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Activity of glutathione peroxidase in cattle blood under different storage conditions

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ABSTRACT

The purpose was to determine glutathione peroxidase (GSH-Px) activities in samples of whole blood of fattening yearlings under different storage conditions. Blood samples from v. jugularis were taken from seven healthy male animals aged 6-8 months into Vacutainer epruvettes. Each sample was separated into nine Eppendorf epruvettes. Four samples from each animal were stored in a refrigerator at 4 °C, while five samples were stored in a freezer at -20 °C. The glutinatione peroxidase activity in samples was determined immediately, and on the first, second, fourth and seventh days after the samples were taken. In samples stored at -20 °C activity was also determined on the 15th day after sample taking. GSH-Px activity was determined by the RANSEL (Randox-Laboratories) method. The average value of enzyme activity increased during the first seven days of measuring in all tested samples ($P>0.05$), although it is pointed out that the increase was lower in the frozen samples. Also, on the fifteenth day, GSH-Px activity in samples stored at -20 °C returned to its starting values ($P<0.05$). The study suggests to determine glutathione peroxidase activity within two days after collection in samples stored at 4 °C, and within 15 days in frozen samples.

Key words: glutathione peroxidase, blood, yearling cattle

Introduction

The measurement of enzyme activity in blood is well established as an integral component of routine clinical diagnosis. Although numerous reports and reviews have appeared in literature, answers to key questions, such as how long and under what conditions enzymes are stable, are still ambiguous and sometimes controversial. Certain

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authors (MITRUKA and RAWNSLEY, 1977; KANEKO, 1980; JONES, 1985) have monitored activity dynamics under different storage regimes.

The purpose of this study, based on previous investigation (HARAPIN, 1996; HARAPIN et al., 2007), was to determine glutathione peroxidase activity in samples of whole blood of fattening yearlings under different storage conditions.

Materials and methods

The activity of GSH-Px in seven samples of whole blood of fattening male calves was examined. The calves were of 6-month-old domesticated Simmental breed. The calves were fed silage, grained grains and commercially prepared food, which contained 10 mg of vitamin E and 0.1 mg of selenium.

Blood samples were taken from the v. jugularis into heparin-treated tubes using the Vacutainer system. Each sample was separated into nine Eppendorf tubes. Four samples from each animal were stored in a refrigerator at 4 °C, while five samples were stored in a freezer at -20 °C. Glutinatione peroxidase activity in the stored samples was then determined on the first, second, fourth and seventh days after the samples were taken. In samples stored at -20 °C, activity was also determined on the 15th day after sample taking. To determine the activity of GLH-Px, the "Ransel" commercial method (Randox Laboratories Ltd., UK) adapted for biochemical autoanalyzer Technicon RA 1000 at a temperature of 37 °C was applied.

Statistical analysis was performed using Statgraphics software version 4.0. Results are presented as mean ± SD. Data were analysed using Student's *t*-test. Significance of mean differences was based on the $P<0.05$.

Results

Table 1. shows values of glutathion-peroxidase in IU/L at 37 °C, obtained from fresh haemolysate in seven control samples of whole blood stored at 4 °C and -20 °C. The presented mean sample values with a standard mean value error (M ± SEM**)** and standard deviation (SD) demonstrate that there are differences at the level of 95% which are statistically significant. The progress of glutathione peroxidase activity is clearly seen in the relative values shown in Fig.1.

Analysis of sample variances stored at 4 °C (F ratio = 6.3; P = 0.001) indicates a significant difference at the level of 95% between individual groups, as it also does in samples stored at -20 °C (F ratio = 3.39; P = 0.021).

Table 1. GSH-Px activity in the blood of fattening calves stored under different regimes

	1st day	2nd day	4th day	7th day	15th day
4 °C		103.7%	109.9%*	114.8%*	
M ± SEM (U/L)	25994 ± 426	26978 ± 549	28577 ± 410	29840 ± 1066	n. d.
SD	1128	1451	1087	2866	
-20 °C		107.1%*	107.1%*	105.4%*	102.8%
M ± SEM (U/L)	n. d.	27125 ± 402	27839 ± 508	27388 ± 361	26732 ± 422
SD		1066	1340	955	1115

*P<0.05; n. d. = not done

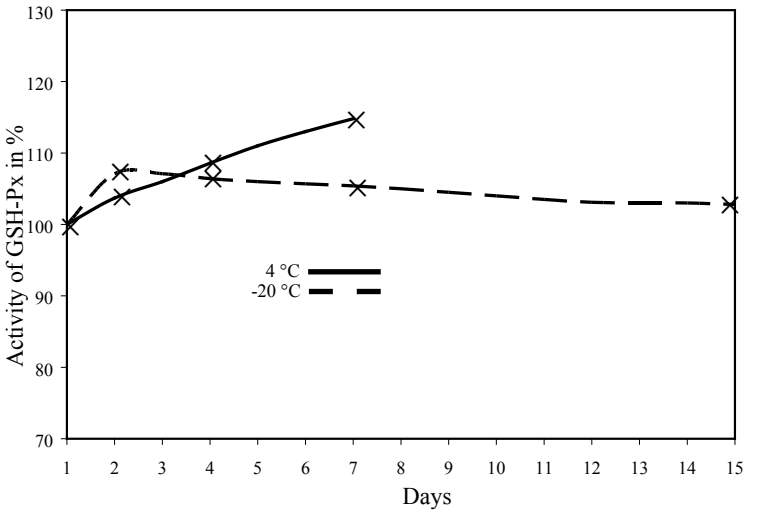

Fig. 1. Activity of GSH-Px in blood of fattening yearlings under different storage regimes

Testing of the average values of samples stored at 4 °C on the 1st and 2nd day yielded no significant differences, whereas testing of average values on the 4th day, as well as on the 7th day, produced a statistically significant difference in relation to the 1st day.

