

## Activity of glutathione peroxidase (GSH-Px) in the blood of ewes and their lambs receiving the selenium-enriched unicellular alga *Chlorella*

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**ABSTRACT:** The effect of supplementation of selenium inorganic and organic forms on the activity of glutathione peroxidase (GSH-Px) was investigated for 7–8 months in three groups of ewes (by five animals each) and in born lambs. The basal feed ration contained 55 µg Se, the ewes of experimental group E1 received a supplement of 180 µg Se in sodium selenite, and the ewes of experimental group E2 were administered a supplement of 180 µg selenium bound in the biomass of the alga *Chlorella*. Control group C was without selenium supplement. The ewes were in the stage of non-pregnancy, pregnancy and lactation during the experiment. The average number of lambs born per ewe was 1.0 in groups C and E1 and 1.8 in group E2. Both forms of selenium supplementation resulted in higher activity of GSH-Px in the whole blood, higher content of Se in the blood serum ( $P < 0.001$ ) and milk of ewes ( $P < 0.001$ ). Average activity of GSH-Px in the whole blood of ewes was as follows: C 697.9 ± 179.3; E1 1 147.4 ± 181.5; E2 1 056.1 ± 267.5 U/g Hb ( $P < 0.001$ ). It reached the highest values in the 5<sup>th</sup> to 6<sup>th</sup> month of Se supplementation while the activity decreased after parturition in connection with lactation. Higher utilisation of Se from the organic form, compared to the inorganic form, was reflected in a higher content of Se in the milk of group E2 ewes ( $P < 0.05$ ). The positive effect of selenium supplementation of ewes contributed to higher activity of GSH-Px in the whole blood of their lambs ( $P < 0.001$ ) and higher Se concentration in the blood serum ( $P < 0.01$ ). The organic form of Se (group E2) was more efficient in this case. Average activity of GSH-Px in the whole blood of lambs was as follows: C 434.1 ± 70.6; E1 1 031.6 ± 172.3 and E2 1 055.6 ± 235.1 U/g Hb.

**Keywords:** selenite; organic selenium; blood selenium; urine; milk; pregnancy; lactation; sheep; glutathione peroxidase; *Chlorella*

The level of selenium supply to animals may be assessed according to its content in blood, urine, tissues, excrements, and in milk in lactating females.

Selenoprotein glutathione peroxidase (GSH-Px) is one of the frequently used indicators. About 11.8% of total Se in the organism is bound in GSH-Px

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(Awadeh et al., 1998). GSH-Px catalyzes the reduction of hydrogen peroxides and organic hydrogen peroxides to water and alcohol. Together with vitamins C, E and other antioxidants it prevents the oxidative damage of cells.

Pavlata et al. (2001) proved a high correlation between Se content in the whole blood and GSH-Px activity in the whole blood ( $r = 0.93$ ). In ewes with the intake of 100  $\mu\text{g}$  Se/kg dietary DM Olivera et al. (2004) reported the activity of GSH-Px to be in the range of 194.38 to 236.51  $\mu\text{kat/l}$  of blood while the simultaneous content of selenium in the blood plasma ranged from 83.44 to 183.90  $\mu\text{g/l}$ . A statistically significantly higher activity was determined in lambs. After a single subcutaneous application of 0.4 mg Se and 5 mg of vitamin E per kg of ewes' prepartal live weight the postpartal activity of GSH-Px increased, amounting to 226 U/g Hb on day 30 *post partum* (Milad et al., 2001). A high correlation between the activities of GSH-Px in calves and in their mothers was reported by Pavlata et al. (2003).

The application of organic forms of selenium usually results in ruminants in higher activity of the enzyme and increased concentration of Se in tissues compared to its inorganic form (Chung et al., 2007; Lee et al. 2007; Qin et al., 2007). Higher activity of GSH-Px and higher concentration of Se in the whole blood and blood serum were measured by Rock et al. (2001) in ewes and in their lambs, supplemented with 300  $\mu\text{g}$  Se/kg of DM of the feed ration in selenised yeast compared to the same dose of Se in sodium selenite. The activity of GSH-Px exceeding 750 U/g Hb in sheep after 3-month supplementation of 278.6  $\mu\text{g}$  Se per day in the form of  $\text{Na}_2\text{SeO}_3$  or Se-yeast was recorded by Boldizarova et al. (2005) while differences in the enzyme activity between groups were not statistically significant. Effects of dietary organic selenium supplementation on the antioxidative status and selenium content of muscles of pigs and rabbits were investigated by Bobček et al. (2004) and Dokoupilová et al. (2007), respectively.

