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BLOOD GLUTATHIONE PEROXIDASE ACTIVITY, SELENIUM AND VITAMIN E CONCENTRATIONS OF RACE HORSES IN SERBIA

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A survey of blood Se and vitamin E concentrations and GSH-Px and AST activities in breeding mares was conducted on two horse farms located in different regions. Horses at the farms were kept on different feeding regimes. There were no significant differences in meann blood plasma Se concentrations in the horses on the farms (73,3 and 72.1 μg/l, respectively), although they were fed diets containing different Se concentrations. High correlation coefficients (0.84) were found between blood GSH-Px activies and plasma Se concentrations in horses at both farms. Horses fed the diet with supplements rich in vitamin E (Ljubičevo horse farm) had several times higher plasma vitamin E concentrations (40.4 mg/l) in comparison to horses fed unsupplemented feed (2.9 mg/l). Plasma AST activities in horses on both farms were slightly below physiological levels.

Key words: Horse, selenium, vitamin E, GSH-Px, blood plasma.

INTRODUCTION

Selenium deficiences in domestic animals have been demonstrated to occur in parts of the world where soils are low in selenium and most frequently on farms using locally grown feeds. Analyses have shown that feedstuffs of Serbian origin are low in Se (Mihailović et al., 1992). Low Se levels in the blood of horses have been associated with nutritional muscular dystrophy (N. M. D.), myocardial failure, or "tying-up" syndrome (Dill and Rebhun, 1985), subclinical hepatic insufficiency and a number of reproductive disorders in the mare including pyometritis, repeat breedings and sudden death in foals (Maylin et al., 1980). Blackmoore et al. (1979) showed that thoroughbreds with unsatisfactory performance had low selenium levels.

Se, as an integral component of the enzyme GSH-Px, plays an importannt role in the prevention of cellular membranes from oxidant damage by toxic lipid peroxides and hydrogen peroxides (Sunde and Hoekstra, 1980). Caple et al. (1978) demonstrated a very highly significant correlation between blood GSH-Px activity and Se concentration in horses Moreover, the enzymic activity is a useful

indicator of Se status, although relationships between Se status and disease in farm animals are still far from certain.

This study was carried out to investigate blood Se and vitamin E concentrations and GSH-Px activity of breeding mares on farms located in two regions in Serbia and held on differennt feeding regimes.

MATERIALS AND METHODS

The two biggest farms (among several in Serbia) were chosen for this survey of blond Se, vitamin E and GSH-Pe. The farms were located in different regions and the mares were fed different diets.

Mares (24 Thoroughbred) in Ljubievo, located about 80 km south from Belgrade, were fed local feedstufts (alfalfa hay, and oats). They received daily supplements of wheat germ (30 g) and vitamin-mineral mixture (Kostovit[1], 20-25 g and Muvisel[2], 3 g). Mares were treated (i/m) with 20-30 ml of Vitoligram[3] monthly. A month before insemination, the supplement of wheat germ was enhanced to 100 g daily. Mares (10 Thoroughbred and 6 Halfbred) in Bečej, located about 130 km north from Belgrade, were fed a pelleted diet prepared from local feedstuffs and localy grown oats.

Heparinized blood samples were taken by jugular vein venipuncture from mares in the middle of March. Whole blood was used for GSH-Px activity determination immediately after the collection according to the method of Günzler et al. (1978). Plasma samples for Se and α-tocopherol determination were stored at - 20°C until they were analyzed. Plasma Se content was determined by a fluorometric method (Lindberg, 1968), using ^{75}Se to count the recovery percentage in each sample. The content of plasma α-tocopherol was determined by a fluorometric micro method (Hansen and Warwick, 1966).

RESULTS AND DISCUSSION

Selenium content in feedstuffs. Selenium contents in the oats were 37.6 μg/kg (Ljubičevo) and 46.9 μg/kg (Bečej); in hay, 53.0 μg/kg and in the pelleted feed 95.7 μg/kg (Table 1). The low Se contents found in grain and roughages is in good agreement with our previous data showinng that feedstuffs grown in Serbia are poor in Se (Mihailović et al., 1992).

[1]Contained the following amounts per kg: Vitamin A, 500,000 IU; vitamin D3, 50,000 IU; vitamin E, 500 mg; vitamin K3, 100 mg; vitamin B1, 50 mg; vitamin B2, 200 mg; pantothenic acid, 400 mg; nicotinic acid, 1,200 mg; vitamin B12, 400 ug; choline chloride, 35.750 mg; CaHPO4 2H2O), 120 g; NaCl, 50 g; CuSO4 5H2O, 300 mg; FeSO4 7H2O, 3g; CoSO4, 74 mg; ZnSO4 7H2O), 2,2 g; KIO3, 36 mg; Mn So4 H2O, 3.6 g; MgSo4 7H2O, 3g.

