# SELENIUM STATUS IN CATTLE AT SLAUGHTER: ANALYSES OF BLOOD, SKELETAL MUSCLE, AND LIVER

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#### Abstract

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The objective of the study was to assess relationships among selenium concentrations in the blood, and liver and diaphragmatic muscle tissues and the activity of glutathione peroxidase (GSH-Px) in whole blood and to decide on the use of these biochemical values in the diagnostics of selenium deficiency in cattle. Samples were collected from 44 cattle at slaughter. Regression and correlation analyses yielded equations of regression lines and correlation coefficients (r) documenting significant (p < 0.01) relationships between whole blood selenium concentration on the one hand and all the other biochemical parameters under study on the other hand. The relations were expressed by the following equations and correlation coefficients: blood Se vs. liver tissue Se y = 1.20x + 31.58, r = 0.78; blood Se vs. muscular tissue Se y = 0.53x + 11.97, r = 83; blood Se vs. GSH-Px y = 8.29x - 68.77, r = 0.93. The equations were used to calculate selenium concentrations in hepatic and muscular tissues and GSH-Px activity corresponding to whole blood selenium concentration of 100 µg.1<sup>-1</sup> and critical concentrations indicating selenium deficiency (calculated value - 10%). Poor selenium status, as assessed from blood, muscle and liver selenium concentrations, was found in 80%, 70% and 73% of the tested animals, respectively. Considering these results and the rather uniform within-herd distribution of the values we can conclude that tissue analyses are suitable for the assessment of selenium status particularly in feeder bulls and grazed beef cattle in which only minor individual differences in selenium supply can be expected.

Glutathione peroxidase, diagnostics, diaphragm, beef cattle

Natural intake of selenium by ruminants depends primarily on the geographical position, or more specifically, on selenium concentration in soil. The general and well documented principle of the control of trace element intake by the link soil-plant-animal applies also to selenium (Groce et al. 1995; Campbell et al. 1995; Kamada et al. 2000). The assessment of selenium status in animals can be based on clinical examination focused on manifestations of selenium deficiency, such as locomotory disorders resulting from muscular dystrophy, disorders of heart activity, increased activity of muscle-specific enzymes in blood plasma, postmortem macroscopic and microscopic lesions in muscle tissue, and, above all, on direct determination of selenium content in feeds, blood, and tissues.

Determination of selenium in whole blood, blood plasma or blood serum is the approach used in most of recent studies of saturation with selenium and the concentration in whole blood of 100  $\mu$ g.l<sup>-1</sup> is regarded as the reference value for the assessment of selenium status most frequently (Van Saun 1990; Fisher et al. 1995). Another criterion is the activity of glutathione peroxidase that contains selenium as its structural component (Enjalbert et al. 1999; Pavlata et al. 2000). In their comparative study of various forms of selenium supplementation, Ortman et al. (1999) used a combination of selenium determination in whole blood and blood plasma and measurement of glutathione peroxidase in whole blood. A similar approach was used also by Pehrson et al. (1999) in their study of selenium status. Selenium concentration in tissues is a criterion used mostly in the assessment of selenium

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metabolism in the dam-offspring system (Van Saun et al. 1989; Kirk et al. 1995). Abdelrahman and Kincaid (1993) and Orr and Blakley (1997) investigated selenium concentrations in foetal liver and kidney tissues. Zust et al. (1996) assessed the selenium status in calves by its concentrations in blood plasma and liver tissue. Also usable for this purpose is muscular tissue (Pavlata et al. 2001). Although the selenium status can be assessed by tissue concentrations, the interpretation of values obtained in various areas, various animal species and categories, and various laboratories is often difficult. Selenium status in the man and in animals can also be assessed from concentrations in hair and urine (Kursa and Kroupová 1975; Kohler et al. 1994; Kvíčala et al. 1995; Shiobara et al. 1998; Kvíčala et al. 1999).

The objective of our investigations was to assess the relations among selenium concentrations and activity of GSH-Px in whole blood and in liver and diaphragmatic muscle tissue and decide on samples suitable for the diagnosis of selenium deficiency, or the assessment of selenium status in cattle.

