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Effect of Se source and dose on selenomethionine and selenocysteine levels in blood and plasma of mature horses

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ABSTRACT - The study comprised 25 mature horses and incorporated five dietary treatments; a negative control (C: 0.085 mg Se/kg DM), 3 levels of Se yeast supplementation, obtained from Saccharomyces cerevisiae CNCM I-3060 (OS2, OS3 and OS4: 0.2, 0.3 and 0.4 mg Se/kg DM respectively) and one positive control supplemented with Na selenite (IS3: 0.3 mg Se/kg DM). Diets were offered for 112 days. Total Se, proportion of total Se comprised as selenomethionine (SeMet) or selenocysteine (SeCys) of pooled samples of blood and plasma for each treatment at d 0.56 and 112 were determined. Total Se and SeCys increased both in blood and plasma during the trial in all treatments supplemented with Se; these increases were proportional to the level of dietary Se supplementation. The SeMet increased only in treatments supplemented with Se yeast, with increases proportional to the level of dietary Se supplementation. In Se yeast horses the proportion of total Se comprised as SeMet accounted for 20 and 14% of total Se increase in blood and plasma respectively; in IS3 only accounted for 5 and 3% respectively. These results seem support the view that SeMet is a non-specific form of Se that is metabolized as a constituent of the methionine pool, and can be considered as a storage form of Se in higher animals.

Key Words: Organic selenium, Selenomethionine, Selenocysteine, Horse.

Introduction - Selenium supplements can be in two forms, inorganic (usually sodium salts of selenite or selenate) or organic forms (Se-yeast), which have recently been authorized within the EU as a feed additive. The two forms of dietary Se supplements are metabolized differently (Surai, 2006). Selenomethionine is actively transported through intestinal membranes during absorption and non-specifically incorporated into tissue proteins (Schrauzer, 2003). In contrast, inorganic Se is passively absorbed as a mineral and little is retained in tissue reserves. Furthermore, Se from inorganic supplements is almost exclusively used for the production of selenoenzymes, whereas organic Se can either be used to produce selenoenzymes or be present in all the proteins containing methionine (Weiss, 2005). The aim of the study was to evaluate the effects of both source (Se yeast vs. sodium selenite) and dose (Se yeast only) of dietary selenium, on the selenomethionine and selenocysteine levels in blood and plasma of mature horses.

Material and methods - The study comprised 25 mature horses (mean age 13.6±4.79 years) sourced from the same herd. The study was of a randomised complete block design and comprised one continuous period of 112 days in which all animals received the same basal diet (on average 6 kg/head per day of grass hay and 3 kg/head per day of concentrate) that differed only in either Se source (organic vs. inorganic) or concentration. Organic Se was obtained from Saccharomyces cerevisiae CNCM I-3060 containing 63% of SeMet and 34-36% of low molecular weight seleno-components (Sel-Plex®). Inorganic Se was provided from sodium selenite. No Se supplements were used in the two month covariate period preceding the start of the study,

during which all horses received the same basal diet. At the end of the covariate period animals were blocked by live weight, gender, age and activity and randomly allocated to one of five dietary treatments. Treatments comprised a negative control (C: background Se only), 3 different levels of Sel-Plex® supplementation (OS2, OS3 and OS4; 0.77, 1.62 and 2.47 mg Se/head per day respectively, to achieve final dietary Se concentrations of 0.2, 0.3 and 0.4 mg/kg DM respectively); one positive control supplemented with sodium selenite (IS3: 1.62 mg Se/head per day to achieve a final dietary Se concentration of 0.3 mg/kg DM). Selenium supplements were offered daily to each horse using a specific premix. Blood samples were obtained before the morning feed (08:00) at d 0, 56 and 112. At each time, blood samples were collected from the jugular vein via venepuncture into two 10-mL Li-heparin treated tubes (Monovet tube Se-free, Sarstedt, Princeton, NJ). Blood samples were immediately placed into an ice-bath prior to processing. The first Li-heparin treated tube of whole blood was pooled with respect to treatment and sampling date (T_0, T_{56} and T_{112}) and freeze dried prior to the determination of total Se and the proportion of total Se comprised as selenomethionine (SeMet) and selenocysteine (SeCys) according to the method of Bierla et al. (2008). The second Li-heparin treated tube was centrifuged (3500 x g for 15 min at 10°C) and the plasma fraction decanted. Plasma samples were pooled and subjected to the determination of the same parameters measured in whole blood. A monthly pooled sample of diets and their compositional ingredients were analyzed to determine Se content (ICP-MS) and to calculate metabolizable energy content (ME). The quantity of feed offered to each individual horse was recorded daily and monthly nutrient intakes were calculated from these data. Results were processed using linear and multiple regressions (SAS Version 9.1, SAS Inst. Inc., Cary, NC), where measured blood parameters were the dependent variable whereas time and Se dose were the independent variables.

