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Effect of sodium selenite or lactate-protein selenium complex supplementation on selenium status in goat kids

A. Pechová[1], L. Antošová[2], L. Pavlata[3], A. Podhorský[2]

[1]Department of Biochemistry and Biophysics, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic
[2]Private veterinary surgeon
[3]Department of Animal Nutrition and Forage Production, Faculty of Agronomy, Mendel University in Brno, Brno, Czech Republic

ABSTRACT: The aim of the study was to compare the effect of selenium (Se) supplementation in organic (lactate-protein complex) or inorganic (sodium selenite) forms on Se concentrations in the blood and organs of goat kids. The experiment involved nineteen male goat kids divided into three groups: C, Se-I, and Se-O. Control group C (n = 5) was without Se supplementation, group Se-I (n = 7) received sodium selenite supplement (0.30 mg Se per animal/day), and group Se-O (n = 7) received lactate-protein selenium complex (0.28 mg Se per animal/day). The supplementation started on the day of weaning and continued for 13 weeks till the day of slaughter. Blood samples for determination of Se concentration and glutathione peroxidase activity were collected on the day of weaning and during weeks 4, 8, and 13 thereafter. Samples of liver tissue, spleen, kidneys, lungs, heart, tongue, diaphragm, shoulder, back, and thigh muscles were taken immediately after slaughter. Significantly higher concentration of Se in group Se-O in comparison with group C was found in thigh muscles (110.4 vs 71.0 μg/kg, $P \leq 0.01$), shoulder (105.0 vs 67.2 μg/kg, $P \leq 0.01$), back (102.9 vs 61.7 μg/kg, $P \leq 0.01$), and heart (180.8 vs 116.7 μg/kg, $P \leq 0.01$). Significantly higher concentration of Se in group Se-I in comparison with group C was found in shoulder (83.2 vs 67.2 μg/kg, $P \leq 0.01$) and diaphragm (93.6 vs 72.8 μg/kg, $P \leq 0.01$). The comparison of the groups Se-I and Se-O showed significantly higher Se concentrations in thigh muscles, heart, back, shoulder, and lungs in group Se-O. Mean Se concentration in tissues of experimental groups was 125.8% in Se-O group and 110.7% in Se-I group in comparison with group C. Our results are suggesting that supplementation of Se in the form of lactate-protein complex is more efficient in comparison with sodium selenite.

Keywords: organic selenium; inorganic selenium; glutathione peroxidase; functional foods

INTRODUCTION

Special attention has recently been given to the effect of trace minerals on animal's health and production. Thanks to its antioxidant functions, one of the most monitored elements is selenium. The clinical manifestation of Se deficiency can be divided into two groups: specific clinical manifestations (white muscle disease, exudative diathesis, hepatosis, mulberry heart disease) and non-specific Se-responsive disorders (Suttle 2010). Non-specific disorders are mostly presented in the form of reproduction disorders, increased perinatal mortality, immunodeficiency, decreased milk production and growth intensity, etc. Se-responsive disorders might have influence on herd health production and reproduction. Apart from the effect of Se supplementation on health of animals and humans, another important aspect is Se influence on food quality and composition,

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e.g. quality of meat, milk, eggs or the spectrum of fatty acids (Skrivan et al. 2010; Wang et al. 2011; Czauderna et al. 2012, 2013).

The sufficient intake of Se in ruminants depends on the content of this element in the soil. Groce et al. (1995) studied correlations between the concentrations of trace elements in soil, plants, and blood of animals, and established high correlation coefficient for Se (r = 0.96). The content of Se in soil varies according to geological areas. In Europe, deficient regions are mainly in Scandinavia (Finland, Norway, Sweden, Denmark), southern Europe, northern England, Scotland, and also in large part of the Czech Republic. The content of Se in soil ranges from 0.5 to 2 mg/kg (McDowell 1992). Soils containing less than 0.5 mg/kg Se in total are classified as Se deficient with a potential risk of Se-deficiency disorder in farm animals. The Se content in ruminant diet should be 0.1–0.3 mg/kg in dry weight but there is a high variability in particular plants according to growing area. Kappel et al. (1984) presents range values of Se concentration in lucerne hay 51–954 μg/kg, in grasses 61–173 μg/kg, and in maize silage 27–100 μg/kg dry matter. For the above reason there is need of Se supplementation into feeding ration. Inorganic forms of Se (sodium selenite, sodium selenate, barium selenate) have been used for many years, however recently there has been an increasing trend to use organic forms of Se with higher utilization despite their higher cost. The mostly studied organic form of Se is selenium-yeast in which the content of selenomethionine (Se-met) is 54–74% of total Se (Rayman 2004). Se-met is the form in which Se is present in natural plant feeds. Cereals and forage crops convert Se mainly into Se-met and incorporate it into protein in place of methionine because $tRNA^{Met}$ does not discriminate between Met and Se-met. In seleniferous corn, wheat, and soybeans, Se-met contents ranged 81–82% of total Se (Schrauzer and Surai 2009). Other Se containing organic forms used for Se supplementation are selenocysteine, Se-methylselenocysteine, selenocystathionine, and selenohomocysteine but most commercial Se supplements have no detailed data on the particular form of Se used. Probably this is the reason why the results of experiments, which compare organic and inorganic forms of Se in ruminants, are not uniform.