#### **Materials and Methods**

Blood and tissue samples were collected on slaughter from 44 animals (21 feeder bulls, 14 heifers, 9 cows) coming from 9 herds. Blood samples were collected from vena jugularis into plastic heparinised tubes and liver (from the incision behind the caudate lobe done within meat inspection) and diaphramatic muscle samples were put into polyethylene bags. The samples were kept in frozen state until further processing. The samples were mineralised by microwave digestion technique in a closed system in the presence of nitric acid and hydrogen peroxide using the apparatus MILESTONE MLS - 120. The mineralised sample was prepared for the determination of selenium by evaporation, dissolution in water and treatment with 20% hydrochloric acid. Selenium was determined in the processed samples using the UNICAM 939 AA spectrometer and the hydride technique AAS. The concentrations are expressed in µg.l<sup>-1</sup> and µg.kg<sup>-1</sup> in blood and wet tissue samples, respectively. The activity of glutathione peroxidase in heparinised whole blood samples, was determined by the method described by Paglia and Valentine (1967) using the Randox set and the automatic analyser COBAS MIRA, and was expressed in µkat.1<sup>-1</sup> of whole blood. Mean values, standard deviations and variation coefficients were calculated for the whole set of animals and for separate herds and categories. Regression line equations and correlation coefficients were calculated to estimate relationships among the results of analyses of various samples. The regression line equations were used to calculate selenium concentrations in liver and muscle tissues corresponding to the concentration of  $100 \text{ }\mu\text{g}.\text{I}^{-1}$  in whole blood. The selenium status was assessed by critical concentrations which were by 10% lower than those calculated for the whole blood concentration given above. All the statistic calculations were done using the EXCEL software.

## Results

The results of analyses are indicative of considerable differences in the selenium status in cattle. Mean values and ranges were as follows: whole blood  $56.6 \pm 36.4 \,\mu g.l^{-1}$  (11.5 to 145.9  $\mu g.l^{-1}$ ); hepatic tissue 99.1 ± 63.9  $\mu g.kg^{-1}$  (29.6 to 235.8  $\mu g.kg^{-1}$ ); muscular tissue 42.2 ± 13.3  $\mu g.kg^{-1}$  (4.1 to 83.1  $\mu g.kg^{-1}$ ). Mean GSH-Px activity in whole blood was 400.0 ± 323.7  $\mu$ kat.l<sup>-1</sup> (13.1 to 1112.7  $\mu$ kat.l<sup>-1</sup>). Correlation analyses for GSH-Px activity and selenium concentrations demonstrated the closest correlation between blood selenium concentration and activity of GSH-Px (r = 0.93; *p* < 0.01). Also highly significant were the correlations among the tissue and blood concentrations and GSH-Px activity (r = 0.76 to 0.83; *p* < 0.01). All the calculated correlation coefficients and data on statistical significance are given in Table 1. Blood selenium concentration and GSH-Px activity

## Table 1

	Se-blood	GSH-Px-blood	Se-liver	Se-diaphragm
Se-blood	1	0.93**	0.78**	0.83**
GSH-Px-blood		1	0.76**	0.82**
Se-liver			1	0.80**
Se-diaphragm				1

correlated better with the concentrations found in muscle samples than with those found in liver samples.

Regression analyses yielded the following equations of regression lines for y (selenium concentrations in tissue samples and GSH-Px activity) corresponding to whole blood selenium concentration (x):

y = 1.20x + 31.58 for liver and blood selenium concentrations;

y = 0.53x + 11.97 for muscle and blood selenium concentrations;

y = 8.29x - 68.77 for GSH-Px activity and blood selenium concentration.

Replacing x by 100  $\mu$ g.l<sup>-1</sup> yielded the following values: liver selenium concentration 151.58  $\mu$ g.kg<sup>-1</sup>; muscle selenium concentration 64.97  $\mu$ g.kg<sup>-1</sup>; GSH-Px activity 760.23  $\mu$ kat.l<sup>-1</sup>.

Results of selenium status assessment by the individual herds and categories of animals are shown in Tables 2 and 3, respectively.

Table	2
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Selenium concentrations and activity of GSH-Px in whole blood and selenium concentrations in tissues of slaughtered animals by herds (B = bulls; H = heifers; C = cows; n = number of animals;  $v_x$  = variation coefficient)