[2]Contained the following amounts per kg: Vitamin A, 20,000 IU; vitamin D3, 1,000 000 IU; vitamin C,20 g; vitamin B5, 18 g; vitamin B3, 0.8 g; vitamin E, 5.25 g; vitamin B2, 2.5 g; vitamin B6,2.0 g; vitamin K3, 1.75 g; vitamin B1, 1.5 g; folic acid, 0.4 g; vitamin B12, 7.5 mg; Se, 33 mg.

[3]Contained the following amounts per ml of solution: Vultamin A, 20,000 IU,; vitamin B1, 1 mg; vitamin B2, 0.1 mg; vitamin B3, 4 mg; vitamin B6, 1 mg; vitamin B7, 1 mg; vitamin B9, 0.04 mg; vitamin B12, 0.05 mg; vitamin C, 10 mg;vitamin D2, 20,000 IU; vitamin D3, 20,000 IU; vitamin E,10 mg; choline, 4 mg; Lysine, 4 mg; Methionine, 4 mg; Thyptophan, 0.5 mg.

Table 1. Selenium content in feedstuffs (μg/kg).

Farm	Oats	Alfalfa hay	Pelleted feed
Ljubičevo	37.6	53.0	–
Bečej	46.9	–	95.7

Blood Se, GSH-Px, AST and vitamin E. The results for Se, GSH-Px, AST and vitamin E are shown in Table 2. We determined Se in plasma, but the Se content of blood plasma and whole blood is about the same (Bergsten et al., 1970). The plasma Se concentrations of mares on both farms were below 90.5 μg/1 (39.9 - 90.5 μg/l). Only one mare (Ljubičevo) had plasma Se below 50.0 μg/l.

Table 2. Whole blood GSH-Px activity, plasma AST activity, and plasma Se annd α-tocopherol concentration of mares.

Farm	Blood GSH-Px (μkat/l)	Plasma Se (μg/l)	Plasmatocopherol (μg/l))	Plasma AST (U/l)
Ljubčevo	320.6 ± 42.6 (24)	73.3 ± 15.0 (9)	40.4 ± 31.9 (9)	202.7 ± 33.7 (9)
	(265.1 ± 424.2)	(39.9 – 90.5)	(5.4 – 89.0)	(145 – 255)
Bečej	301.9 ± 69.4 (16)	72.1 ± 8.4 (6)	2.9 ± 1.8 (6)	163.5 ± 26.7 (6)
	(169.7 – 445.5)	(58.4 – 86.1)	(1.5 – 6.3)	(123 – 186)
	P > .05	P > .05	P < .001	P < .05

The categorization of whole blood Se concentrations into ranges that can be associated with adequate, marginally deficient or deficient conditions in the horse is not well established (Carmel et al, 1990; Baalsrud and Overnes, 1986; Gabbedy and Richards, 1970; Schougard et al., 1972; Andersonn et al., 1978; Higuchi et al., 1989; Willson et al., 1976). For this survey, plasma Se concentrations greater than or equal to 0.1 mg/l were considered adequate; levels less than 0.1 mg/l but above 0.05 mg/l were considered marginally deficient; Se levels below 0.05 mg/l were considered deficient (Carmel et al., 1990; Baalsrud and Overnes, 1986). Thus, mares at both farms, except one in Ljubičevo (with a plasma Se of 39.9 μg/l) were marginally deficient. During long term observation (two years) we did not register any clinical case of muscular dystrophy in mares or foals at the farms.

We did not find any significant difference between plasma Se concentrations of mares at the two farms (73.3 and 72.1 μg/l) although they were fed rations with different cotents of Se (145 μg/kg in Ljubičevo and 71.3 μg/kg in Bečej). When the plasma Se values are related to the Se content in the ration at the farm in Bečej, it appears that 72.1 μg/l plasma corresponds to 71.3 μg/kg feed. This indicates that the correlation between blood Se and Se in the feed is close. Similar results were found by Bergsten et al. (1970). However, the Se content of the ration for mares in Ljubičevo (145 μg/kg) was much higher than the mean plasma Se level (73.3 μg/1). The reason for this might be that the Muvisel supplement which was available for horses at this farm, was not well homogenized inn the ration and some selenium was lost. Namely, selenium availability im the ration,as fed, may have been hauer.

Simitarly to plasma Se concentration, there was no significant difference in whole blood GSH- Px activities between horses at the two farms (320.6 and 301.9 μkat/l).

