Selenium content in the blood serum and urine of ewes receiving selenium-enriched unicellular alga Chlorella

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ABSTRACT: The effect of selenium supplementation in inorganic and organic form was investigated in three five-member groups of ewes and their lambs in the course of 7 months. The basal feed ration contained 55 μg Se, ewes of experimental group E1 received a supplement of 180 μg Se in sodium selenite, and ewes of experimental group E2 were applied a supplement of 180 μg selenium bound in the biomass of the alga Chlorella. Control group C did not receive any selenium. The ewes were in the stage of non pregnant, pregnancy and lactation during the experiment. Average contents of Se in the blood serum of ewes were as follows: E1 114.2 ± 23.6; E2 103.1 ± 20.3; C 68.6 ± 16.8 μg/l ($P < 0.001$). A decrease in serum Se was recorded in all groups in the last third of pregnancy and in the first week *post partum*. Average contents of urinary selenium contents were also higher in experimental groups: E1 25.4 ± 13.5; E2 18.7 ± 9.6; C 13.3 ± 4.5 μg/l ($P < 0.001$). The positive effect of supplementation of Se to ewes was reflected in its higher average concentration in the blood serum of born lambs: E1 48.5 ± 7.3; E2 53.5 ± 3.4; C 30.3 ± 7.4 μg/l ($P < 0.05$). The effect of selenium bound in Chlorella biomass was higher and persisted until 30 days of lamb age: E1 52.9 ± 3.4; E2 59.0 ± 7.0; C 35.5 ± 5.8 μg/l ($P < 0.01$). The average number of lambs born per ewe was 1.0 in group C and E1, and 1.8 in group E2.

Keywords: sodium selenite; organically bound selenium; ewes; pregnancy; lactation; lambs

Selenium deficiency occurs mainly in young categories of farm animals and in high-performing animals (Kursa, 1969; Pavlata et al., 2001a). In the majority of the production herds of farm animals general Se supplementation is a condition for maintaining their health (Pavlata et al., 2004) and a sufficient amount of Se in animal products (Travnicek et al., 2006).

A low selenium content in soils (Pavlata et al., 2002) causes selenium deficiency in the Czech Republic. The blood serum of the CR population contains only 42–63 μg Se/l (Kvicala et al., 2003). In some areas of Southern Bohemia Kvicala and Kroupova (1999) reported only 2–5 μg Se/l in the blood plasma of cattle. The required daily selenium intake in ruminants varies from 100 to 300 μg/kg DM according to feed digestibility, age and performance (Schenkel and Flachowsky, 2000) while in the Czech Republic an amount of 200 μg Se/kg DM is recommended for all categories of sheep. The supply of Se to animals is evaluated according to its content in blood, urine and/or excrements and tissues, indirectly according to the activity of the enzyme glutathione peroxidase (GSH-Px). According to Stowe and Herdt (1992) an optimum selenium level in the blood serum of sheep is 120–150 μg/l

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whereas Alazzeh and Abu-Zanat (2004) reported the value 100–200 µg/l. Selenium deficiency is indicated by the values from 25 to 50 µg/l (Underwood and Suttle, 2001).

A long-term intramuscular application of 150 µg selenium in the form of sodium selenite (once a week) increased selenium content in the whole blood of lambing ewes to values exceeding 200 µg/l while in lambing ewes without supplementation Se content did not exceed 70 µg/l (Travnicek et al., 2001). After an intravenous application of sodium selenite Boldizarova et al. (2005) recorded an increase in Se in the blood serum of ewes from 0.39 to 7.83 µmol/l (30.7–616.5 µg/l) and in urine from 0.07 µmol/l to 18.53 µmol/l. In the blood of slaughter cows from various localities of the CR Pavlata et al. (2001b) measured 83.2 µg/l on average and in the blood of heifers only 39.0 µg/l.