The aim of the study was to compare the influence of Se supplementation in organic (lactate-protein complex) or inorganic (sodium selenite) form on Se concentrations in the blood and tissues of small ruminants.

MATERIAL AND METHODS

Experiment design. The experiment was conducted on 19 castrated male kids of white short haired breed. It started on the day of weaning. Kids were divided into three groups: group C (n = 5) was a control group without Se supplementation, group Se-I (n = 7) received Se in inorganic form (sodium selenite), and group Se-O (n = 7) received Se in organic form (Selene chelate; Karel Gebauer AGROBAC, Třemešné, Czech Republic). It is characterized as a lactate-protein complex produced by cultivation of bacteria *Lactobacillus acidophilus* on substrate enriched with sodium selenite. The kids groups were formed according to mother's nutrition. Mother goats had been supplemented with Se in appropriate forms since 4 months before delivery. The average age of kids at the beginning of supplementation was 114.6 ± 14.6 days in group C, 112.4 ± 16.5 days in group Se-I, and 115.9 ± 5.1 days in group Se-O. The average weight of kids was 19.96 ± 3.57 kg in group C, 21.79 ± 3.70 kg in group Se-I, and 19.61 ± 2.38 kg in group Se-O.

Table 1. Composition of granulated feed mixture

Nutrients per 1 kg of feed mixture	
Dry matter (g)	886.05
Net energy of lactation (MJ)	6.31
Total protein (g)	118.40
Metabolizable energy (MJ)	10.44
Fibre (g)	78.24
Fat (g)	29.06
Calcium (g)	7.90
Phosphorus (g)	7.96
Magnesium (g)	2.19
Sodium (g)	2.96
Potassium (g)	9.17
Iron (mg)	79.33
Cobalt (mg)	0.60
Iodine (mg)	1.51
Manganese (mg)	36.69
Copper (mg)	29.42
Zinc (mg)	114.62
Chlorides (g)	4.34
Selenium (mg)	0.14

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Table 2. Selenium concentration in whole blood of kids (μg/l) (mean ± standard deviation)

Group	Weaning	4 weeks	8 weeks	13 weeks
C	141.0 ± 35.7[a]	124.0 ± 29.1[a]	130.3 ± 18.2[a]	129.8 ± 23.2[a]
Se-I	168.9 ± 32.1	158.5 ± 44.9	171.2 ± 21.7[b]	179.9 ± 22.1[b]
Se-O	187.9 ± 15.7[b]	177.5 ± 23.8[b]	207.3 ± 16.8[c]	197.0 ± 16.3[b]

C = control (n = 5), Se-I = kids supplemented with sodium selenite (n = 7), Se-O kids supplemented with lactate-protein complex (n = 7)

[a–c]different letters in one column show statistically significant differences between groups

$P \leq 0.05$

Feeding ration for kids consisted of granulated feed mixtures with different Se content and they had *ad libitum* access to hay and drinking water. Granulated feed mixture was fed three times a day in the total amount of 300 g per animal and day. The appropriate amount of supplementary diet (150 g per one feeding twice a day) was added to the individual groups into common feeders large enough to enable all animals to eat at the same time. The composition of granulated feed mixture is stated in Table 1. Group C received unsupplemented feed mixture with natural content of Se (0.14 mg/kg). Experimental group Se-I received feed mixture containing 1.0 mg/kg Se in sodium selenite form and group Se-O received feed mixture containing 0.94 mg/kg Se in lactate-protein complex form.