Herd		Se-blood µg.l <sup>-1</sup>	GSH-Px μkat.l <sup>-1</sup>	Se-liver µg.kg <sup>-1</sup>	Se-diaphragm µg.kg <sup>-1</sup>
1 - B	mean ± S.D.	$69.0 \pm 15.3$	457.1 ± 96.1	$128.5 \pm 33.1$	$40.1 \pm 14.9$
(n = 4)	v <sub>x</sub> (%)	22	21	26	37
2 - B	mean ± S.D.	$12.7 \pm 1.1$	$29.9 \pm 9.8$	$41.7 \pm 5.4$	$6.7 \pm 2.7$
(n = 5)	v <sub>x</sub> (%)	9	33	13	40
3 - B	mean ± S.D.	$113.7 \pm 19.9$	831.8±134.2	$173.7 \pm 47.9$	$72.0 \pm 6.2$
(n = 6)	v <sub>x</sub> (%)	18	16	28	9
4 - B	mean ± S.D.	$28.9 \pm 5.8$	$129.1 \pm 26.2$	$52.9 \pm 10.6$	$22.0\pm4.6$
(n = 6)	v <sub>x</sub> (%)	20	20	20	21
5 - H	mean ± S.D.	$55.4 \pm 14.6$	$267.9\pm78.0$	$62.8\pm9.2$	$34.3 \pm 14.3$
(n = 5)	v <sub>x</sub> (%)	26	29	15	42
6 - H	mean ± S.D.	$35.2 \pm 23.9$	$254.2 \pm 193.9$	$109.4\pm24.0$	$45.7\pm8.8$
(n = 5)	v <sub>x</sub> (%)	68	76	22	19
7 - H	mean ± S.D.	$23.2 \pm 5.9$	$143.3\pm50.8$	$40.2 \pm 7.1$	$28.3 \pm 5.6$
(n = 4)	v <sub>x</sub> (%)	25	35	18	20
8 - C	mean ± S.D.	$76.4 \pm 19.7$	$672.7 \pm 183.2$	$129.2\pm25.2$	$63.3 \pm 13.1$
(n = 5)	v <sub>x</sub> (%)	26	27	20	21
9 - C	mean ± S.D.	91.6±19.3	827.9±287.8	$159.3 \pm 50.3$	67.9±11.5
(n = 4)	v <sub>x</sub> (%)	21	35	32	17

Table 3 Selenium concentration and activity of GSH-Px by categories of slaughtered animals (mean  $\pm$  S.D.)

	Se-blood µg.1 <sup>-1</sup>	GSH-Px μkat.l <sup>-1</sup>	Se-liver µg.kg <sup>-1</sup>	Se-diaphragm µg.kg <sup>-1</sup>
Bulls $(n = 21)$	$56.9 \pm 43.2$	368.7 ± 343.4	99.1±63.9	36.1 ± 26.7
Heifers $(n = 14)$	$39.0 \pm 20.8$	$227.4 \pm 130.8$	$73.0 \pm 33.1$	36.6±12.2
Cows (n = 9)	$83.2\pm20.0$	$741.7 \pm 233.5$	$142.6\pm38.9$	$65.3 \pm 11.9$

Variation coefficients were calculated to estimate within-herd differences in selenium intake. As can be seen in Table 2, the selenium status was fairly uniform in the individual herds. Minimum differences were observed in liver selenium concentrations, followed by muscle and blood concentration and GSH-Px activity.

The concentrations calculated by regression analysis reduced by 10%, i.e. 137  $\mu$ g.kg<sup>-1</sup> and 58  $\mu$ g.kg<sup>-1</sup> for liver and muscle concentrations, respectively, were taken as critical values for the diagnosis of selenium deficiency. Further critical values were blood selenium concentration of 90  $\mu$ g.l<sup>-1</sup> and GSH-Px activity 680  $\mu$ kat.l<sup>-1</sup>. The results of selenium status assessment are shown in Table 4.

				1
Herd	Se-blood	GSH-Px	Se-liver	Se-diaphragm
	< 90 µg.1 <sup>-1</sup>	< 680 µkat.1 <sup>-1</sup>	< 137 µg.kg <sup>-1</sup>	< 58 µg.kg <sup>-1</sup>
	(number of animals)	(number of animals)	(number of animals)	(number of animals)
	(11111001 01 1111111)	(inumber of unitials)	(inumber of unitials)	(indicer of diminus)
1 (n = 4)	4	4	3	4
2 (n = 5)	5	5	5	5
3 (n = 6)	0	0	1	0
4 (n = 6)	6	6	6	6
5 (n = 5)	5	5	5	5
6 (n = 5)	5	5	4	5
7 (n = 4)	4	4	4	4
8 (n = 5)	4	2	3	1
9 (n = 4)	3	2	1	1
1  to  9 (n = 44)	36	33	32	31
Selenium deficiency	82 %	75 %	73 %	70 %

Table 4 Assessment of selenium deficiency by results of blood and various tissues analyses

The data show a high degree of agreement in the demonstration of insufficient selenium intake by cattle. Discrepancy was observed in 8 animals showing blood selenium concentrations lower than 90  $\mu$ g.l<sup>-1</sup> and higher-than-critical concentrations in liver and muscle tissues (n = 3), liver tissue (n = 3), or muscle tissue (n = 2). The data given in Table 4 also indicate a high percentage of selenium deficient animals. Determination of blood, liver and muscle concentrations and activity of GSH-Px branded as selenium-deficient 36 (82%), 32 (73%), 31 (70%) and 33 (75%) animals.