The majority of the used mineral feed additives contains selenium in the form of inorganic salts. In recent years the supply of mineral supplements with organically bound selenium (mostly on the basis of selenium enriched yeast biomass) has increased because its better resorption is assumed (Ortman and Pehrson, 1999; Kim and Mahan, 2001; Sustala et al., 2003). Unicellular algae of the genus Chlorella that can absorb up to 500 mg selenium per 1 kg algal biomass from selenium solutions during cultivation in solar bioreactors (Doucha and Livansky, 1999) are an alternative source of organically bound selenium. In laboratory cultivated algae dimethylselenonium propionate, Se-allylselenocysteine and selenomethionine were detected (Larsen et al., 2001).

Although a lot of extensive studies were aimed at the evaluation of efficiency of organic and inorganic form of selenium in relation to parameters of antioxidative and immunity capacity, the results are not explicitly clear. The results of experiments focused on the utilization of various selenium sources proved differences between the species in metabolism and resorption of selenium in combination with other substances of selenium-enriched feed. E.g. Knowles et al. (1999) reported a higher transmission of selenium into milk when Se was applied in an organic form compared to its application in an inorganic form.

The objective of this study was to evaluate the efficiency of selenium supplementation in infertile, pregnant and lactating ewes and in their lambs when selenium was applied in an organic form in the biomass of fresh-water alga Chlorella and in an inorganic form as sodium selenite. The experimental testing of efficiency of selenium organic form in ruminants is necessary due to the existence of microorganisms in proventriculi the action of which may influence the molecular structure of selenium-containing compound and final products of rumen microorganisms.

MATERIAL AND METHODS

An experiment was conducted on fifteen ewes of the Sumava sheep breed at 18 months of age. The ewes were divided into three groups of five animals: control C and experimental ones E1 and E2 (Table 1). Feed rations for the groups of ewes differed only in selenium content in a mineral feed additive. Average feed ration formulation per head per day: 1 180 g hay, 240 g dried lucerne pellets, 270 g oat groats, 6 g mineral feed additive. The mineral feed additive for group C did not contain any selenium, for group E1 it contained 180 µg selenium in the form of sodium selenite (Na_2SeO_3) and for group E2 180 µg selenium bound in the biomass of the alga Chlorella. The mineral feed additive for group C and E1 contained the same amount of Chlorella biomass as for group E2, but without selenium. Table 2 shows the total dietary intake of selenium according to the groups.

The technology of controlled cultivation in solar bioreactors was used for the production of algal biomass in Microbiological Institute of the Academy of Sciences of the Czech Republic in Trebon according

Table 1. Live weight of ewes, number of lambs born and litter weight

Group (n = 5)	Live weight of ewes at the beginning of experiment	Live weight of ewes 2 months after parturition	Number of lambs born per ewe	Average live weight of lambs born (kg)	Average live weight of the litter (kg)
C	37.0 ± 2.6	50.0 ± 2.7	1	4.6	4.6
E1	40.0 ± 3.6	52.8 ± 3.9	1	4.7	4.7
E2	39.3 ± 6.0	52.3 ± 3.6	1.8	4.4	7.7

Table 2. Average composition of the daily ration per ewe and selenium intake

Component	C			E1			E2		
	amount (g)	DM (g)	Se (µg)	amount (g)	DM (g)	Se (µg)	amount (g)	DM (g)	Se (µg)
Hay	1 180	1 010	40	1 180	1 010	40	1 180	1 010	40
Lucerne	240	218	6	240	218	6	240	218	6
Scraped oat	270	236	9	270	236	9	270	236	9
Mineral mixture	6	6	0	6	6	180	6	6	180
Total	1 696	1 470	55	1 696	1 470	235	1 696	1 470	235
Selenium content (µg/kg DM)			37			160			160

to Patent Doucha and Livansky (1999). Selenium content per 1 kg of dry matter of produced algal biomass was 255 mg.

The experiment was conducted from July 2005 to March 2006. The tupping of ewes by rams of the same breed started in September 2005. The ewes were in the stage of infertility, pregnancy and lactation during the experiment. All ewes became pregnant and parturitions took place in the course of six weeks. Blood and urine for selenium analysis were collected in monthly intervals, the last collection was done on average 4 weeks before the expected parturition. After parturition blood samples were taken from lambing ewes and born lambs on Day 1, 3, 10 and 30. Selenium in blood serum and urine was detected by neutron activation analysis and spectrofluorimetry (Kvicala et al., 1995). Statistical processing of data included the calculation of mean values ($\bar{x}$), standard deviations (SD), coefficient of variation (V%), minimum and maximum values, median, 25 and 75 percentile, statistical significance was determined by the ANOVA – Tukey's test.

