Effect of Dietary Supplementation with Vitamin E and Selenium in Thoroughbred Horses on the Concentration of F_2-isoprostanes in the Blood Plasma as a Marker of Lipid Peroxidation

H. HÄRTLOVÁ[1], R. RAJMON[1], A. DÖRFLEROVÁ[1], L. ZITA[1], D. ŘEHÁK[2], J. ROSMUS[3], M. ŠINDELÁŘ[4], P. KLABANOVÁ[1]

[1]Czech University of Life Sciences Prague
[2]Institute of Animal Science Prague-Uhříněves
[3]State Veterinary Institute Prague
[4]Private veterinary surgeon

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Abstract

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The objective of this study was to assess the effect of vitamin E and selenium (Se) supplementation on the plasma levels of F_2-isoprostanes as a marker of oxidative stress in horses in their training period. Twelve healthy 3-year-old English thoroughbred horses were divided into two groups: control (n = 6) and experimental (n = 6). Feeding rations were adapted to a moderate workload. The horses of the experimental group received supplements of DL-α-tocopheryl acetate E (2 250 mg/day/horse) and of sodium selenite (0.5 mg/day/horse). The plasma concentrations of both antioxidants and F_2-isoprostanes were monitored on days 0, 44 and 70. After 70 days of supplementation, the concentrations of selenium in the experimental group were significantly higher ($P < 0.05$) compared to the beginning of the experiment (mean ± SE: 135.81 ± 10.19 $\mu g \cdot l^{-1}$ vs. 98.70 ± 10.88 $\mu g \cdot l^{-1}$), as well as to the control group (day 0: 101.78 ± 11.06 $\mu g \cdot l^{-1}$, day 70: 108.18 ± 7.77 $\mu g \cdot l^{-1}$). In the horses of the experimental group, plasma α-tocopherol levels significantly increased from the 44th day of supplementation compared to the beginning of the study as well to the control group (5.23 ± 0.52 $mg \cdot l^{-1}$ vs. 2.45 ± 0.25 $mg \cdot l^{-1}$ or 3.46 ± 0.34 $mg \cdot l^{-1}$, respectively). The plasma concentration of F_2-isoprostanes tended to be lower in the experimental group at the end than at the beginning of monitoring (156.8 ± 12.89 $pg \cdot ml^{-1}$ vs. 170.3 ± 60.8 $pg \cdot ml^{-1}$), although the control group showed the opposite trend (181.2 ± 15.67 $pg \cdot ml^{-1}$ vs. 137.0 ± 47.05 $pg \cdot ml^{-1}$). Nevertheless, none of these differences were significant because of the large variability of the individual values. It can be stated that supplementation of the diet used with selenium and vitamin E caused a non-significant decrease of F_2-isoprostane concentration in the blood plasma only, and a significant increase of plasma concentrations of these antioxidants. The variation of isoprostane levels probably reflected rather the individual responses of the horses' organisms to the training workload.

Horse, free radicals, exercise, antioxidants, nutrition

Excessive production of free radicals, reactive oxygen species (ROS), is dangerous to the organism because these species attack nucleic acids and proteins, degrade carbohydrates and cause lipid peroxidation in cell membranes (Sen 1995). The organism is protected against overproduction of ROS by an effective antioxidant defence system (Sen 1995; Sacheck and Blumberg 2001; Deaton et al. 2004; Kinnunen et al. 2005). Vitamin E and selenium are considered to be the key antioxidant components in the feed ratio. An insufficient intake of either of these substances leads to nutritional myodegeneration, namely in the foals (Löfstedt 1997). Recently, Ludvíková et al. (2005ab) confirmed a negative correlation between plasma vitamin E and selenium levels and myopathy occurrence at particular horse farms, as well as poor blood level of these antioxidants in horses at Czech horse farms in general.

Address for correspondence:
MVDr. Helena Härtlová, CSc.
Department of Veterinary Science
Faculty of Agrobiology
Food and Natural Resources Czech University of Life Sciences Prague
165 21 Prague 6, Czech Republic

Phone: +420 224 382 799
Fax: +420 234 381 841
E-mail: hartlova@af.czu.cz
http://www.vfu.cz/acta-vet/actavet.htm

An adequate intake of vitamin E and selenium is important for maintaining membrane integrity during exercise (Bejma and Ji 1999; Williams et al. 2003, 2004; Konda at al. 1998). Intensive exercise generates free radicals, and without an adequate rest period this may cause accumulation of these dangerous substances (Chiaradia et al. 1998). Strenuous training on a daily basis may increase the rate of lipid peroxidation and protein oxidation, leading to an inflammatory reaction and muscle disease (Kinnunen et al. 2005; Sacheck and Blumberg 2001). The protective role of antioxidant supplementation against excessive oxidative damage of trained horses was confirmed (Avellini et al. 1999; White et al. 2001; de Morffarts et al. 2005).

