Direct and indirect assessment of selenium status in sheep – a comparison

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ABSTRACT: The aim of this study was to determine the relationship between selenium concentration and activity of glutathione peroxidase (GSH-Px) in the whole blood of sheep and reference ranges for the activity of GSH-Px for evaluation of the selenium status of sheep in the Czech Republic. Selenium concentration and activity of glutathione peroxidase were determined in whole blood samples collected from 92 sheep in six herds. The GSH-Px activity in the samples was measured using the photometric method. Selenium in the sample was measured using the hydride technique atomic absorption spectrometry. Data on both parameters were processed using correlation and regression analysis in order to obtain reference values of GSH-Px for the indirect evaluation of the selenium status of sheep. The two variables showed a close and significant correlation ($r = 0.95$; $P < 0.01$). The regression line, defined by the equation $y = 7.5857x - 121.87$ (linear) or $y = -0.0167x^2 + 11.993x - 355.57$ (polynomial), allowed us to determine the GSH-Px activity of 637, resp. 677 μkat/l as equivalent to selenium concentration in whole blood of sheep 100 μg/l. Mean selenium concentration and mean GSH-Px activity found in whole blood samples were 123.42 ± 57.84 μg/l and 814.34 ± 463.64 μkat/l, respectively. In this study, a close dependence of GSH-Px activity on the selenium concentration in the blood of sheep was found. Activity values of GSH-Px were determined for use in the diagnosis of sufficient selenium status in sheep in the Czech Republic (GSH-Px activity greater than 600 μkat/l of whole blood).

Keywords: glutathione peroxidase; whole blood; trace elements; diagnostics; ruminants; white muscle disease

Selenium is one of the essential trace elements which protects organisms from oxidative damage. Many biological functions of this element, which is present in various selenoproteins, have been described. The most important of them are glutathione peroxidases, iodothyronine deiodinases, selenoproteins P, W, R, T, N, thioredoxine reductase and others (Birringer et al. 2002).

More exact methods for the diagnostics of selenium status include direct determination of selenium in blood and tissues or hair and indirect assessment of selenium status by measurement of the activity of glutathione peroxidase in whole blood (Verde et al. 1995; Pavlata et al. 2000, 2005, 2011a). Since GSH-Px becomes a component of erythrocytes already at the stage of erythropoiesis, its activity in whole blood indicates the long-term selenium status, while blood plasma selenium concentration rather reflects the actual status (Gerlof 1992). However, as every researcher uses different kinds of units, each laboratory ought to have its own reference values (Andres et al. 1996).

Many authors confirmed a positive correlation between GSH-Px activity and selenium concentration in whole blood. In the Czech Republic, for example, this has been demonstrated by Pavlata et al. (2000) in cattle, Pavlata et al. (2005) and Misurova et al. (2009a) in goats and Ludvikova et al. (2005) in horses. In the Czech Republic a correlation between concentration of selenium and activity of glutathione peroxidase in whole blood has not been described for sheep. There exist articles from other countries on sheep, in which some authors describe a positive correlation (Anderson et al. 1978; Verde

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et al. 1995), whilst others have not found any correlation (Rosen et al. 2009).

The objective of our study was to assess the correlation between the activity of GSHPx and selenium concentration in the whole blood of sheep, to calculate by regression analysis the activity of GSH-Px corresponding to selenium concentrations of between 70 µg/l and 100 µg/l of whole blood regarded as the reference value for sheep, and to evaluate the measurement of GSH-Px as a method for the determination of selenium status or diagnosis of selenium deficiency in the Czech Republic.

MATERIAL AND METHODS

Blood samples were collected from 92 sheep of the Suffolk or Merinolandschaft breeds on six farms in various parts of the Czech Republic. The farms were selected to represent herds differing in selenium supply (animals fed non-supplemented feed vs. animals receiving a seleniumcontaining mineral supplement) to obtain the widest possible range of low and sufficient blood selenium concentrations and the corresponding GSH-Px activities. The samples were collected from *v. jugularis* into heparinised disposable test tubes. The GSH-Px activity in the samples was measured by the method developed by Paglia and Valentine (1967), using a set supplied by Randox and the automatic analyser COBAS MIRA, and expressed in terms of µkat/l of whole blood. Selenium concentration was determined in the same whole blood sample. Prior to analysis, the samples were mineralised in a closed system of microwave digestion in the presence of nitric acid and hydrogen peroxide using the Milestone Ethos TC (Milestone, Italy) apparatus. After removal of nitric acid from the digested sample in the evaporation rotor of the same apparatus, the water solution was mixed with 20% hydrochloric acid. Selenium in the processed sample was measured using HG-AAS (Hydride Generation Atomic Absorption Spectrometry) according to the method described by Pechova et al. (2005) in an AAS Solaar M6 (Unicam, Great Britain). Selenium concentrations were expressed in terms of µg/l of whole blood.