Results and conclusions - The mean background Se content of the diet was 0.085 mg/kg DM, whereas the Se content during the same period for treatments OS2, OS3, OS4 and IS3 were 0.181, 0.290, 0.395 and 0.288 mg/kg DM respectively. The mean crude protein content of the diets was 121.6±12.40 g/kg DM and the mean calculated ME content of the diets was 2.16 Mcal/kg DM with values ranging between 2.12 and 2.20 Mcal/kg DM.

Total Se in blood and plasma measured at T_0 in all treatments, and in the control group (C) during the trial, are indicative of marginal Se status. As expected, the total Se content of blood and plasma increased during the trial in all treatments supplemented with Se (Table 1). A dose effect was observed and the increase of total Se during the trial, both in blood and plasma, was proportional to the level of Se supplementation. Although no source effect was apparent for total Se in blood and plasma, due to limited replication, it should be noted that values of total Se observed on d 56 and d 112 were numerically higher in those animals receiving organic Se sources when compared to those receiving a comparable dose of selenite (OS3 vs IS3).

Selenocysteine was the predominant Se species in blood and plasma and accounted for, on average, 79.1 and 71.4% of total Se in blood and plasma respectively, whereas Se comprised as SeMet accounted for, on average, 15.2 and 10% of total Se in blood and plasma respectively.

The proportion of Se comprised as SeCys in blood and plasma increased in all treatments supplemented with Se. In both blood and plasma increases of SeCys were proportional to the level of Se supplementation within the diet. Although no source effect was apparent, due to limited replication, SeCys values were marginally higher in the plasma of OS3 animals when compared to those receiving a comparable dose of selenite (IS3). In both blood and plasma the proportion of Se comprised as SeMet increased in treatments supplemented with Se yeast, increases being proportional to the level of Se supplementation. The SeMet in OS2 at d 112 was 1.5 times the value observed at d 0, and in OS4 was 2.5 times. There was only a very slight increase in the proportion of total Se comprised as SeMet in the blood and plasma of IS3 animals. In Se yeast horses the proportion of total Se comprised as SeMet accounted for 20 and 14% of total Se increase in blood and plasma respectively; in IS3 accounted only for 5 and 3% respectively. These increases in the SeMet content of blood and plasma in animals supplemented with Se yeast were probably the consequence of: i) SeMet of Se yeast being absorbed non-specifically within the intestinal tract; ii) SeMet remaining intact and thus available for protein synthesis in place of methionine. Burk et al. (2001) demonstrated that Se from SeMet, but not from selenate or selenocysteine, can be incorporated into albumin, presumably as

SeMet within the methionine pool. Conversely, in horses supplemented with selenite the SeMet in plasma did not increase, probably because higher animals have no efficient mechanism for Met synthesis and are also unable to synthesize SeMet (Schrauzer, 2003).

Table 1. Total Se and proportion of Se comprised as SeMet and SeCys (ng Se/g dry sample).

Item		Time[1]	C	OS2	OS3	OS4	IS3	Linear dose effect, per mg of Se/kg DM2
Blood	Total Se	T_0	590	630	670	610	570	
	(ng Se/g)	T_{56}	600	790	980	1090	860	1389***±347
		T_{112}	840	960	1140	1240	980	
	SeMet	T_0	80	90	81	95	94	
	(ng Se/g)	T_{56}	84	121	146	165	104	271***±66
		T_{112}	92	140	198	250	124	
	SeCys	T_0	419	465	461	494	466	
	(ng Se/g)	T_{56}	511	604	754	888	694	876***±237
		T_{112}	496	707	901	830	836	
Plasma	Total Se	T_0	1030	1170	1130	1100	1090	
	(ng Se/g)	T_{56}	890	1510	1890	2150	1640	2805***±773
		T_{112}	840	1600	2100	2100	1760	
	SeMet	T_0	106	110	96	104	110	
	(ng Se/g)	T_{56}	106	158	158	240	117	279***±87
		T_{112}	105	197	215	250	147	
	SeCys	T_0	797	810	809	783	763	
	(ng Se/g)	T_{56}	704	1043	1446	1451	1246	1745***±552
		T_{112}	622	1151	1437	1446	1066	

**: P<0.10; **: P<0.05; ***: P<0.01. [1]T_0 to T_{112} = at d 0 to 112 from the start of the Se supplementation period. [2]Includes OS2, OS3, and OS4.*

In conclusion, selenite and Se yeast supplementation increased total Se and SeCys in blood and plasma with increases proportional to the level of dietary Se supplementation, but only Se yeast increased SeMet in blood and plasma. These results seem support the view that SeMet is a non-specific form of Se that is metabolized as a constituent of the methionine pool. Consequently SeMet can be considered as a storage form of Se in higher animals and can contribute to endogenously synthesized selenoproteins, particularly during periods of low Se supply.

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