The supplementation of Se was carried out for 13 weeks. The experiment was terminated by slaughtering the kids. Weight of kids was monitored in one-week intervals during the whole experiment. Blood samples were collected from *v. jugularis* on the day of weaning and during weeks 4, 8, and 13 of the experiment. After slaughter, samples of liver, spleen, kidneys, lungs, myocardium, tongue, diaphragm, thigh, shoulder, and back muscles were taken.

Laboratory methods. Glutathione peroxidase (GSH-Px) was assessed in whole heparinized blood according to the method described by Paglia and Valentine (1967) with the use of the Ransel-Randox set and an automatic biochemical analyzer Cobas Mira (Roche, Basel, Switzerland).

Se was measured in whole blood and individual tissues using the HG-AAS method and the AAS Solar M6 (Unicam, Witchford, UK) device after microwave mineralization of samples in the Milestone Ethos TC (Milestone, Sorisole BG, Italy) unit using the method by Pechova et al. (2005). The Se concentration is stated in μg/kg of fresh tissue and in μg/l of whole blood.

Statistical analysis. The data were statistically analyzed by the F-test to evaluate the variance of the individual sets and according to the results Student's t-test was used for sets with equal/non equal variances for results between experimental groups. MS Excel (Version 14.0, 2010) software was used for the evaluations.

RESULTS

Selenium concentration and glutathione peroxidase activity in blood. The concentration of Se in blood of kids was significantly influenced

Table 3. Glutathione peroxidase activity in whole blood of kids (μkat/l) (mean ± standard deviation)

Group	Weaning	4 weeks	8 weeks	13 weeks
C	1019.2 ± 242.8	873.6 ± 221.0[a]	653.4 ± 173.1[a]	739.8 ± 111.7[a]
Se-I	1175.0 ± 71.6	1109.3 ± 172.6[a]	1020.3 ± 82.8[b]	1195.0 ± 40.1 [b]
Se-O	1242.5 ± 80.1	1300.5 ± 83.9[b]	1059.1 ± 74.7[b]	1188.5 ± 141.7[b]

C = control (n = 5), Se-I = kids supplemented with sodium selenite (n = 7), Se-O kids supplemented with lactate-protein complex (n = 7)

[a,b]different letters in one column show statistically significant differences between groups

$P \leq 0.05$

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Table 4. Selenium concentration in organs and tissues of kids (μg/kg fresh tissue) (mean ± standard deviation) at the end of the experiment

Group	C	Se-I	Se-O
Liver	416.9 ± 148.4	500.6 ± 124.2	379.6 ± 124.2
Kidney	933.2 ± 225.7	943.9 ± 153.5	1058.1 ± 111.7
Spleen	268.3 ± 26.3	267.5 ± 93.4	256.1 ± 34.3
Lung	181.6 ± 13.1[a]	137.8 ± 14.6[b]	198.2 ± 35.1[a]
Heart	116.7 ± 7.9[a]	126.6 ± 15.3[a]	180.8 ± 27.4[b]
Diaphragm	72.8 ± 12.2[a]	93.6 ± 15.3[b]	82.8 ± 9.2
Tongue	107.1 ± 12.9	108.4 ± 15.0	108.9 ± 20.8
Thigh	71.0 ± 6.4[a]	84.1 ± 11.3[a]	110.4 ± 18.0[b]
Back	61.7 ± 9.4[a]	80.0 ± 15.3[b]	102.9 ± 10.7[c]
Shoulder	67.2 ± 8.5[a]	83.2 ± 7.4[a]	105.0 ± 12.0[b]

C = control (n = 5), Se-I = kids supplemented with sodium selenite (n = 7), Se-O kids supplemented with lactate-protein complex (n = 7)

[a–c]different letters in one column show statistically significant differences between groups

$P \leq 0.01$

by Se supplementation in the feeding ration (Table 2). Significantly higher Se concentration in group Se-O at the time of weaning was found and it was increasing moderately during the experiment. Also a higher concentration of Se in blood of kids in group Se-I was found at the start of the experiment but the difference was not significant. During the experiment the Se concentration in Se-I group further increased and at the end the difference was significantly higher when compared with group C. Glutathione peroxidase activity (Table 3) was also influenced by the Se supplementation. In both supplemented groups, significantly higher activities were found 8 and 13 weeks after the start of the experiment.