## Discussion

The close correlation between blood selenium concentration and activity of GSH-Px (r = 0.93; p < 0.01) has confirmed the conclusions of Pavlata et al. (2000), Thompson et al. (1981), Stevens et al. (1985), Erskine et al. (1987), and Mass et al. (1993) that selenium status in cattle can be assessed by the activity of GSH-Px. The two methods are also used most frequently for the assessment of selenium status.

Analyses of tissue samples are used both in diagnostics and in experimental studies. Close correlations between blood and tissue concentrations of selenium were demonstrated in our preceding study (Pavlata et al. 2001) in which the effects of organic and inorganic selenium compounds were compared in 15 calves. Statistical processing of analytical data demonstrated close and highly significant (p < 0.01) correlation between blood and liver concentrations (r = 0.85), blood and skeletal muscle concentrations (r = 0.77). Also high

were the correlation coefficients for selenium concentrations among the tested tissues (r = 0.78 to 0.94).

The present study has extended the number of tissues suitable for investigations of selenium status, have confirmed our previous results, and completed them by data on correlation between blood and tissue concentrations and activity of GSH-Px. Liver and diaphragmatic muscular tissues were selected because of simple sampling with minimum damage to carcasses and organs.

Any comparison of absolute values of selenium concentrations is rather difficult, because published data are expressed in different units (fresh tissue, dry matter) and were obtained in different regions from various animal species and categories. Nevertheless, there were efforts to assess selenium status, or diagnose selenium deficiency, on the basis of tissue concentrations. Stowe and Herd (1992) found that blood serum concentrations 50 to 80 ng.ml<sup>-1</sup> in calves and lambs and 70 to 100 ng.ml<sup>-1</sup> in adult cattle corresponded to liver selenium concentration 1200 to 2000 µg.kg<sup>-1</sup> dry matter irrespective of animal species and age. Zust et al. (1996) assessed selenium status in calves by blood plasma and liver concentrations regarding the values 30  $\mu$ g.l<sup>-1</sup> of blood plasma and 300  $\mu$ g.kg<sup>-1</sup> of liver dry matter as minimum. Stussy et al. (2000) assessed selenium status in Oregon elk by liver concentration regarding 0.120 ppm as the critical value, but concluded that blood samples should be preferred for this purpose. Galgan and Frank (1995), who monitored selenium status in Sweden by testing wild moose (Alces alces L.), found liver concentrations ranging from 0.03 to 3.1 mg.kg<sup>-1</sup> fresh tissue and used the concentration 0.1 mg.kg<sup>-1</sup>, recommended for cattle, as the critical value for the diagnosis of selenium deficiency. The corresponding concentration calculated from our results (137 µg.kg<sup>-1</sup>) is very close to this critical value. Our results are congruent with data published by Grace et al. (2000) who demonstrated positive correlation (r = 0.86; y = 1.25 + 71.6) between liver and blood concentrations of selenium in red deer (Cervus elaphus). Selenium concentrations in diaphragmatic muscles found in our investigations are similar to results published by Jorhem et al. (1996) who reported concentrations ranging from 0.030 to 0.18 mg.kg<sup>-1</sup> fresh tissue in beef imported to Sweden. Data similar to our results were published also by Van Vleet (1975) who tested fresh tissues collected from clinically normal weaned calves after slaughter and found selenium concentrations 0.12 ppm and 0.05 ppm in liver and muscle samples, respectively. Venalainen et al. (1997) within their study of selenium saturation in the Finnish population demonstrated the dependence of selenium concentrations in bovine tissues on the level of soil treatment with selenium-containing fertilisers. Mean selenium concentration in bovine liver samples was  $0.28 \pm 0.05$  mg kg<sup>-1</sup> fresh tissue during the period of low-level fertilisation and  $0.51 \pm 0.18$  mg.kg<sup>-1</sup> fresh tissue during the period of high-level fertilisation. Salisbury et al. (1991) found in liver tissue of slaughtered cattle selenium concentration 280 µg.kg<sup>-1</sup>.