The basic statistical parameters of the set of the results (mean, standard deviation, minimum, maximum, median) obtained from individual animals and the relationship between the results of the two methods were tested by calculating the correlation coefficient (r) using Microsoft EXCEL 2010 software, and regression analysis and calculating the regression line equation allowed the determination of presumed values of y (GSH-Px) for various values x (selenium).

RESULTS

The basic statistics of the obtained data are presented in Table 1 and the relationship between selenium concentration and GSH-Px activity in the whole blood of sheep is depicted in Figures 1 and 2. A positive correlation between both variables was expressed by the coefficient of correlation ($r = 0.95$; $P < 0.01$; $n = 92$).

The linear relation of the selenium concentration and the GSH-Px activity expressed by the equation $y = 7.59x - 121.87$ is highly significant ($P < 0.01$) (Figure 1). Apart from the linear regression relation, a 2nd degree polynomial regression between both variables was found. This relationship is shown in Figure 2. This type of regression better illustrates the relationship of the two parameters, which is also documented by the higher value of reliability (R^2). From the graph, it is clear that rising concentrations of selenium (greater than 200–250 µg/l) no longer caused a linear increase in GSH-Px activity, but that activity values plateaued (at 1500–1800 µkat/l). Theoretical values of the activity of GSH-Px for the threshold selenium concentrations were calculated using these linear and polynomial equations (Table 2). On the basis of these results the ranges given in Table 2 were used for the purposes of deriving the GSH-Px activity in the diagnostics of selenium deficiency in sheep.

Table 1. Basic descriptive statistics of the selenium concentrations and the activity of GSH-Px in whole blood of sheep ($n = 92$)

	Se (µg/l)	GSH-Px (µkat/l)
Mean	123.4	814.3
Standard deviation	57.8	463.6
Coefficient of variance (%)	46.8	56.9
Minimum	35.3	29.4
Maximum	258.4	1694.0
Median	105.7	698.4

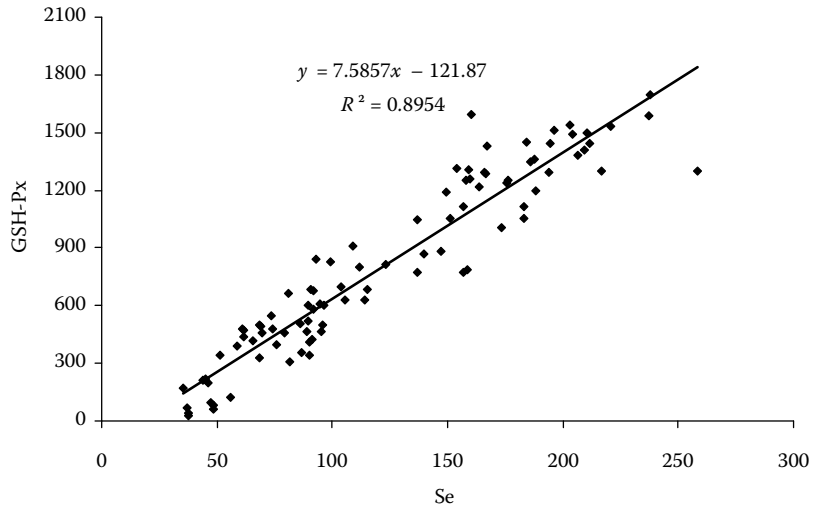

Figure 1. Relation between the selenium concentration (µg/l) and the activity of GSH-Px (µkat/l) in whole blood of sheep – the method of linear regression

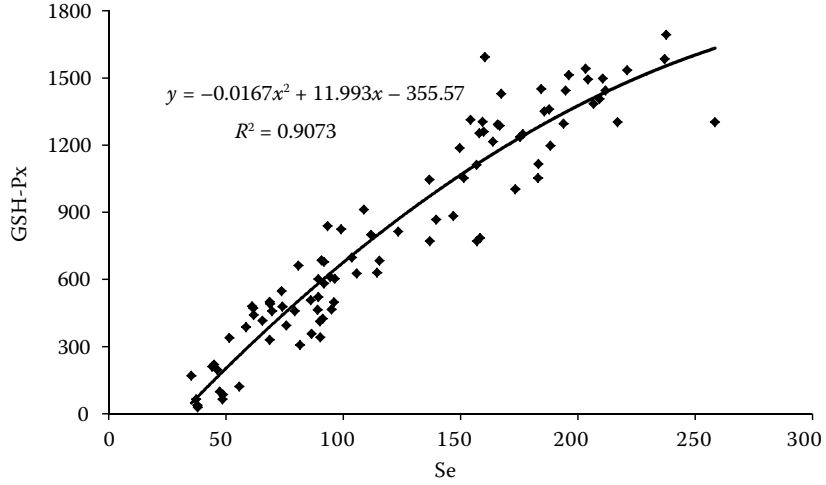

Figure 2. Relation between the selenium concentration (µg/l) and the activity of GSH-Px (µkat/l) in whole blood of sheep – the method of the 2nd degree polynomial regression