Selenium concentrations in organs and tissues. Se supplementation with organic and inorganic Se forms increased Se concentration especially in muscle tissues (Table 4). Significantly higher Se concentrations in Se-O group when compared with control group were found in myocardium, thigh, back, and shoulder muscles. In group Se-I the only significantly higher concentrations were found in diaphragm and back muscles.

The results showed that the group supplemented with lactate-protein complex had significantly

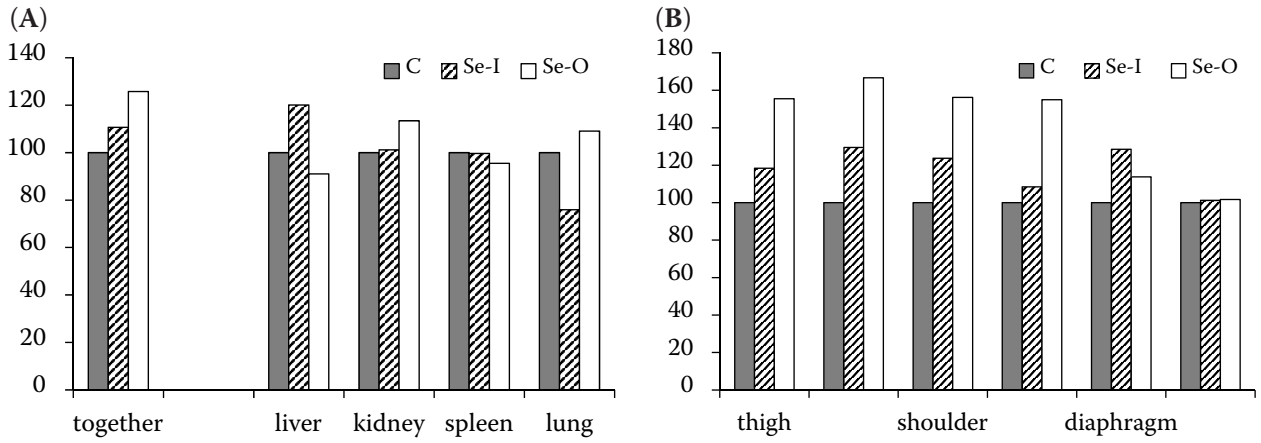

Figure 1. Relative increase of Se concentration (%) in all examined tissues and in individual organs (**A**) and in muscle tissues (**B**) of kids from experimental groups (Se-I, Se-O) compared to control group (C)

C = control (n = 5), Se-I = kids supplemented with sodium selenite (n = 7), Se-O = kids supplemented with lactate-protein complex (n = 7)

doi: 10.17221/7907-CJAS

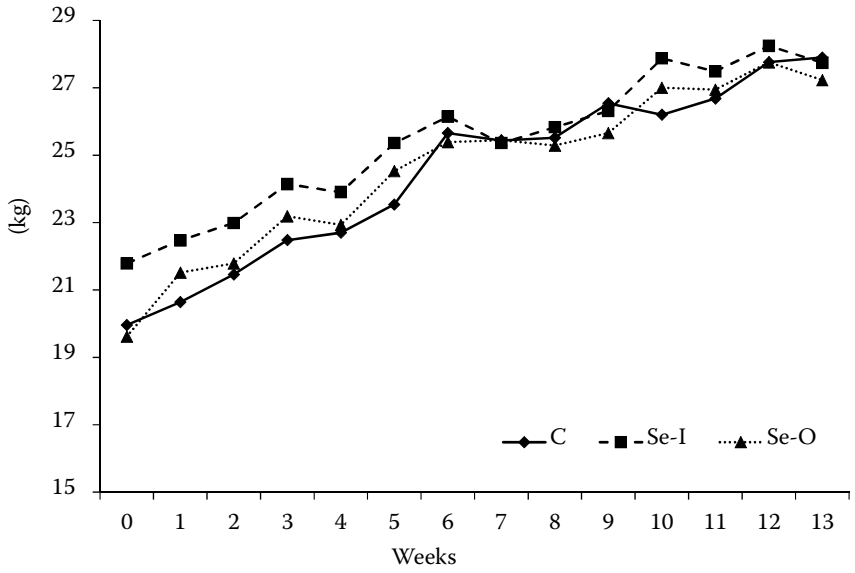

Figure 2. Weight of kids during the experiment

C = control (n = 5), Se-I = kids supplemented with sodium selenite (n = 7), Se-O = kids supplemented with lactate-protein complex (n = 7)

higher Se concentrations in shoulder, back and thigh muscles, and myocardium when compared with group supplemented with inorganic form of Se.

