Effect of evening primrose oil on biochemical parameters of thoroughbred horses under maximal training conditions

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ABSTRACT: The antioxidative effect of evening primrose oil (EPO) administration on the oxidative stress of race horses during their regular training period was determined. The eight-week experiment was performed on ten clinically healthy thoroughbred horses. All the horses were enrolled in a regular training program. Eight weeks before the experiment, the horses were fed a diet which remained the same for the following eight weeks, only supplemented with 150 ml EPO (blood samplings 3 times). Total antioxidant reactivity (TAS), thiobarbituric acid reacting substances (TBARS), uric acid (UA) levels, activities of muscle enzymes – aspartate aminotransferase (AST), creatine kinase (CK), and parameters of fatty acid metabolism such as triacylglycerols (TAG) and nonesterified fatty acids (NEFA) were determined. Average values of TAS after supplementation with EPO rose gradually and were detected at significantly higher levels ($P \leq 0.05$) in the sixth week in comparison with the control. The concentration of malondialdehyde, measured as TBARS, decreased significantly ($P \leq 0.05$) compared with the untreated control. The activities of AST and CK fluctuated, but no disturbance was demonstrated in muscle homeostasis. The present results indicate that the total antioxidant activity of the thoroughbred horses fed a diet supplemented with EPO was higher, and it helped stabilize the permeability of the muscle cell membranes in the horses at full workload.

Keywords: horse training program; oxidative stress; tissue damage; antioxidative effect

INTRODUCTION

Physical exercise increases tissue demand for oxygen and cell respiration, resulting in the generation of free radicals and reactive oxygen species (Jenkins 1998). Overproduction of oxidants exceeding the cellular antioxidant capacity results in oxidative stress (Gomes et al. 2012). Oxidative stress leads to tissue damage of a wide range of biomolecules and causes metabolic changes that consequently influence performance (Kirschvink et al. 2002). To protect against oxidative stress, the body has an effective antioxidant defence system, including non-enzymatic and enzymatic components (Deaton and Marlin 2003; Kinnunen et al. 2005; Shaliutina-Kolesova et al. 2013).

Animals do not have such a high concentration of non-enzymatic antioxidants in comparison to humans. Horses in particular have a relatively low concentration of systemic non-enzymatic antioxidants. For this reason it is necessary to supplement the antioxidants perorally. The most common antioxidants added to horse diets are vitamin E and C, alternatively a combination of both (White et al. 2001; Williams et al. 2004), or vitamin E and some forms of selenium (de Mof-

farts et al. 2005; Hartlova et al. 2008). Human, as well as veterinary medicine, is currently focused on natural substances with a distinct antioxidative effect. One of these is oil from *Oenothera biennis* – evening primrose. The effect of evening primrose oil (EPO) on oxidative stress includes the inhibitory effect on lipid peroxidation and also its enhancement of the glutation-dependent antioxidative defence system (Benatti et al. 2004).

EPO contains a high amount of unsaturated fatty acids – 74.6% of linoleic acid, 9.6% of gamma linoleic acid, 6.6% of palmic acid, and 6.1% of oleic acid. These fatty acids create a substrate for cyclooxygenase and lipooxygenase enzymes on plasma membranes that convert these into local hormones – eicosanoids. Eicosanoids subsequently influence a range of metabolic activities of the organism, such as platelets aggregation, inflammation, bleeding, vasoconstriction, vasodilatation, blood pressure or immune functions (Christie 1999; Benatti et al. 2004; Lisiak et al. 2013). EPO is unique because it contains a high percentage of gamma-linoleic acid and many antioxidants, such as catechin, epicatechin, gallic acid, and α-tocopherol (Christie 1999).

It has been shown that supplementing PMO to the diet of laboratory animals can increase the antioxidative ability of organisms (Kanbur et al. 2011; Zaitone et al. 2011). For this reason it is also used as an alternative therapy in humans when treating systemic illnesses (O'Connor et al. 2012).

Dietary administration of essential oils and unsaturated fatty acids was used for the treatment of canine atopic dermatitis and other serious illnesses (hyperlipidaemia, cardiovascular, renal, and neurological diseases), with positive results (Tretter and Mueller 2011; Gunal et al. 2013; Kubelkova et al. 2013; Lenox and Bauer 2013). Furthermore, the positive effect of PMO on ruminal fermentation activity was monitored in beef cattle, sheep, and goats (Varadyova et al. 2007; Cieslak et al. 2009; Szumacher-Strabel et al. 2009), and in addition its effect on fatty acids in muscle tissue, sperm fertility and motility was observed in poultry (Cerolini et al. 2003).