F_2-isoprostanes can be used as a marker of oxidative injury. They are isometric to prostaglandin $F_{2\alpha}$ derived from cyclooxygenase reaction but they are produced by free radical-induced peroxidation of lipoprotein arachidonic acid in cellular membranes independently of cyclooxygenase activity *in vivo* (Morrow et al. 1990; Roberts and Morrow 2002). F_2-isoprostanes are associated with oxidant injury and appear to be a much more specific biomarker of lipid peroxidation than thiobarbituric acid-reactive substances (Meydani 1997; Reckelhoff et al. 1998). The measurement of F_2-isoprostanes was found to be one of the most suitable approaches to assessing the oxidative stress status *in vivo* (Montuschi et al. 2004).

The objective of this study was to assess the effect of supplementation with vitamin E and selenium on the plasma levels of F_2-isoprostanes as a marker of oxidative stress in the thoroughbred horses during the first half of the training period.

Materials and Methods

A total of 12 English thoroughbred horses 3 years old, weighing 500 ± 30 kg, were used in our study. Each of these horses had been in a regular training programme prior to the commencement of the study and was considered physically fit. The training workload was moderate. The study was performed during a 70-day period from March to May. Feeding rations used in the experiment were related to a moderate workload (Table 1). Analysis of the feeds, including vitamin E and selenium contents, was performed at the State Veterinary Institute in Prague using the same methods as in the case of plasma examination.

Six horses of the experimental group received supplements of DL-α-tocopheryl acetate and sodium selenite in the grain feed - 2 250 mg/horse/day and 0.5 mg/horse/day, respectively. The feeding doses contained 2806.6 mg of vitamin E and 3.38 mg of selenium per horse and day in the experimental group, in the control group it was 556.6 mg of vitamin E and 2.88 mg of Se (Table 2).

Table 1. Composition of the basic diet

Ingredient	kg/day
Hay	6
Oat	5
Pellets	2.5
Bran	0.5
Oil	0.2
Salt block	0.08

Blood samples were collected at the beginning of the experiment (day 0) and then on days 44, and 70. Peripheral venous blood was collected at 08:00 h, i.e., 1.5 h after feeding, in heparinized polyethylene tubes (Vacutainer system), and kept on crushed ice to ensure complete inhibition of cyclooxygenase enzymes. The samples were immediately transferred to the laboratory, where blood plasma was separated by centrifugation at 1930 × *g* for 10 min and immediately stored at -70 °C until analysis.

Selenium plasma concentrations (Se) were measured by atomic absorption spectrophotometry (AAS) by Varian SpectrAA 220Z with generation of hydrides in biological samples after mineralization, in accordance with Bye (1989). The vitamin E status was assessed by measuring plasma levels of α-tocopherol by reverse-phase high-presure liquid chromatography with fluorimetric liquid detection (Zonta et al. 1982) using the Waters 2695 with fluorescent detector Waters 2475. F_2-isoprostanes were determined by ACE™ Elisa kit (Cayman Chemical Company). The analyses were performed in cooperation with the State Veterinary Institute in Prague in their laboratories.

Table 2. Nutrient contents analyzed in the basic and supplemented diets (b.d., s.d.) and corresponding NRC (1989) values

Nutrient	Day	NRC (1989)
Dry matter (kg)	12.673	12.195
N-subst. (g)	1105.4	1059.6
DE (MJ)	150.7	157.9
Se (mg) – b.d.	2.88	0.5 – 1
Se (mg) – s.d.	3.38	
Vit. E (mg) – b.d.	556.6	650
Vit. E (mg) – s.d.	2806.6	

The statistical analysis of the data on variables was performed by two-way analysis of variance with treatment and time interactions using the GLM procedure of SAS (SAS Institute Inc. 2003).

Results

At the beginning of the experiment, no differences between concentrations of selenium, α-tocopherol, and F_2-isoprostanes (Table 3) were found in the horses of either the experimental or the control group.

Table 3. Effects of selenium and vitamin E supplements on the concentrations of selenium, α-tocopherol and F_2-isoprostanes in the blood plasma

Indicator	Time (day)	n	Control group	Experimental group	Main effects (Multiple-factor ANOVA)		
			LS mean	LS mean	Time	Treatment	Time vs. Treatment
Selenium μg·l^{-1}	0	4	101.78$^{a, 1}$	98.70$^{a, 1}$	NS	**	NS
	44	4	95.54$^{a, 1}$	121.60$^{a, b, 1,2}$			
	70	6	108.18$^{a, 1}$	135.81$^{b, 2}$			
α-tocopherol mg·l^{-1}	0	6	2.81$^{a, 1}$	2.45$^{a, 1}$	**	**	**
	44	6	3.46$^{a, 1}$	5.23$^{b, 2}$			
	70	6	3.80$^{a, 1}$	4.94$^{b, 2}$			
Isoprostanes pg·ml^{-1}	0	6	137.0$^{a, 1}$	170.3$^{a, 1}$	NS	NS	NS
	44	6	147.3$^{a, 1}$	194.5$^{a, 1}$			
	70	6	181.2$^{a, 1}$	156.8$^{a, 1}$			