RESULTS AND DISCUSSION

Table 2 shows average daily selenium intake. In experimental group E1 and E2 the supplementation of 180 µg selenium in inorganic or organic form per head/day resulted in the intake of 235 µg Se per head/day (160 µg/kg DM). This amount is in the range of the standard (Schenkel and Flachowsky, 2000) but it does not reach the upper limits of recommended doses. The low Se content in the feed ration of control group (group C) reflects its low content in plant feeds.

Statistical evaluation of the effect of Se supplementation on its serum and urinary content is documented in Table 3 and 4. The average Se concentration in the blood serum of ewes of group E1 (114.2 ± 23.6 µg/l) and E2 (103.1 ± 20.3 µg/l) was 1.7 and 1.5 times higher, respectively, than in the ewes of control group C (68.6 ± 16.8 µg/l) (Table 3). The differences were statistically highly significant. Comparing the experimental groups, the average serum Se content was by 10.8% higher in group E1 than in group E2. The difference between experimental groups was not statistically significant. Similarly, Boldizarova et al. (2005) did not prove any statistically significant differences in Se content in plasma, whole blood and red blood cells after supplementation of 300 µg Se/kg DM as sodium selenite and Se-enriched yeast.

The urinary selenium content (Table 4) was also statistically significantly higher in experimental groups: group E1 25.4 ± 13.5 µg/l, group E2

Table 3. Average selenium content in the blood serum of ewes (µg/l)

Group	n	$\bar{x}$	SD	V(%)	Min	Max	25 percentile	Median	75 percentile
C	48	68.6[a]	16.8	24.5	41.7	114.2	58.2	63.4	81.9
E1	47	114.2[b]	23.6	20.7	70.3	175.0	97.0	114.3	127.6
E2	47	103.1[b]	20.3	19.7	63.5	154.0	87.8	101.9	116.2

[a:b]$P < 0.001$

Table 4. Average selenium content in the urine of ewes (µg/l)

Group	*n*	$\bar{x}$	SD	*V*(%)	Min	Max	25 percentile	Median	75 percentile
C	23	13.3[a]	4.5	33.8	6.7	22.9	9.2	13.0	16.6
E1	25	25.4[b]	13.5	53.1	4.4	56.2	16.6	20.5	31.9
E2	22	18.7[b]	9.6	51.3	3.6	35.0	10.2	19.3	26.0

[a:b]$P < 0.001$

18.7 ± 9.6 µg/l and control group C 13.3 ± 4.5 µg/l. The difference in urinary Se content between experimental groups was not statistically significant. The variability in urinary Se content was higher in experimental groups (*V*% 53.1 and 51.3, respectively).

Compared to the reference range (Stowe and Herd, 1992) of selenium in blood serum 120–150 µg/l, the average values in experimental groups supplied 180 µg and with total intake of 235 µg were lower. Only individual values and partial means in groups E1 and E2 reached the reference range (Table 3).

The highest Se content in the serum of ewes of group E1 was recorded after 3–4 months of selenium supplementation (126.5 ± 26.6 µg/l) while in group E2 it was highest about a month later, i.e. in month 4–5 of the experiment (123.3 ± 12.9 µg/l). Serum Se dropped in all groups in connection with pregnancy and lactation stress in the last third of pregnancy and in the first week after parturition. This drop persisted until about Day 30 *post partum* in ewes of group E2 (Figure 1). A decrease in serum selenium between Day 100 and 150 of ewe pregnancy was also reported by Gurdogan et al. (2006). Selenium content in the urine of ewes of group C and E2 also dropped in the stage of pregnancy (Figure 2).