The diet supplementation with oils in sport and racing horses is common (Harris and Harris 2005; Nielsen 2009). Supplementing the horse diet exclusively with EPO has not yet been objectively monitored. In our study, we investigated the effect of EPO supplementation on the degree of lipid peroxidation and antioxidant activity measured by thiobarbituric acid reacting substances (TBARS) and total antioxidant reactivity (TAS) of thoroughbred horses during intensive training.

MATERIAL AND METHODS

Animals. The experiment was performed from June to October 2012 on 10 clinically healthy thoroughbred horses (5 male, 5 female, age 3–5 years, weight 470 ± 30 kg). All the horses had been in a regular training program prior to the study, with maximal workload.

EPO treatment. The diet dosage, 8 weeks prior to and 8 weeks during the experiment, corresponded with the requirements of NRC (2007) for thoroughbred horses of a certain weight and maximal physical load. All the horses were fed the same diet, supplemented with 150 ml of EPO (Solio Kft., Fadd, Hungary; digestible energy for horses (DEh) 35.7 MJ) from day 0 of the experiment to 8 weeks (scheme of EPO supplementation, blood sample collection, and parameters analyzed in blood samples is given in Figure 1). The diet was based on oats, meadow hay, and extruded supplemental feed mixture for horses: Fitmin Horse House müsli, Fitmin Horse Opti, Fitmin Horse Energy (all Dibaq a.s., Helvíkovice, Czech Republic) and a supplement of vitamins and minerals Equistro (Vétoquinol s.r.o., Nymburk, Czech Republic) (Table 1). The examination of the diet compounds was performed by the laboratory of

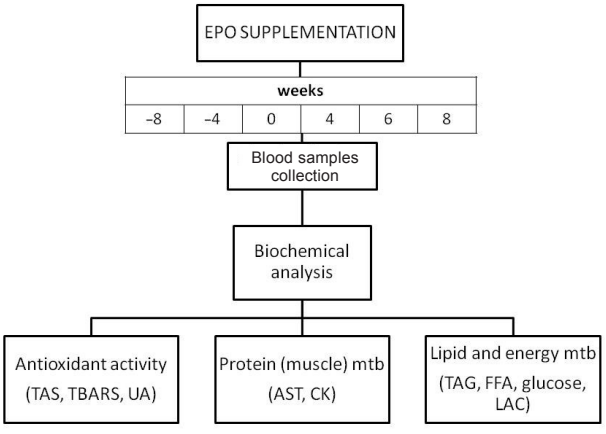

Figure 1. Evening primrose (EPO) supplementation schedule and parameters analyzed in horse blood samples

TAS = total antioxidant reactivity, TBARS = thiobarbituric acid reacting substances, UA = uric acid, AST = aspartate aminotransferase, CK = creatine kinase, TAG = triacylglycerols, FFA = free fatty acids, LAC = lactate, mtb = metabolism

Table 1. Composition of feed ratio for horses in experiment

	Meadow hay	Oats	Horse Opti	Horse Energy	Horse House Müsli	EPO	Σ Total	Σ Total + EPO
Nutrients (kg)	7.00	4.00	0.50	0.80	1.00	0.15		
Dry matter (g)	6 521.20	3 569.20	468.85	744.56	905.00	0.00	12 208.81	12 208.81
Crude protein (g)	398.30	472.00	75.00	64.00	100.00	0.00	1 109.30	1 109.30
DEh (MJ)	7.90	50.20	7.00	12.40	13.00	3.60	90.50	94.10
Ash (g)	364.00	128.00	40.65	10.80	59.20	0.00	602.65	602.65
Ether extract (g)	114.10	157.20	33.50	81.60	49.20	0.00	435.60	435.60
Fibre (g)	2 695.00	389.20	26.00	14.56	55.00	0.00	3 179.76	3 179.76
Vitamin A (I.U.)		176.00	7 610.00	672.00		0.00	8 458.00	8 458.00
Vitamin E (I.U.)			60.00	80.00	9.00	0.00	149.00	149.00
Se (mg)		0.84	0.04	0.02		0.00	0.90	0.90

EPO = evening primrose oil, DEh = digestible energy for horses

the Czech University of Life Sciences Prague. The horses had free access to water. Feeding doses were related to the rate of maximal workload.

Blood sample collection. The blood samples were collected into polyethylene tubes (Vacutainer BD, Heidelberg, Germany) 8 and 4 weeks before the experiment, on day 0, and 4, 6, and 8 weeks after the EPO application, at 6.30 h. The samples were immediately processed in the laboratory and serum was stored at –70°C until analysis.