** = significant, ($P \leq 0.05$) NS = non significant

$^{a, b}$ - means in the line with common superscript do not differ significantly determined by Scheffe's test

$^{1, 2}$ - means in the column with common superscript do not differ significantly determined by Scheffe's test

After 70-day supplementation with sodium selenite, the plasma concentrations of selenium in the horses of the experimental group were significantly higher ($P < 0.05$) compared to the control group (mean ± SE: 135.81 ± 10.19 μg·l^{-1} vs 108.18 ± 7.77 μg·l^{-1}) as well as the Se levels at the beginning of the experiment - control group 101.78 ± 11.06 μg·l^{-1}, experimental group 98.70 ± 10.88 μg·l^{-1} (Table 3).

In the horses of the experimental group, the plasma concentrations of α-tocopherol (Table 3) were significantly higher ($P < 0.05$) from the 44th day of the experiment than the levels at the beginning of the experiment (5.23 ± 0.52 mg·l^{-1}.vs 2.45 ± 0.25 mg·l^{-1}). Also, the plasma α-tocopherol levels in the horses of the experimental group were significantly higher ($P < 0.05$) than those in the horses of the control group (5.23 ± 0.52 mg·l^{-1} vs. 3.46 ± 0.34 mg·l^{-1}) (Table 3).

At the end of the dietary supplementation, the plasma concentrations of F_2-isoprostanes (Table 3) in the horses of the experimental group tended to be lower than those in the horses of the control group but the differences were not significant (156. 8 ± 12.89 pg·ml^{-1} vs. 181.2 ± 15.67 pg·ml^{-1}). The plasma concentration of F_2-isoprostanes tended to be lower in the experimental group at the end than at the beginning of monitoring (156.8 ± 12.89 pg·ml^{-1} vs. 170.3 ± 60.8 pg·ml^{-1}), although the control group showed the opposite trend (181.2 ± 15.67 pg·ml^{-1} vs. 137.0 ± 47.05 pg·ml^{-1}). Nevertheless, none of these differences were significant because of the large variability of the individual values. A considerable variation was found among the levels of F_2-isoprostanes in some horses. The concentrations varied from 42 pg·ml^{-1} to 449 pg·ml^{-1}.

Discussion

The initial plasma concentrations of the monitored antioxidants were above the reference levels thought to be critical for the risk of nutritional myodystrophy in horses, i.e., above 70 μg·l^{-1} of Se and 1.1 mg·l^{-1} of vitamin E (Valberg and Hodgson 2002). There were no significant differences found between the two groups of horses. Nevertheless, the initial

plasma concentrations of Se were below the reference range of 130–160 μg·1^{-1} by Stowe and Herdt (1992). Therefore, with respect to the current training workload, the Se content in the basic feed ration was set above the level recommended by the NRC (1989).

Despite this fact, no increase of plasmatic Se concentration appeared in horses of the control group during the course of the experiment. On the contrary, the plasma Se level in the experimental group was significantly increased after a 70-day supplementation. A non-significant tendency of the Se concentration increase was already apparent on day 44. Indeed, Richardson et al. (2006) noticed a significant increase of the Se plasma level from similar beginning values as early as after day 28 of supplementation but in their study the Se addition was higher, 4.7 mg of inorganic Se/day/horse vs. 3.38 mg inorganic Se/day/horse in our experiment.

Also, vitamin E supplementation led to a significant increase in its plasma concentrations in the experimental group, although it was not as pronounced as Avellini et al. (1999) observed in their study - after a 70-day supplementation of 40 mg of vitamin E/kg/day in horses, they found 8-times higher tocopherol concentrations than at the beginning. On the other hand, the results obtained by Pagan et al. (2005) in thoroughbred horses supplemented with 5000 IU/day for 56 days or with doses increased every two weeks (500, 1000, 2000, 8000 IU/day, respectively), were even non-significant in the case of synthetic vitamin E.