Relative changes in Se concentrations in individual groups are presented in Figure 1. A relative increase of Se concentration in tissues and organs was calculated comparing to the control group which was taken as a basis (100%). In total assessment of all organs and tissues the relative increase in group Se-O (125.8%) comparing to group Se-I (110.7%) was found. This better result of supplementation with organic form of Se is based on Se concentration increase in particular muscle tissues where the values range from 155 to 167%. In the group supplemented with inorganic form, the Se content increased in muscle tissues by about 109% (heart) to 130% (back muscles) while there was no increase in the tongue muscles.

Growth intensity of kids. The Se supplementation did not significantly influence the growth intensity of kids (Figure 2). An average weight gain of kids was 86 ± 25 g/day in group C, 65 ± 18 g/day in group Se-I, and 83 ± 13 g/day in group Se-O. From the stated values it is obvious that the lowest weight gains were in group Se-I. However, differences among particular groups were not statistically significant because of the relatively high variability within groups.

DISCUSSION

Se concentration in blood of kids was relatively high in all experimental groups during the whole experiment, which shows the Se intake in the feeding ration was sufficient. Deficiency is described in animals with blood Se concentration below 80 µg/l (Bickhardt et al. 1999). Limit for a sufficient supplementation is 100 µg/l (Pugh 2002; Pavlata et al. 2012). However, other authors report whole blood Se concentrations ranging 150–250 µg/l as the reference values (Van Metre and Callan 2001).

Differences in Se concentrations among particular groups at the beginning of experiment were caused by the origin of kids from mothers supplemented with Se in appropriate forms since 4 months before delivery. Both experimental groups of kids had higher Se concentration in blood than the control, but significant difference was only in group supplemented with organic form of Se. Se concentrations in blood of kids at the time of weaning are influenced by supplementation of mother goats at the time of pregnancy and lactation. Content of Se in milk is influenced not only by Se dose contained in feed but also by the form in which Se is administered to animals. Se concentration in milk is significantly increased by preparations containing Se-met (Givens et al. 2004; Juniper et al. 2006). Increased content of Se in milk is caused by incorporation of Se-methionine directly into milk protein. In one of our previous trials (Pechova et al. 2008), the effect of Se supplementation in various organic forms on excretion of Se in milk was studied. Milk Se concentration increased after 5 days of the experiment only in groups supplemented with Se-yeast. The other groups supplemented with lactate-protein complex

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and proteinate showed no increase in Se excretion by milk. We assume that this is the reason why in this experiment there was not a significant difference between groups Se-O and Se-I at the time of weaning. Similarly, in another experiment (Pechova et al. 2012) in goat kids at the time of weaning, the significant difference in the concentration of Se in blood in groups supplemented with sodium selenite and lactate-protein complex was not found, but supplementation of mothers with selenium-yeast significantly increased Se in comparison with other groups.

The differences among groups were increasing during the experiment and after 13 weeks of supplementation both experimental groups had significantly higher Se concentrations in blood than control. There was higher Se concentration in group supplemented with organic form of Se than in group supplemented with inorganic form but the difference was not significant. A higher effect of organic form of Se was confirmed on the basis of Se concentration assessment in particular organs and tissues where the total increase in group Se-O was 125.8% while in group Se-I it was only 110.7%. The increase of Se concentration in organs and tissues of Se-supplemented kids was relatively low and was detected only in muscle tissues but not in organs. In a similar study by Sevcikova et al. (2011) the influence of different forms of Se supplementation in kids in the period of weaning was monitored, and the increase of Se concentration to 161% in the group which received lactate-protein complex and to 144% in the group which received sodium selenite was found. The cause of this difference was the different content of Se in the basic feeding ration because deficiency of Se was detected in control group, while there was no deficiency found in our experiment. Sufficient intake of Se is proved by Se concentration in blood and in liver tissue.