The positive effect of supplementation of selenium to ewes was reflected in its higher concen-

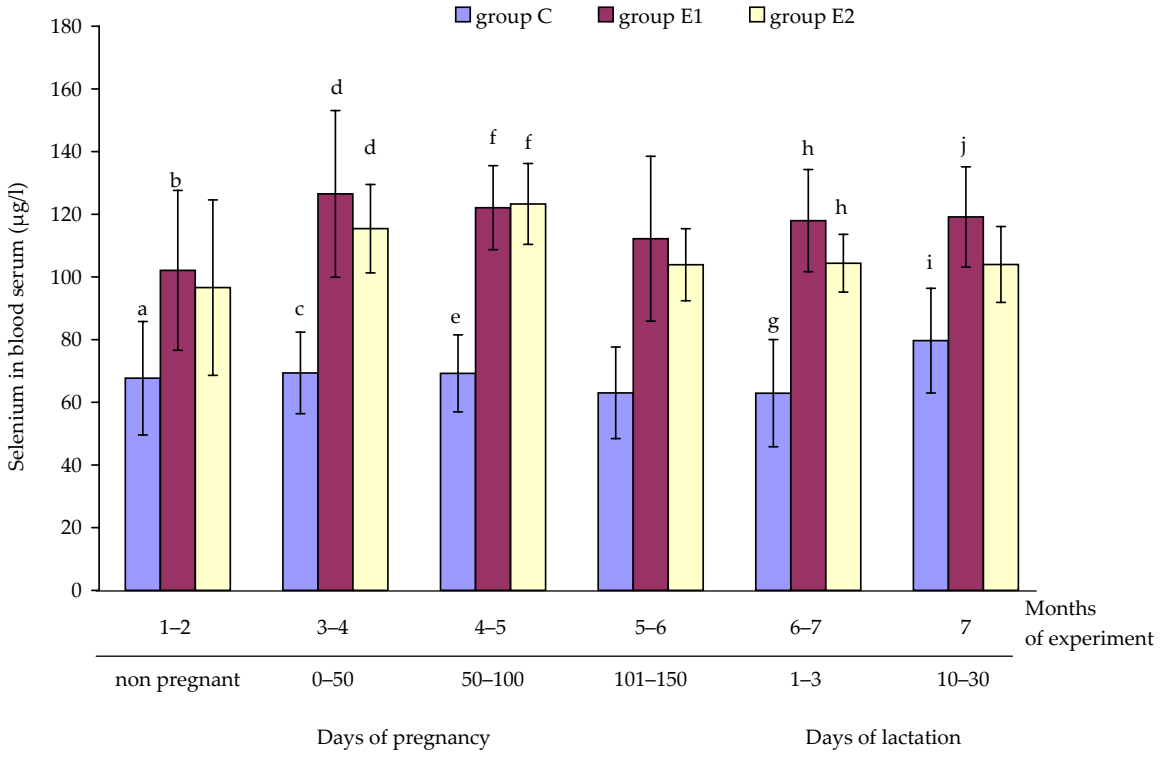

Figure 1. Selenium content in the blood serum of ewes (µg/l)

Statistical significance: [a:b]$P < 0.5$; [c:d]$P < 0.001$; [i:j]$P < 0.01$

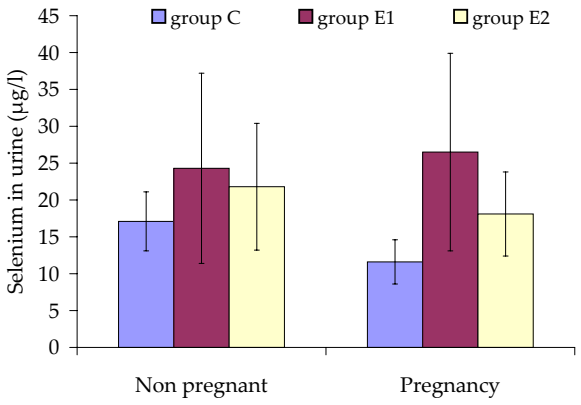

Figure 2. Selenium content in the urine of ewes (µg/l)