On the basis of the increase in detected antioxidants in the blood plasma of horses in the experimental group, one could expect a higher antioxidative capacity of the horse organism demonstrated by lowered plasma concentrations of F_2-isoprostanes. Such trend was actually shown, but it was non-significant. The main reasons for this were apparently the sizeable fluctuation of F_2-isoprostane levels in individual horses during the course of time as well as a large variation of this variable values at the beginning of the study in both the monitored groups (from 42 pg·ml^{-1} to 449 pg·ml^{-1}, SE representing about 25% of the mean value). A similar variability in F_2-isoprostane concentration in the blood plasma was shown, for example, in a study by Jacob et al. (2003), investigating the influence of diet on this marker levels in smokers. In our observation, horses with low initial F_2-isoprostane concentrations demonstrated an increase in these values during the experiment, and horses with high initial values subsequently stabilized at medium levels in general (data not shown). Therefore, the data variability was considerably lower at the end of the study. It is known that the production of F_2-isoprostanes as markers of lipid peroxidation (Roberts and Morrow 2002) does not only depend on the level of antioxidants reached in the organism. It is known, for example, that activity of glutathione peroxidase is correlated to selenium concentration, but its response to selenium concentration increase is rather delayed (Ludvíková et al. 2005a). Also, individual accommodation to the training workload is important (Art and Lekeux 2005). It is probable that under conditions of a similar workload, environment and uniform feeding in each of the two groups, the fluctuation of F_2-isoprostane levels was caused just by the different degree of individual horse's adaptation to the training workload. The lower variability of data at the end of the experiment indicates that a longer experimental period might be useful.

The extent of the supplementation effect of the diet used could be negatively influenced by a higher Se content in the basic diet. Although it led neither to any increase in plasmatic Se levels in the control horses during the course of our experiment, nor to the achievement of the recommended range of Se plasma values of 0.130-0.160 μg·ml^{-1} (Stowe and Herdt 1992), it resulted in a Se saturation of the control horses at least above the standard of the Czech Republic (Ludvíková et.al. 2005ab).

Finally, we can state that supplementation of the diet used with selenium and vitamin E caused a non-significant decrease of F_2-isoprostane concentrations in the blood plasma, and resulted in a significant increase of plasma concentrations of these antioxidants. The

variation of isoprostane levels probably reflected rather the individual responses of the horses' organisms to the training workload. An interesting observation was that the selenium supplement of 2.88 mg/horse/day was sufficient only in horses with a medium degree of training workload for the maintenance of plasma concentrations of this microelement at the initial but not high values.

Vliv přídavku vitaminu E a selenu v krmné dávce plnokrevných koní na koncentrace F_2-isoprostanů jako ukazatelů lipidové peroxidace

Cílem práce bylo posoudit vliv dotace vitaminu E a selenu (Se) na plazmatické hladiny F_2-isoprostanů coby ukazatelů oxidativního stresu u plnokrevných koní v tréninku. Dvanáct zdravých tříletých plnokrevných koní bylo rozděleno do 2 skupin - kontrolní (n = 6) a pokusné (n = 6). Krmné dávky odpovídaly střední pracovní zátěži. Koně pokusné skupiny dostávali doplněk DL-α-tokoferyl acetátu (2250 mg/den/kůň) a seleničitanu sodného (0,5 mg/den/kůň). Plazmatické koncentrace obou antioxidantů a F_2-isoprostanů byly sledovány 0., 44. a 70. den. Koncentrace selenu u pokusné skupiny byla po 70-ti dnech medikace signifikantně vyšší ($P < 0,05$) oproti počátku sledování (135,81 ± 10,19 $\mu g \cdot l^{-1}$ vs. 98,70 ± 10,88 $\mu g \cdot l^{-1}$) i vůči kontrolní skupině (den 0: 101,78 ± 11,06 $\mu g \cdot l^{-1}$, 70. den: 108,18 ± 7,77 $\mu g \cdot l^{-1}$). Koncentrace α-tokoferolu v krevní plazmě koní pokusné skupiny byla od 44. dne významně zvýšena ($P < 0,05$) - v porovnání s počátkem pokusu i vůči kontrolní skupině (5,23 ± 0,52 $mg \cdot l^{-1}$ vs. 2,45 ± 0,25 $mg \cdot l^{-1}$, resp. 3,46 ± 0,34 $mg \cdot l^{-1}$). Plazmatické koncentrace F_2-isoprostanů byly u pokusné skupiny na konci sledování nižší než na počátku (156,8 ± 12,89 $pg \cdot ml^{-1}$ vs. 170,3 ± 48,6 $pg \cdot ml^{-1}$), zatímco kontrolní skupina vykazovala opačný trend (181,2 ± 15,67 $pg \cdot ml^{-1}$ vs. 137,0 ± 47,05 $pg \cdot ml^{-1}$). Nicméně žádný z těchto rozdílů nebyl statisticky průkazný pro velkou variabilitu jednotlivých hodnot. Závěrem lze konstatovat, že použitý přídavek selenu a vitaminu E navodil neprůkazný pokles koncentrace F_2-isoprostanů v krevní plasmě, jakkoli vedl k signifikantnímu navýšení plasmatických koncentrací těchto antioxidantů. Kolísání hladin isoprostanů pravděpodobně odráželo spíše individuální odezvu organismu koní na tréninkovou zátěž.

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