In earlier studies a high correlation between whole blood Se and GSH-Px activity in horses was reported (Caple et al., 1978; Maylin et al., 1980; Roneus, 1987. In the ranges of plasma Se levels from 39.9 to 90.5 μg/l and GSH-Px activities from 169.7 to 445.5 μkat/l a significant cerrelation between these two parameters in mares at the two farms was found (Figure 1). Higuchi et al. (1989) revealed a similar correlation between whole blood GSH-Px activity and plasma Se level.

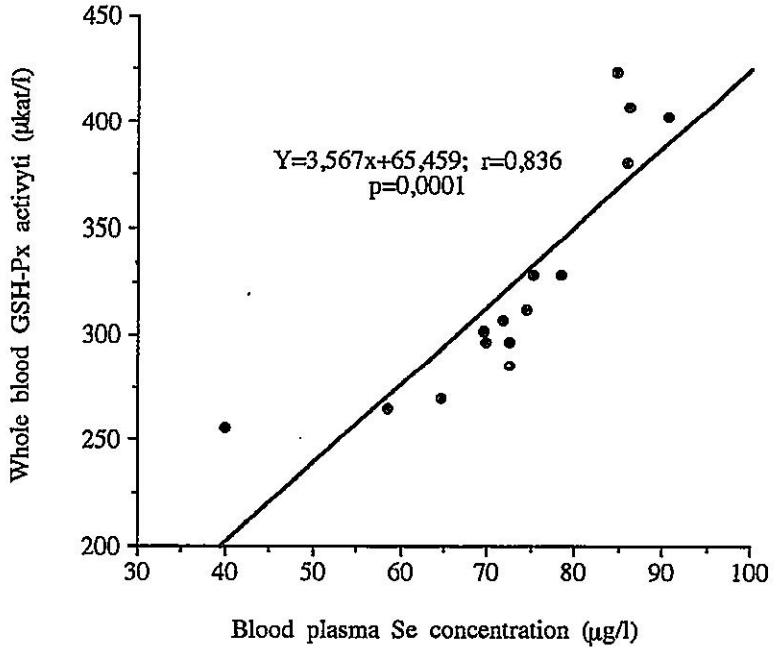

Figure 1. Relationship between whole blood GSH-Px activity and plasma selenium concentration.

Plasma AST activites of mares at both farms (202.7 and 163.5 U/l, respectively) were slightly below the physiological level for horses (226-366 U/l (Kaneko, 1989). An increase in serum enzyme activity (AST, CPK and LDH) was often used for diagnosis of N. M. (Higuchi et al., 1989).

The mean plasma tocopherol level of 6 mares in Bečćej was 2.9 $\pm$ 1.8 mg/1. Two mares (33%) had plasma tocopherol below 2,0 mg/l (the level considered as normal). Higuchi et al. (1989) found low levels of serum tocopherol (below 2.0

mg/l) in mares of foals affected with N. M. D. We did not register any case of N. M. D. in foals at this farm. The mean plasma tocopherol level of mares in Ljubičevo (40.4 ± 31.9/mg/l) was much higher than in mares in Bečej. The high plasma tocopherol level in the mares might be the result of supplemental vitamin E. During the last two years at this farm three cases of mares with twins were registered.

The findings in this study suggest that there is risk of incidence of N. M. D. in foals on horse farms i Serbia due to a deficiency of Se and tocopherof. For prevention of N. M. D. an adequate supplay of Se and tocopherol should be given to pregnant mares.