Laboratory analysis. Biochemical parameters were determined using commercial kits (Erba Lachema s.r.o., Brno, Czech Republic), TAS was determined with a commercial kit (Randox Laboratories Ltd., Crumlin, UK) on an automatic analyser XL-200 (Erba Lachema s.r.o.). TBARS were detected by an OxiSelect™ TBARS Assay kit (Cell Biolabs, Inc., San Diego, USA), based on the method principle presented by Esterbauer and Zollner (1989).

Statistical analysis. The data was first subjected to repeated measures, split-plot design analysis of variance using the SAS software package (Statistical Analysis System, Version 9.1, 2009), and the treatment alignments were conducted using the Least Squares significant difference method (Duncan's Multiple Range Test). The level of significance was 0.05 for all the statistical analyses.

RESULTS

EPO supplementation results in increased antioxidant activity in race horses. To test the antioxidant effect of EPO, TAS was measured 8 and 4 weeks before and 4, 6, and 8 weeks after EPO treatment (Figure 1). The values of TAS showed a significant ($P \leq 0.05$) increase after 6 weeks of EPO supplementation. The longer period of supplementation did not show any significant change of TAS compared with the previous experimental weeks, indicating that EPO treatment reached its saturation level after 6 weeks of supplementation (Table 2). Next, we determined cell membrane lipoperoxidation by detection of malondialdehyde in serum by TBARS assay. We discovered that EPO supplementation gradually reduced the TBARS level within a period of 8 weeks of the supplementation, showing a significant ($P \leq 0.05$) reduction

Table 2. Serum oxidant and antioxidant parameters in racehorses (n = 10) in intense training before (week 8, 4, day 0) and after (week 4, 6, 8) the application of 150 ml of evening primrose oil (EPO) (means ± SD)

Measurements	Sampling					
	8 weeks before	4 weeks before	day 0	4 weeks EPO	6 weeks EPO	8 weeks EPO
TAS (mmol/l)	0.65 ± 0.14^{B}	0.69 ± 0.12^{B}	0.89 ± 0.08^{B}	0.79 ± 0.11^{B}	1.25 ± 0.49^{A}	1.16 ± 0.42^{A}
TBARS (µm)	56.39 ± 1.007^{C,D}	80.48 ± 18.46^{A,B}	92.94 ± 19.13^{A}	74.45 ± 20.52^{B,C}	69.65 ± 32.99^{B,C}	42.90 ± 12.47^{D}
UA (µmol/l)	7.70 ± 6.58^{B}	7.40 ± 2.76^{B}	12.90 ± 6.20^{A}	10.70 ± 2.79^{A,B}	10.50 ± 2.55^{A,B}	11.00 ± 4.66^{A,B}

$^{A–D}$different superscripts within the lines indicate statistically significant differences ($P \leq 0.05$)

TAS = total antioxidant activity, TBARS = substances reacting with thiobarbituric acid, UA = uric acid

Table 3. Serum activity enzymes aspartate aminotransferase (AST) and creatine kinase (CK) in racehorses (n = 10) at intense training before (week 8, 4, day 0) and after (week 4, 6, 8) application of 150 ml of evening primrose oil (EPO) (means ± SD)

Measurements	Sampling					
	8 weeks before	4 weeks before	day 0	4 weeks EPO	6 weeks EPO	8 weeks EPO
AST (μkat/l)	5.94 ± 1.01^{B}	7.01 ± 0.97^{A}	5.83 ± 1.06^{B}	7.33 ± 0.89^{A}	5.96 ± 0.88^{B}	6.51 ± 0.91^{A,B}
CK (μkat/l)	1.54 ± 0.30^{B}	2.03 ± 0.77^{B}	3.99 ± 1.08^{A,B}	3.21 ± 0.85^{A,B}	3.68 ± 1.21^{A,B}	3.89 ± 1.21^{A,B}

^{A,B}different superscripts within the lines indicate statistically significant differences ($P \leq 0.05$)

of cell membrane lipid peroxidation (Table 2). To monitor the metabolic activity of the horses, we measured the uric acid (UA) levels. The levels of UA did not change in comparison with untreated controls (Table 2).

EPO does not disturb muscle homeostasis in race horses. To examine the muscle homeostasis of race horses after EPO supplementation, the levels of muscle enzymes AST and CK in the serum of race horses with or without EPO supplementation were compared. There were no significant differences in AST activities after EPO supplementation, whereas CK levels were slightly higher compared with the control, indicating that muscle homeostasis was not disturbed (Table 3).

