5, Figures 1–3). Animals from both groups showed increased Se levels and activity of GSH-Px in blood ($P < 0.01$) after feeding the mixture with high concentration of selenium. Similarly, Faixová et al. (2007) observed higher Se concentration and GSH-Px activity in lambs that received increased doses of Se.

Although Se concentration and GSH-Px activity in blood did not significantly differ between the groups, the animals receiving selenite tended to show higher values of these parameters. Higher GSH-Px activity can be caused by the fact that

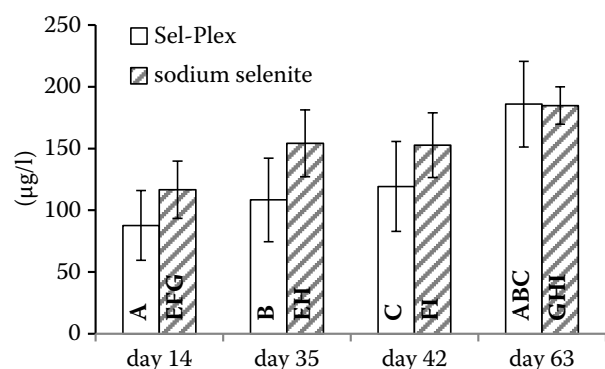


Figure 1. Dynamics of Se concentration in blood of sheep receiving organic selenium (Sel-Plex) or inorganic selenium (sodium selenite). Samples were taken when feeding optimum dose of Se (day 14), deficient dose of Se (day 35), 2 h after the first feeding of high-selenium diet (day 43), and during feeding increased dose of Se (day 63)

Groups labeled by the same letters show statistically highly significant differences ($P < 0.01$)

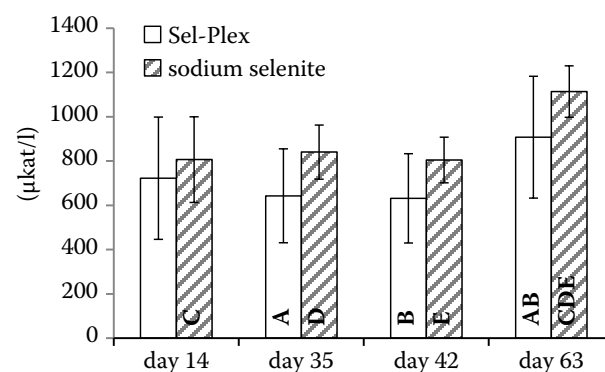


Figure 2. Dynamics of GSH-Px activity in blood of sheep receiving organic selenium (Sel-Plex) or inorganic selenium (sodium selenite). Samples were taken when feeding optimum dose of Se (day 14), deficient dose of Se (day 35), 2 h after the first feeding of high-selenium diet (day 43) and during feeding increased dose of Se (day 63)

Groups labeled by the same letters show statistically highly significant differences ($P < 0.01$)

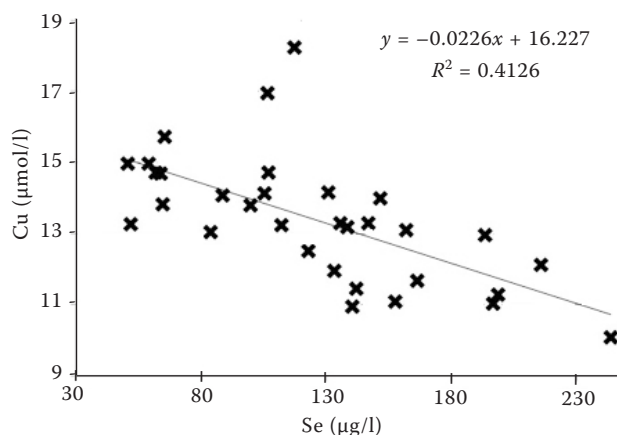


Figure 3. Relation between concentration of selenium and copper in blood of sheep receiving organic selenium (Sel-Plex) throughout the experiment, irrespective of the amount of Se added in the feeding ration ($r = 0.642$, $n = 32$)

organically bound selenium (selenomethionine) becomes embedded in proteins and thus it is less available for production of biologically active selenium compounds such as GSH-Px (Pavlata et al., 2011b). The similar effect of inorganic and organic selenium was observed also by Misurova et al. (2009) in goats. Monterrosa et al. (2011), who compared the effect of supplementation of selenium products on the digestive function of lambs, did not find any differences in selenium absorption and digestibility between animals receiving sodium selenite or selenomethionine. On the contrary, Rock et al. (2001) in lambs and Davis et al. (2008) in wethers observed higher concentrations of Se in experimental groups receiving Se-yeast (i.e. organic selenium).