The majority of the used mineral feed additives contains selenium in the form of inorganic salts.

In recent years there has been an increase in the supply of mineral supplements with organically bound selenium (mostly on the basis of selenium-enriched yeast biomass), in which better resorption is anticipated (Ortman and Pehrson, 1999; Kim and Mahan, 2001; Šustala et al., 2003). Unicellular algae of the genus *Chlorella* are an alternative source of organically bound selenium: during cultivation in solar bioreactors they may absorb from selenium solutions up to 500 mg selenium per 1 kg of dry matter of algal biomass (Doucha and Livansky, 1999). Dimethylselenonium propionate (dimethylselenopropionat), Se-allylselenocystein and selenomethionine were detected in laboratory cultures of algae (Larsen et al., 2001). A positive effect of the dietary Se-enriched alga *Chlorella* on Se concentration of hen eggs was reported by Skřivan et al. (2006). Trávníček et al. (2007) found out a higher content of Se in the blood serum of lambs when the selenium-enriched alga *Chlorella* was applied to their mothers.

The objective of the present paper was to evaluate the effects of supplementation of organic form of selenium in the biomass of alga of the genus *Chlorella* and inorganic form ( $\text{Na}_2\text{SeO}_3$ ) on the activity of GSH-Px in the whole blood of ewes and their lambs.

## MATERIAL AND METHODS

An experiment was conducted with three groups of ewes of the Šumava sheep breed while each group comprised five animals: control group C and experimental group E1 and E2 (Table 1).

Feed rations for the groups of ewes differed only in selenium content in a mineral feed additive. The mineral feed additive for group C did not contain any selenium, for group E1 it contained 180  $\mu\text{g}$  selenium in the form of sodium selenite ( $\text{Na}_2\text{SeO}_3$ ) and for group E2 180  $\mu\text{g}$  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

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

Group (number of ewes = 5)	C	E1	E2
Live weight of ewes at the beginning of experiment (kg)	37.0 $\pm$ 2.6	40.0 $\pm$ 3.6	39.3 $\pm$ 6.0
Number of lambs born per ewe	1	1	1.8
Average live weight of lambs born (kg)	4.6	4.7	4.4

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

Component	Group								
	C			E1			E2		
	DM (%)	DM (g)	Se (µg)	DM (%)	DM (g)	Se (µg)	DM (%)	DM (g)	Se (µg)
Hay	145.5	1 010	40	145.5	1 010	40	145.5	1 010	40
Lucerne	14.8	218	6	14.8	218	6	14.8	218	6
Scraped oat	18.3	236	9	18.3	236	9	18.3	236	9
Mineral mixture	0.4	6	0	0.4	6	180*	0.4	6	180**
Total		1 470	55		1 470	235		1 470	235
Selenium content (µg/kg DM)			37			160			160

\*selenium in the form of sodium selenite ( $\text{Na}_2\text{SeO}_3$ )

\*\*selenium bound in the biomass of the alga *Chlorella*

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 CR in Trebon according to the Patent of Doucha and Livanský (1999). Selenium content per 1 kg of dry matter of produced algal biomass was 255 mg.

The ewes were in the stage of non-pregnancy, pregnancy and lactation during the experiment. Blood and urine for selenium analysis were collected in monthly intervals, and on the day of parturition and on day 10, 30 and 60 *post partum*. The blood of lambs was collected on the day of birth and on day 3, 10 and 30 of age. Milk samples were taken from ewes on the day of parturition, on day 3, 10, 30 and 60 *post partum*. The activity of GSH-Px was determined in the whole heparinised blood by Ransel kit from Randox Laboratories (Crumlin, UK) on Olympus AU 400 Analyzer (Olympus, Mishima, Japan). The results were expressed in units per gram of haemoglobin (U/g Hb). Selenium in the blood serum was detected by neutron activation

analysis (Kvíčala et al., 1995). Selenium content in milk and feeds was determined after microwave mineralisation with a UNICAM 939 AA spectrometer applying the hydride technique of AAS.