EPO supplementation reduces lipolysis in race horses. To observe the effect of EPO supplementation on lipid and energy metabolism, we determined the levels of triacylglycerols (TAG), free fatty acids (FFA), glucose, and lactate (LAC). As a result, we discovered significantly ($P \leq 0.05$) increased TAG and glucose levels after physical activities, but without any effect of EPO (Table 4). On the other hand, levels of FFA and LAC significantly ($P \leq 0.05$) decreased after physical exercise supplementation with EPO. Moreover, EPO supplementation almost diminished FFA levels in serum, showing the decreased effect of EPO on lipolysis (Table 4).

DISCUSSION

The aim of this study was to examine the antioxidant effect of EPO supplementation in the diet of racehorse at full workload, and thus its ability to lower oxidative stress. Maximal workload is associated with an increase in the production of free radicals and reactive oxygen species, which can consequently lead to imbalance between pro-oxidants and antioxidants and the formation of oxidative stress (Art and Lekeux 2005, Kinnunen et al. 2005).

Antioxidant activity measuring by TBARS showed a statistically significant reduction in lipid peroxidation after 8 weeks of EPO administration in monitored thoroughbreds under the maximal workload. These results indicate that EPO as a very important antioxidant effectively reduced the rate of oxidative stress generated during the workload of racehorses. Consistent with our study, Chiaradia et al. (1998) showed that the elimination of lipid peroxidation is a slow process, and if the workload is not compensated with e.g. a sufficient rest period, it can lead to the accumulation of these dangerous substances and ultimately to tissue damage. Moreover, the effect of EPO with a higher content of gamma-linoleic acid and several antioxidants (Lu and Foo 1995; Wettasinghe and Shahidi 2002) significantly increased TAS in weeks 6 and 8 of EPO saturation.

Table 4. Blood parameters of fat and energy metabolism in racehorses (n = 10) at intense training before (week 8, 4, day 0) and after (week 4, 6, 8) application of 150 ml of evening primrose oil (EPO) (means ± SD)

Measurements (mmol/l)	Sampling					
	8 weeks before	4 weeks before	day 0	4 weeks EPO	6 weeks EPO	8 weeks EPO
TAG	0.41 ± 0.11^{A,B}	0.52 ± 0.15^{A}	0.38 ± 0.06^{B}	0.53 ± 0.15^{A}	0.42 ± 0.11^{A,B}	0.50 ± 0.15^{A,B}
NEFA	0.03 ± 0.02^{B}	0.07 ± 0,03^{A}	0.05 ± 0.01^{A}	0.07 ± 0.04^{A}	0.02 ± 0.01^{B}	0.01 ± 0.01^{B}
Glucose	5.95 ± 0.32^{B,C}	6.14 ± 0.31^{B}	5.38 ± 0.48^{D}	6.05 ± 0.39^{B,C}	5.56 ± 0.30^{B,C}	7.14 ± 1.12^{A}
Lactate	0.87 ± 0.20^{D}	1.40 ± 0.25^{B,C}	1.81 ± 0.64^{A}	1.32 ± 0.47^{C}	1.74 ± 0.39^{A}	1.17 ± 0.18^{A,B}

$^{A–D}$different superscripts within the lines indicate statistically significant differences ($P \leq 0.05$)

TAG = triacylglycerols, NEFA = nonesterified fatty acids

A positive correlation among products of lipid peroxidation and activities of blood muscle enzymes AST and CK is well documented (Valberg 2009). Despite the variability in the activities of AST and CK enzymes during the experiment, these values did not show a disturbance in muscle homeostasis (Williams et al. 2004).

The lipid and energy metabolism of the racehorses was monitored, too. Increased serum levels of TAG of racehorses that were not compensated by the EPO supplementation were found. The increased levels of TAG had a small impact on energetic metabolism of skeletal muscle, which could be a result of the length and the intensity of the exercise correlating with the intensity of lipolysis activity. Nevertheless, the other marker of lipolysis, NEFA, was found significantly decreased after the EPO supplementation. This data suggest that the extent of lipolysis was not affected by the physical activity of horses (Turcotte 1999).

The serum levels of glucose and lactate correlated with the workload of the racehorses and were not affected by the EPO treatment. The individual differences in the values of serum glucose could have been affected by the time of the last meal (Frank et al. 2010).

In summary, our study has shown that the intake of the antioxidant EPO is beneficial and can compensate the oxidative stress generated during the maximum physical workload, without significant disruption of musculoskeletal disorders of thoroughbred horses.

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