Table 2. Evaluation of the selenium status in sheep using the calculated GSH-Px activity in whole blood – values recommended for the evaluation

Se status	Se (µg/l)	GSH-Px (µkat/l)[a]	GSH-Px (µkat/l)[b]
Deficient	< 70	< 409	< 461
Marginal	70–100	409–637	461–677
Adequate	> 100	> 637	> 677

[a]calculated by linear regression
[b]calculated by polynomial regression

DISCUSSION

Our results confirm the existing dependencies between the activity of glutathione peroxidase and the concentration of selenium in whole blood of sheep as described previously by other authors in cattle, goats and camels (Pavlata et al. 2000, 2005; Gerloff 1992; Rahim 2005). We can thus indirectly assess the selenium status in sheep too.

In assessing the selenium status we can use three basic stages of evaluation: adequate (higher than 100 µg of selenium per liter of whole blood), marginal (70–100 µg/l) and deficient (less than 70 µg/l) (Pavlata et al. 2000). Scholz and Stober (2002) described as also adequate a blood level of selenium greater than 100 µg/l. Guard (2008) considers the normal value of selenium in the blood to be more than 120 µg/l, values between 80–120 µg/l as mar-

ginal selenium status, and selenium concentrations lower than 80 μg/l as deficient selenium status.

For practical use, in our laboratory we can recommend the lower limit of the reference value of glutathione peroxidase activity in whole blood of sheep of 600 μkat/l, which is very similar to the values recommended by Pavlata et al. (2000) for cattle (600–700 μkat/l in whole blood).

Ludvikova et al. (2005) defined for the practical establishment of selenium deficiency in horses the marginal level of glutathione peroxidase activity as 200–300 μkat/l in whole blood when the deficiency level for selenium is set as 75–100 μg/l of Se in the blood. Misurova et al. (2009a) found that a selenium concentration of 100 μg/l corresponds with a glutathione peroxidase activity 700 μkat/l in the blood of adult goats and 720 μkat/l in the blood of newborn kids.

It is obvious from the figures that the tightest correlation between glutathione peroxidase activity and selenium is found at Se concentration values up to 70 μg/l. In contrast, the lowest correlation is found with Se concentration values above 200 μg/l. These results confirm was has previously been reported by Neve (2000). He stated that a very significant correlation between glutathione peroxidase activity and Se concentration exists at relatively low levels of selenium. This correlation is less significant with higher Se concentrations and there is even no correlation when Se concentrations are very high. This marginal level is indicative of a situation where the body is supplied with adequate amounts of selenium for the normal function of glutathione peroxidase, and, therefore, a plateau of GSH-Px activity is reached.

Our findings demonstrates that the correlation between GSH-Px activity and selenium concentration in the whole blood of sheep is very close and that GSH-Px activity could be used as a diagnostic tool for the determination of selenium status in sheep. However, when interpreting results we need to take into consideration possible changes in selenium supplementation, the form of selenium supplement used and other factors that could affect the GSH-Px activity. For example, it was found that with different forms of selenium supplementation the speed or growth of activity can differ. Misurova et al. (2009b) reported that sodium selenite and selenium lactate-protein complexs significantly increased Se levels in the whole blood and colostrum of adult goats as compared with the control group. In newborn kids, however, a significant increase in blood Se levels and GSH-Px activity was observed only in the kids of mothers supplemented with sodium selenite. Pavlata et al. (2011b) found in an experiment on goats, that the effects of supplementation with selenite and a lactate-protein selenium complex is similar from the standpoint of the ability to supply the organism with selenium, but that the speed of GSH-Px activity increase was better with selenite, which therefore appears to be a biologically better available form of selenium for the production of glutathione peroxidases – functional biologically active selenium proteins. Other authors, for example, Schrauzer (2000), reported that the utilisation of selenomethionine for GSH-Px synthesis also depends on methionine status. In methionine-deficient rats that were supplemented with selenomethionine GSH-Px activity was lower than in selenomethionine-supplemented animals with adequate methionine supply.

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