Statistical processing of data included the calculation of mean values ( $\bar{x}$ ), standard deviations (SD), coefficient of variation (CV %), minimum and maximum values, median, 25 and 75 percentiles; statistical significance was determined by the ANOVA – Tukey's test.

## RESULTS AND DISCUSSION

Table 1 shows the basic characteristics of the control and experimental groups. Table 2 documents data on the content of dietary selenium and its average intake. The supplementation of 180 µg selenium in inorganic or organic form per head/day resulted in the average daily intake of 235 µg Se (160 µg/kg DM) in experimental groups E1 and E2. This amount is within the limits defined by the standard (Schenkel and Flachowsky, 2000), but it does not reach the upper limit values of recom-

Table 3. Average GSH-Px activities in the whole blood of ewes (U/g Hb)

Group	<i>n</i>	$\bar{x}$	SD	CV (%)	Min.	Max.	25 percentile	Median	75 percentile
C	44	697.9 <sup>a</sup>	179.3	25.7	407.3	1 081.3	589.8	678.1	808.0
E1	40	1 147.4 <sup>b</sup>	181.5	15.8	737.5	1 544.3	1 017.6	1 160.0	1 271.6
E2	39	1 056.1 <sup>b</sup>	267.5	25.3	604.8	1 722.7	886.3	982.8	1 258.5

*n* = number of examinations

<sup>a,b</sup>*p* < 0.001

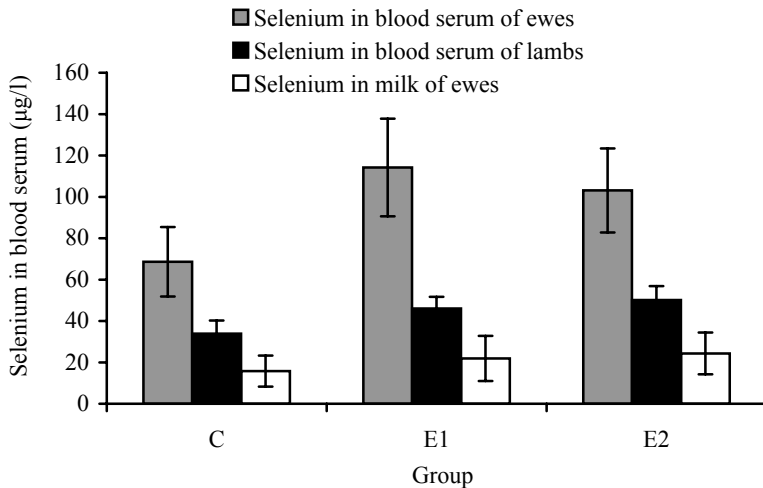


Figure 1. Average selenium content in the blood serum of ewes and lambs and selenium content in the milk of ewes

mended doses. The average daily intake of Se in the control group C was only 55 µg, being connected with its low content in bulky feeds. Differences in selenium intake were reflected in Se content in the blood serum of ewes and born lambs (Figure 1). The statistically insignificantly lower content of Se in the blood serum of group E2 ewes can be explained by a higher demand for selenium in connection with a higher number of nursed lambs. On the other hand, the higher content of Se in the blood serum of lambs of this group confirms higher conversion and utilisation of Se from organic forms (Qin et al., 2007). Detailed data on Se dynamics in the serum and urine of ewes and serum of lambs was published previously (Trávníček et al., 2007).

The supplemental feeding of selenium to ewes also had a positive effect on its concentration in the milk of ewes (Figure 1). Differences were statistically significant. A higher content of selenium in the milk of ewes of the group receiving an organic

form of selenium (group E2) corresponds to similar results obtained with the yeast form of selenium (Knowles et al., 1999; Kim and Mahan, 2001).

The maintenance of a higher Se output with milk in the second month of lactation in group E2 (Figure 2) reflects a higher level of utilisation of selenium organic forms.