The high percentage of animals in which selenium deficiency was demonstrated after slaughter is consistent with results of our earlier investigations in which deficiency was demonstrated in 64% of the 326 cattle from various regions of the Czech Republic (Pavlata et al. 2000). Selenium deficiency was observed more frequently in slaughtered heifers and bulls than in dairy cows. This difference apparently resulted from ration composition and supply of minerals which is controlled in dairy cows more carefully.

Assessment of selenium status by all the four parameters (whole blood selenium concentration, activity of GSH-Px, selenium concentrations in liver and diaphragmatic muscle tissues) showed a relatively high agreement, which was almost absolute in the herds 1 through 7 (differences found at most in one sample within one herd). The agreement was weaker in the herds 8 and 9 in which the whole blood selenium concentration lay near the critical value decisive for the diagnosis of deficiency. In spite of this among-herd difference it is apparent that the critical tissue selenium concentrations can be used for the assessment

of selenium status, or diagnosis of severe selenium deficiency, in particular in beef and fattened cattle. Findings of critical or near-critical concentrations indicate the necessity to assess the selenium status by tests of more samples.

Our results further showed a fair within-herd uniformity of selenium status. Hence, results obtained in individual animals can be regarded as representative for the whole herd. Results of tissue analyses were even more uniform that those of blood analyses. These findings facilitate herd diagnostics of deficiencies, because results of tests of representative samples, including those collected from slaughtered animals, allow the assessment of herd selenium status and, when necessary, elaboration and timely implementation of prophylactic and therapeutic measures. Tests of tissue samples can become a suitable alternative to blood testing above all in grazing cattle and feeder bulls. Handling of beef cattle reared all the year round on pastures without shelters is very difficult and blood sampling may be hazardous to the personnel. Stress associated with blood sampling affects not only the sampled individuals, but the whole herd. In seleniumdeficient animals, stress can induce clinical or subclinical manifestations of nutritional myodystrophy. Tissue samples are less suitable for testing of dairy cow herds in which nutrition is not so uniform as in other cattle categories and in beef cattle in general. Great variations in intake of concentrates and mineral supplements in various phases of lactation and stages of the reproductive cycle can lead to considerable differences in selenium saturation. This applies particularly to the dry period and first weeks of the lactation period when selenium deficiency develops most frequently (Anonymous 1998; Illek et al. 1999).

# Hodnocení stavu zásobení selenem u jatečného skotu na základě vyšetření krve, svaloviny a jater

Cílem sledování bylo vyhodnotit vztah hodnot koncentrací selenu v krvi, játrech, svalovině bránice a aktivity GSH-Px v plné krvi a posoudit možnost použití těchto hodnot v diagnostice karence selenu u skotu. Vzorky k vyšetření byly získány od 44 kusů poráženého skotu. Mezi koncentrací selenu v plné krvi a aktivitou glutathionperoxidázy (GSH-Px) v plné krvi, koncentrací selenu v játrech i ve svalovině bránice byly regresní a korelační analýzou získány rovnice regresní přímky a korelační koeficienty (r) dokládající těsný, statisticky významný vztah (p < 0.01). Pro vztah koncentrace selenu v krvi a koncentrace selenu v játrech y = 1,20x + 31,58; r = 0,78, pro hodnotou selenu v krvi a koncentraci selenu ve svalovině bránice y = 0.53x + 11.97; r = 0.83 a pro vztah mezi hodnotou selenu v krvi a aktivitou GSH-Px y = 8,29x - 68,77; r = 0,93. S použitím těchto rovnic byly vypočteny hodnoty selenu v játrech, svalovině bránice a aktivity GSH-Px odpovídající koncentraci selenu v plné krvi 100  $\mu$ g.l<sup>-1</sup>. Za pomoci vypočtených hraničních hodnot deficitu (vypočtená hodnota - 10 %) pak bylo provedeno vyhodnocení stavu zásobení selenem u vyšetřených zvířat. Nízká úroveň zásobení selenem byla nalezena u 82 % zvířat, dle koncentrace selenu v krvi a u 70 %, resp. 73 % zvířat dle koncentrací selenu ve svalovině bránice, resp. játrech. Vzhledem k uvedeným výsledkům a poměrně vysoké vyrovnanosti hodnot u zvířat stejného chovu lze konstatovat, že stav zásobení selenem lze vyšetřením uvedených tkání poražených zvířat posoudit. Tato vyšetření jsou použitelná především pro posouzení stavu zásobení selenem u vykrmovaných býků a pastevně chovaného masného skotu, kde je velká vyrovnanost krmné dávky u zvířat celého stáda a tím i obdobná saturace selenem.

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