Suggested concentration of Se in liver for Se deficiency diagnosis in ruminants is 110–140 μg/kg of liver fresh tissue (Galgan and Frank 1995; Pavlata et al. 2001b). In our study the liver Se concentration in the control group was 417 μg/kg. This proves a very good level of kids supply with Se from natural sources. There was also a relatively high Se concentration in kidneys with average values in particular groups from 933 to 1058 μg/kg. A similar concentration of Se in kidneys was reported by Salisbury et al. (1999) and Pavlata et al. (2001a). The Se supplementation influenced primarily the Se concentration in the muscle tissues where it ranged from 62 to 110 μg/kg with the highest values found in group Se-O. Similarly Smrkolj et al. (2005) reported significant influence of Se supplementation on Se concentration in muscles of bulls. In the above study the Se concentration of 35 ± 3 μg/kg was found in group receiving 0.4 mg Se per animal and day and of 143 ± 8 μg/kg in group receiving 4.4 mg per animal and day. A similar Se concentration in muscles was published by Murphy and Cashman (2001) who reported 61–105 μg/kg in beef meat, 82–129 μg/kg in pork meat, and 71–109 μg/kg in lamb meat. Sevcikova et al. (2011) reported Se concentration of 34–83 μg/kg in muscles of weaned kids, which is lower than showed the values in our study. In the literature, where most studies compared selenium yeast with sodium selenite forms, the higher efficiency was found for the organic form (Fisher 1995; Pavlata et al. 2001a; Juniper et al. 2009a, b). In our experiment we used a lactate-protein complex about which there is a lack of information in literature. The lactate-protein complex is prepared by cultivation of *Lactobacillus acidophilus* on the substrate with high content of sodium selenite. The producer of this Se supplement does not declare the detailed composition of the Se compound used; therefore we had to use published works as a source of information. Alzate et al. (2008) tested which forms of Se are produced during the lactate fermentation at the presence of *Lactobacillus* bacteria. They found that predominant forms synthesized during this type of fermentation were the selenocysteine (Se-cys) and Se-methylselenocysteine. These amino acids are, similarly as Se-met, absorbed by Na^+ dependent amino acids transport system in small intestine, while the inorganic form is absorbed passively (Schrauzer 2000). However, after absorption the metabolism of Se-met is different from that of Se-cys. Se-cys is not incorporated into tissue protein but it is degraded to selenide which is used for specific selenoproteins synthesis or excreted from the body. Similarly, the inorganic form of Se – the sodium selenite is firstly reduced to selenide. Se-met can be directly incorporated into body proteins or the second option is degradation to selenide with following utilization for functional selenoprotein synthesis (Suzuki and Ogra 2002). Juniper et al. (2009b)

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found that Se-cys is a dominant Se-amino acid in glandular organs, while Se-met is dominant in muscles of animals. In the study of Juniper et al. (2008) the high increase of Se-met concentration (about 167%) in blood after yeasts supplementation was found; while the concentration of Se-cys was only 90–100% when compared with control, which was independent of the Se supplement form used. The accumulation of Se occurs in some organs such as muscles, testicles, and brain in the form of Se-met as a storage substance that can be released to meet the body needs when necessary (Schrauzer 2003). Se-cys is more quickly degraded in organism than Se-met (Windish 2002). When organic and inorganic form of Se was compared in the present study, the results show moderately better effect of the organic form. As the metabolism of Se-cys and selenite starts similarly by the reduction to selenide, higher values of Se are probably caused by differences in absorption. The absorption of Se in ruminants is influenced by composition of feeding ration and also by rumen microbial activity (Koenig et al. 1997). In the rumen selenite can be reduced to insoluble selenide and thus the absorption of Se may be decreased. Panev et al. (2013) found that when supplementing organic or inorganic forms of Se, the ratio of Se in biomass and in rumen fluid is similar. The microorganisms in the rumen would influence Se-containing proteins if the protein is susceptible to microbial degradation and this could change the bioavailability of the supplied Se (Mynhardt et al. 2005). Rumen microbial fermentation can influence the final absorption of Se by changing Se form.

CONCLUSION

The results of the experiment show that in kids the Se supplementation in the form of lactate–protein complex has higher bioavailability than the supplementation of sodium selenite form. Another important finding is that even when the animals have a sufficient intake of Se, it is possible to increase the concentration of Se in muscle tissues by supplementation of Se mainly in organic form. This finding is particularly important in regard to the production of functional foods.

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Corresponding Author

doc. MVDr. Alena Pechová, CSc., University of Veterinary and Pharmaceutical Sciences Brno,
Faculty of Veterinary Hygiene and Ecology, Department of Biochemistry and Biophysics,
Palackého 1-3, 612 42 Brno, Czech Republic
Phone: +420 541 562 600, e-mail: pechovaa@vfu.cz