Table 3 shows the statistical evaluation of GSH-Px activity according to groups. The average activity of GSH-Px in the whole blood of ewes of group E1 ( $1\ 147.4 \pm 181.5$  U/g Hb) and E2 ( $1\ 056.1 \pm 267.5$  U/g Hb) was 1.6 and 1.5 times higher, respectively, than in the control group C ( $697.9 \pm 179.3$  U/g Hb) ( $P < 0.001$ ). These ratios of GSH-Px activity between the groups are similar to the ratios of selenium contents in the blood serum between the groups (Trávníček et al., 2007). Differences in the activity of GSH-Px between the control and experimental groups were statistically highly significant but differences between the experimental groups were not

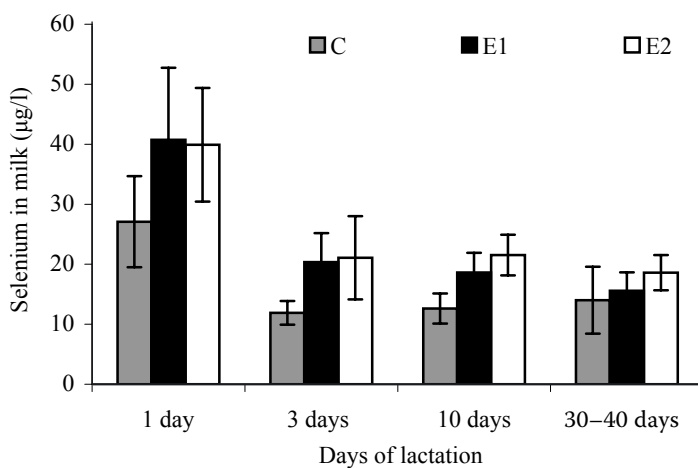


Figure 2. Selenium content in the milk of ewes until 60 days of lactation (µg/l)

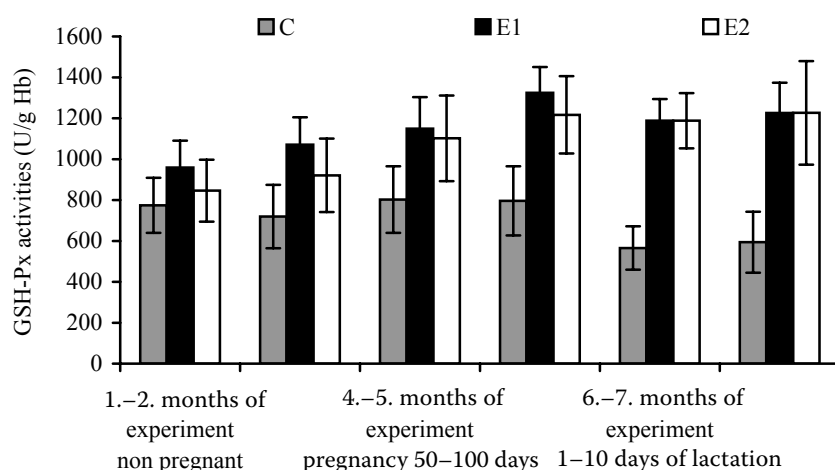


Figure 3. GSH-Px activities in the blood of ewes

significant. In comparison with data on the GSH-Px activity in ewes after single *s.c.* application (Milad et al., 2001) or after 3-month supplementation of both the organic and inorganic form of Se (Boldizarova et al., 2005) our values of GSH-Px activity are substantially higher. It is possible to derive a positive effect of the long-term supplementation and selected level of Se supplement on the activity of GSH-Px. A statistically significant difference in the GSH-Px activity after 56-day supplementation of selenised yeast and sodium selenite at a dose of 160 µg/kg DM of feed ration was also reported by Qin et al. (2007).

The dynamics of GSH-Px activity (Figure 3) is similar in both experimental groups. It reached the highest values in the 5<sup>th</sup> to 6<sup>th</sup> month of supplementation (E1 1 324 ± 126.0, E2 1 216.7 ± 188.8 U/g Hb). Ewes were in the last third of pregnancy in that period. In all groups the activity of GSH-Px decreased after parturition while the lowest values were recorded on day 10 *post partum*. The activity of GSH-Px on days 1–10 *post partum* was lower by 30.6% in group C compared to the prepartal period, in group E1 it was lower by 22.2% and in group E2 only by 6.7%.