Testing of average values of samples stored at -20 °C resulted in a statistically significant difference between values obtained on the 1st, 2nd and 4th days, and the 7th day, but there was no statistically significant difference in activity between the sample from the 1st and 15th days.

Discussion

WILSON and JUDSON (1976) determined glutathion-peroxidase activity in relation to the quantity of selenium in the whole blood of 80 sheep and 94 cattle, and established that in the erythrocytes frozen at -15 °C, and in whole blood stored at -10 °C, enzyme activity was stable for a period of up to 6 days. LANGLANDS et al. (1980) compared a spot test and the spectrophotometric measuring of glutathion-peroxidase activity. The activity of the measured samples stored at a temperature of between 3 °C and -8 °C remained unchanged, but found that enzyme activity depended on the temperature at which measuring was carried out. HUSSEIN and JONES (1981) measured the activity of glutathion-peroxidase in the whole blood of cattle, goats and horses. They established that sample storage at room temperature (20 °C), or in a refrigerator (at 5 °C), considerably reduced enzyme activity within 3 days, particularly in the blood of horses. AGERGAARD and JENSEN (1982) measured the GSH-Px activity in samples of cattle blood following storage at 22 °C, 4 °C and -22 °C**.** In all samples values increased slightly on the 1st day. On the 2nd day they returned to the initial levels, after which they remained stable for the next 7 days at temperatures of 22 °C and 4 °C, and for about 1 month at a temperature of -22 °C. KOLLER et al. (1984) established that glutathion-peroxidase activity in fattening cattle was less stable and reliable in whole blood than was the concentration of selenium, and that GSH-Px activity in blood remained stable for 7 days at a temperature of 4 °C. DAVIDSON et al. (1990) researched glutathion-peroxidase stability in the plasma of cattle, pigs and sheep, with and without the addition of glutathione to the samples. They established that glutathion-peroxidase activity was decreased at a temperature of 4 °C and 20 °C, and that sheep and cattle enzymes were significantly less stable than those of pigs. The addition of glutathione in a concentration of 2 mmol/L to plasma immediately before storage reduces loss of glutathion-peroxidase activity in both cattle and sheep. The authors recommended that glutathion-peroxidase activity should be measured immediately after samples are taken, or that samples be frozen at -20 °C. JONES (1985) found that GSH-Px was relatively stable in whole blood but, unless frozen rapidly, lost activity approximatly 20% in aqueos lysates. The minimum stability of GSH-Px in whole blood at 4 °C was 28 days and 42

days in frozen samples, but only one day in a 4 °C sample and also 42 days in frozen blood lysate.

The results obtained in our study show a slight increase in GSH-Px activity, probably due to dehydration of the sample. This result corresponds to results achieved at by AGERGAARD and JENSEN (1982). We recomended to determine activity within two days after collection in samples stored at 4 °C it is recommended, and in frozen samples within 15 days.

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SAŽETAK

Određivana je aktivnost glutation-peroksidaze u punoj krvi tovne junadi pri različitim uvjetima pohranjivanja uzoraka. Pretraženi su bili uzorci od sedam zdravih bičića u dobi 6-8 mjeseci. Krv je uzimana iz jugularne vene u Vacutainer epruvete. Svaki od sedam krvnih uzoraka bio je razdijeljen u 9 Eppendorf epruveta. Četiri uzorka od jedne životinje bila su pohranjena u hladnjak na 4 °C, a pet uzoraka u zamrzivač na -20 °C. Aktivnost glutation peroksidaze u uzorcima pohranjenima na 4 °C i -20 °C određivana je netom nakon uzimanja, zatim prvoga, drugoga, četvrtoga i sedmoga dana nakon uzimanja, a u uzorcima pohranjenim na -20 °C još i petnaestoga dana nakon uzimanja krvi. Aktivnost GSH-Px određivana je RANSEL (Randox-Laboratories) metodom. Dobivenim vrijednostima izračunata je srednja vrijednost i analiza varijance. U svim istraživanim uzorcima srednja vrijednost aktivnosti enzima blago se povećala u prvih 7 dana mjerenja, s time da je manje rasla u uzorcima koji su bili zamrznuti. Blagi porast izmjerene aktivnosti može se pripisati dehidraciji pohranjenoga uzorka. U uzorcima pohranjenima na -20 °C aktivnost GSH-Px petnaesti se dan vratila na početne vrijednosti. Istraživanje upućuje na potrebu da se uzorci pohranjeni na 4 °C pretraže unutar dva dana, a zamznuti na -20 unutar 15 dana od vađenja krvi.

Ključne riječi: glutation peroksidaza, krv, tovna junad