tration in the blood serum of born lambs (Table 5, Figure 2). The effect of selenium bound in Chlorella biomass was higher than that of selenium in the inorganic form. The average selenium content in the serum of lambs of group E2 ewes was by 8.9% higher than in lambs of group E1 and by 47.8% higher than in group C lambs (Table 5). The serum selenium content at the day of birth was 48.2 ± 7.3, 53.5 ± 3.4 and 30.3 ± 7.4 µg/l in lambs of group E1, E2 and C, respectively; the respective values at 30 days of age in group E1, E2 and C were 52.9 ± 3.4, 59.0 ± 7.0 and 35.5 ± 5.8 µg/l (Figure 3). The effect of selenium supplementation to mothers on Se content in the blood of the born young was proved by Pavlata et al. (2003) in calves after an injection application of Se product to their mothers; and also e.g. by Abdelrahman and Kincaid (1995) after the application of selenium bolus to the rumen of cows – mothers. Higher levels of selenium in the blood serum of lambs from birth to Day 30 of age in groups E1 and E2 are explained by higher transplacental transmission of selenium (Van Saun et al., 1989; Pavlata et al., 2004) and probably by higher selenium output through colostrum and milk in relation to dietary intake of selenium (Grace et al., 2001; Pavlata et al., 2004). The differences between experimental group E1 and E2 are influenced by higher retention of selenium from organic forms in the organism and higher excretion into milk (Knowles et al., 1999) compared to inorganic selenium. The differences in Se content in the blood

Table 5. Average selenium content in the blood serum of lambs until 30 days of age (µg/l)

Group	*n*	$\bar{x}$	SD	*V*(%)	Min	Max	25 percentile	Median	75 percentile
C	13	33.9[a]	9.3	27.4	20.9	57.8	29.7	35.9	40.0
E1	18	46.0[b,c]	5.7	12.4	39.9	60.9	43.3	45.7	51.8
E2	32	50.1[b,d]	6.8	13.6	39.7	72.6	47.1	49.9	53.2

[a:b]$P < 0.01$, [c:d]$P < 0.05$

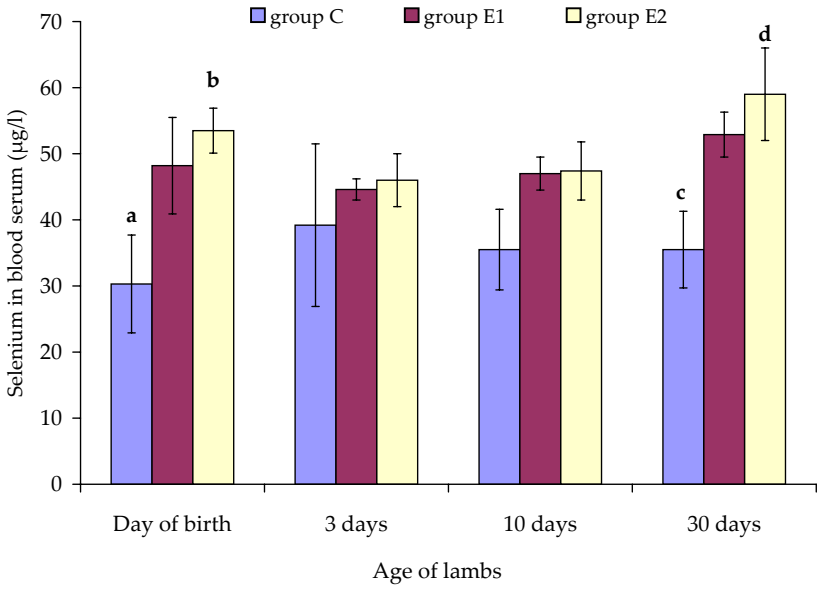

Figure 3. Selenium content in the blood serum of lambs until 30 days of age (µg/l)

Statistical significance: [a:b]$P < 0.05$; [c:d]$P < 0.01$

serum and urine of ewes of groups E1 and E2 in the period of pregnancy and especially lactation are connected with higher requirements for selenium in the ewes of group E2 providing nutrition to a higher number of fetuses and subsequently of born lambs (Table 1, Figures 1 and 2).

Markedly higher fertility of ewes receiving Se-enriched algae (group E2) is an interesting finding. An increase in fertility by 38% in selenium deficient areas after Se supplementation was reported for a large group of sheep by Balicka-Ramisz et al. (2006).

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