The positive effect of selenium supplementation in ewes was also reflected in the higher activity of

Table 4. Average GSH-Px activities in the whole blood of lambs until 60 days of age (U/g Hb)

Group	<i>n</i>	$\bar{x}$	SD	CV (%)	Min.	Max.	25 percentile	Median	75 percentile
C	18	434.1 <sup>a</sup>	70.6	16.2	316.8	561.2	370.9	450.7	475.3
E1	18	1 031.6 <sup>b</sup>	172.3	16.7	707.4	1 357.4	891.6	1 041.1	1 174.4
E2	34	1 055.6 <sup>b</sup>	235.1	22.3	748.2	1 968.9	865.5	1 007.2	1 193.3

*n* = number of examinations

<sup>a,b</sup>*P* < 0.001

Table 5. Correlations between Se content in the blood serum and GSH-Px activities in the whole blood

Group	Ewes non-pregnant and pregnant		Ewes until 60 days of lactation		Lambs until 60 days of age	
	<i>n</i>	<i>r<sub>xy</sub></i>	<i>n</i>	<i>r<sub>xy</sub></i>	<i>n</i>	<i>r<sub>xy</sub></i>
C	25	0.29	19	0.63*	18	0.56*
E1	25	0.26	17	0.31	18	0.37
E2	25	0.27	16	0.60	31	0.53*

*n* = number of examinations

\**P* < 0.05

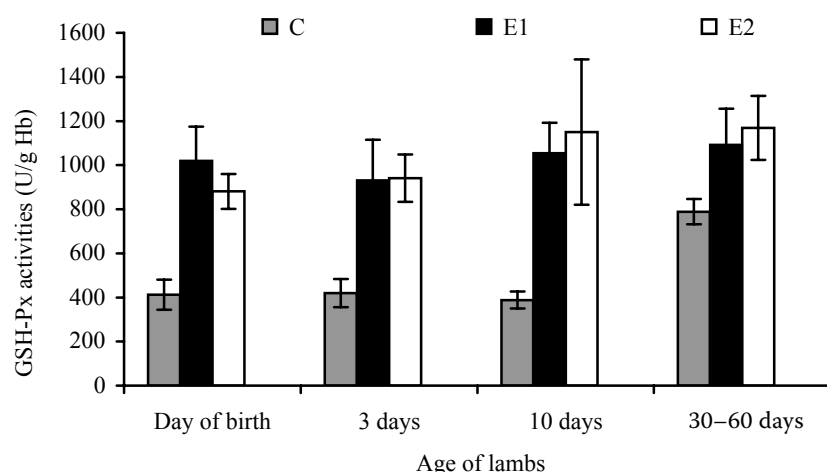


Figure 4. GSH-Px activities in the blood of lambs until 60 days of age

GSH-Px in born lambs (Table 4, Figure 4) whereas the organic form of Se (group E2) was more efficient. The higher activity of GSH-Px in lambs from birth to day 60 of age in group E1 and E2 with supplementation of selenium can be explained by its higher transplacental transmission (Saun van et al., 1989; Pavlata et al., 2004) and increased intake in milk (Figures 1 and 2) (Grace et al., 2001; Pavlata et al., 2004) and its previous retention in the organism of ewes during pregnancy. Higher activity of GSH-Px in the blood of ewes and higher content of Se in the blood of born lambs whose mothers were supplemented with yeasts as an organic form of selenium compared to sodium selenite were also reported by Qin et al. (2007).

The coefficients of correlation between Se content in the blood serum and GSH-Px activity in the whole blood expressed in U/g Hb (Table 5) do not reach the values that were determined for Se and GSH-Px in the whole blood by Pavlata et al. (2001). A higher correlation is documented in ewes in the postpartal period and in lambs. In the group with the inorganic form of Se supplementation it was lower in these cases compared to group C and E2.

Qin et al. (2007) explained the higher conversion of Se from its organic forms such as selenised yeast which was reflected in higher content of Se in tissues and blood and higher activity of GSH-Px by direct utilisation of selenoamino acids during proteosynthesis. The positive effect of the feeding of selenium enriched alga *Chlorella* can be explained in a similar way.

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