In the present study, statistically highly significant correlation ($r = 0.83$, $n = 60$, $P < 0.001$) was found between blood Se concentration and blood GSH-Px activity. Correlation between Se concentration and GSH-Px activity was observed also by other authors: Pavlata et al. (2000, 2001b) in cattle, Pavlata et al. (2011a) in goats, Rock et al. (2001) in lambs, Pavlata et al. (2012b) in sheep, and Ludvíková et al. (2005) in horses. Furthermore, significant correlations ($P < 0.001$, $n = 45$) were found between Se concentration in ruminal fluid, or ruminal biomass, and blood concentration of Se ($r = 0.60$; $r = 0.68$) or blood GSH-Px activity ($r = 0.55$; $r = 0.58$).

After three weeks of feeding the increased dose of Se, concentration of copper in blood plasma dropped (Table 5) below physiological limit of $12 \mu\text{mol/l}$ (Pavlata, 2009) both in animals fed organic ($P < 0.01$) and inorganic ($P < 0.05$) selenium. Moreover, organic Se group showed significant negative correlation ($r = -0.64$, $P < 0.001$, $n = 32$) between blood Se concentration (Figure 3), or blood GSH-Px activity and blood Cu concentration ($r = -0.63$, $P < 0.001$, $n = 32$). No negative correlation was found in animals receiving sodium selenite. These results indicate possible negative interaction between organically bound selenium and copper. Antagonism between Cu and Se has been documented in animal studies (Hill, 1974). Some studies demonstrated that copper can inhibit selenite-induced cytotoxicity and apoptosis in HT-29 cells. Zeng and Botnen (2004) described that selenite and selenocysteine can cause cell cycle arrest via distinct mechanisms, and suggest that

Table 5. Concentration of zinc and copper in blood plasma of sheep during feeding optimum dose of Se (day 14), deficient dose of Se (day 35), 2 h after the first feeding of high-selenium diet (day 43), and during feeding increased dose of Se (day 63)

Sampling day	Group	Zinc ($\mu\text{mol/l}$)	Copper ($\mu\text{mol/l}$)
14	A	$11.37 \pm 1.45^{\text{C}}$	$13.89 \pm 1.40^{\text{a}}$
	B	$12.70 \pm 0.48^{\text{EF}}$	$13.35 \pm 2.24^{\text{b}}$
35	A	$9.99 \pm 0.91^{\text{a}}$	$13.41 \pm 1.54^{\text{C}}$
	B	$12.11 \pm 0.93^{**\text{b}}$	$12.65 \pm 1.60^{\text{c}}$
43	A	$8.97 \pm 1.0^{\text{CD}^{\text{a}}}$	$14.37 \pm 1.83^{*\text{D}}$
	B	$10.92 \pm 0.83^{**\text{Eb}}$	12.40 ± 1.24
63	A	$10.00 \pm 1.02^{\text{D}}$	$11.93 \pm 1.07^{\text{aCD}}$
	B	$11.04 \pm 1.28^{\text{F}}$	$11.70 \pm 1.53^{\text{bc}}$

A = sheep fed organic selenium ($n = 8$), B = sheep receiving inorganic selenium ($n = 7$)

C:C to E:F in one column $P < 0.01$, ^{a:a} to ^{c:c} in one column $P < 0.05$

* $P < 0.05$, ** $P < 0.01$ (significantly higher value as compared with the other group at the same sampling day)