REFERENCES

1. *Anderson, P. H., Patterson, D. S. P., annd Barret, S.* 1978. Seleium deficiency. *Vet. Rec. 103,* 145-146.
2. *Baalsrud, K. J. and Overnes, G.* 1986. Influence of vitamin E and selenium supplement on antibody production in horses. Equine. *Vet. J. 6,* 472-474.
3. *Bergsten, G., Holmback, R. and Lindberg, P.* 1970. Blood selenium in naturally fed horses and the effect of selenium administration. *Acta Vet. Scand. 11,* 571-576.
4. *Blackmoore, D. J., Wilett, K. and Agness, D.* 1979. Selenium and gamma-glutamyl transferase activity in the serum of thoroughbreds. *Res. Vet. Sci. 26,* 76-80.
5. *Caple, I. W., Edwards, J. A., Forsyth, W. M., Whiteley, P., Selth, R. H. and Fulton, Lj.* 1978. Blood glutathione peroxidae activity in horses in relationn to muscular dystrophy and selenium nutrition. *Aust. Vet. J. 54,* 57-60.
6. *Carmel, D. K., Crisman, M. V., Ley, W. B., Irby, M. H. annd Edwards, G. H.* 1990. A survey of whole blood selenium concentration of horses in Maryland. *Cornell Vet. 80, 251-258.*
7. *Dill, S. G. and Rebhun, W. C.* 1985. White muscle disease in foals. Comp. Cont. *Ed. Pract. Vet. 11,* 627-635.
8. *Gabbedy, B. J. and Richards, R. B.* 1970. White muscle disease in a foal. *Aust. Vet. J.* 46, 111-112.
9. *Günzler, W. A, Kremers, H. and Flohe,* F. 1974. An improved coupled test procedure for glutathione peroxidase (EC 1.11.1.9) in blood. *Z. Klin. Chem. Biochem. 12,* 444-448.
10. *Hansen, L. G. and Warwick, W. J.* 1966. A fluorometric micro method for serum tocopherol. *Amer. J. Clin. Path. 46,* 133-138.
11. *Higuchi, T., Ichijo, S., Osame S. ad Ohishi, H.* 1989. Studies on serum selenium and tocopherol in white muscle disease of foal. *Jpn. J. Vet. Sci. 51,* 52-59.
12. *Kaneko, J.* 1989. Clinical biochemistry of domestic animals. *4th ed. Academic Press. London. p.* 886.
13. *Lindberg, P.* 1968. Selenium determination in plant and aimal material, and in water. *Acta Vet. Scand. Suppl. 23,* 1-41.
14. *Lindholm, A and Asheim, A.* 1973. Vitamin E and certain muscular enzymes in the blood serum of horses. *Acta Agric. Scannd. 19,* 40-49.
15. *Maylin, G. A., Rubin, D. S. and Lein, D.H.* 1980. Selenium and vitamin E in horses. *Cornell Vet. 70,* 272-289.
16. *Mihailović, M., Lindberg, P., Jovanović, I. and Tešić, M.* 1992. Sadržaj selena u hranivima uzgajanim na teritoriji Republike Srbije. V. Savetovanje veterinnara Srbije. *Kopaonik, 2-5 Septembar. Kratki sadržaji radova, 178.*
17. *Roneus, B.* 1982. Glutathione peroxidase and selenium in the blood of healthy horses and foals affected by muscular dystrophy. *Nord. Vet. Med. 34,* 350-353.
18. *Schougard, H., Basse, A, Gissel-Nielsen, G. and Simesen, M. G.* 1972. Nutritional muscular dystrophy (NMD) i foals. *Nord. Vet. Med. 24,* 67-84.

19. *Sunde, R. A. and Hoekstra, W. G.* 1980. Structure, synthesis and function of glutathion peroxidase. *Nutr. Rev. 38,* 265-273.

20. *Willson, T. M., Morrison, H. A., Palmer, N. C., Finley, G. G. and Breumel,* A. A. 1976. Myodegeneration and suspected selenium/vitamin E deficiency in horses *J. Amer. Vet. Med. Assoc. 169,* 313-217.

AKTIVNOST GLUTATION PEROKSIDAZE, KONCENTRACIJE SELENA I VITAMINA E KOD TRKAĆIH KONJA U SRBIJI

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SADRŽAJ

Određivai su aktivnost glutation peroksidaze u krvi i koncetracije selena i vitamina E u plazmi kod kobila u reprodukciji u dve ergele trkaćih konja. Za ispitivanja su odabrane dve najveće ergele u Srbiji locirane u dva različita regiona i držanim na različitim režimima ishrane. Kobile u ergeli Ljubičevo hranjene su senom i ovsom lokalnog porekla uz dnevne dodatke pšeničnih klica i vitaminsko mineralnih smeša (Kostovit i Muvisel). Kobile su jedanput mesečno bile tretirane (i/m) sa Vitoligamom. Kobile u ergeli Bečej su bile hranjene peletiranom hranom i ovsom lokalnog porekla. Mada su kobile u ergeli Ljubičevo hranjene hranom koja je sadržavala znatno veću količinu Se (zbog dodatka Muvisela) njihove koncenacije Se u plazmi i aktivnosti GSH-Px u krvi nisu se značajno razlikovale od onih kod kobila u ergeli Bečej. Razlog ovome može biti što dodatak Muvisela kobilama u Ljubičevu nije bio dobro homogenizovan u hrani te je ostao nepojeden. Ustanovljena je signifikantna korelacija između aktivnosti GSH-Px u punoj krvi i koncentracije selena u krvnoj plazmi kod kobila u obe ergele. Koncentracije vitamina E u plazmi kobila u ergeli Ljubičevo bile su višestruko veće zbog visokog sadraja vitamina E u dodacima hrani (pšenične klice, Kostovit i Muvisel) i tretmana sa Vitoligamom (jedanput mesečno). Aktivnost AST u plazmi u kobila iz obe ergele bile su neznatno ispod fizioloških vrednosti za konje.