Cu may interact with selenite extracellularly, which represents the basis on antagonism between copper and selenite. Wang et al. (2010) described interaction between selenomethionine and copper ions. The copper coordinated with selenomethionine by the formation of Cu-Se and Cu-OCO bonds or by the formation of Cu-N and Cu-OCO bonds. Selenium interactions with other elements rate among the factors bearing on selenium metabolism in an organism. There are descriptions of interactions of selenium and sulfur but also of selenium and cadmium, arsenic, copper, cobalt, manganese, lead, iron, and others (Shamberger, 1983). Increasing the sulfur content in the feedstuffs has impact on lowering the plasma concentration of selenium in dairy cows (Ivancic and Weiss, 2001). Pavlata et al. (2005b) described negative effect of higher doses of iodine on the metabolism of selenium in kids.

Furthermore, significant decrease of Zn concentration in blood plasma in both experimental groups was noted after feeding Se-deficient grain mixture (Table 5). In group A, Zn blood concentration dropped from 11.37 ± 1.45 to 8.97 ± 1.00 $\mu\text{mol/l}$ ($P < 0.01$) and in group B from 12.70 ± 0.48 to 10.92 ± 0.83 $\mu\text{mol/l}$, though Zn levels in feed mixture did not change throughout the experiment (Table 2). This fact indicates possible positive effect of Se on utilization of dietary Zn. However, this trend has not been confirmed by provable correlation of both parameters.

Positive correlation was also found between blood Se concentration and albumin level ($n = 32$, $r = 0.63$, $P < 0.001$) and between GSH-Px activity and albumin level ($n = 32$, $r = 0.68$, $P < 0.001$) in animals fed organic selenium. In wethers receiving inorganic selenium these provable correlations were not observed. These results suggest that organic Se in blood is more strongly bound with albumin than inorganic selenium. This could be due to the fact that selenomethionine is absorbed through absorption system of amino acids. Ingested selenomethionine is either metabolized directly into reactive forms of selenium or stored in place of methionine in body proteins. Selenomethionine metabolism is closely linked to protein turnover (Schrauzer, 2000, 2003).

Other monitored biochemical parameters of blood remained in the physiological range throughout the trial (total protein 62.3 ± 3.9 g/l, albumin 36.4 ± 2.9 g/l, urea 3.85 ± 1.29 mmol/l, total bilirubin 6.37 ± 0.93 $\mu\text{mol/l}$, AST activity 1.72 ± 0.38 $\mu\text{kat/l}$, GMT activity 1.20 ± 0.27 $\mu\text{kat/l}$) and

were not significantly affected by different forms and doses of Se. This corresponds with the results of Cristaldi et al. (2005) in lambs and Davis et al. (2006) in reproducing ewes, who did not observe any changes of blood biochemistry (albumin, ALP, ALT, AST, CK, GMT) even after one year of feeding high doses of selenium (10 and 20 mg/kg respectively). If high doses of selenium were toxic, increase of enzymatic activity (AST, GMT, etc.) would be expected. In fact, activity of enzymes increases when cells are damaged and enzymes are released from intracellular space into blood. From the above-given it follows that levels of selenium that were used in the present study did not have any toxic effect on sheep.

CONCLUSION

The actual intake of selenium by sheep is reflected by Se concentration and GSH-Px activity in their blood as well as Se levels in ruminal fluid and, more importantly, in ruminal biomass. The form of supplemented selenium has neither significant effect on the total content of Se in ruminal biomass and blood, nor activity of GSH-Px in blood. However, animals receiving inorganic selenium tend to have higher Se concentration and GSH-Px activity in blood, whereas sheep fed organic selenium tend to show higher levels of Se in ruminal biomass. In ruminal fluid of animals receiving high doses of selenium, significant decrease of infusoria count as well as provable increase of GMT, LDH, ALP, and AST activity were observed. Other monitored parameters of ruminal fluid were not affected by the application of selenium. After administration of high Se doses, animals of both experimental groups showed decreased concentration of copper in blood. Decrease of Cu concentration was more marked in animals fed organic selenium, in which negative correlation between blood Se and Cu concentrations was seen. This indicates possible negative interaction between copper and selenium. On the contrary, addition of low doses of selenium in the feeding ration led to significant decrease of zinc levels in the blood of experimental sheep.

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