



Effect of dietary polyunsaturated fatty acids and Vitamin E on serum oxidative status in horses performing very light exercise

Domenico Bergero¹, Nicoletta Miraglia², Achille Schiavone¹,
Mimmo Polidori², Liviana Prola¹

¹Dipartimento di Produzioni Animali, Epidemiologia ed Ecologia. Università di Torino, Italy

²Dipartimento di Scienze Animali, Vegetali e dell'Ambiente. Università del Molise, Italy

Corresponding author: Dr. Achille Schiavone. Dipartimento di Produzioni Animali, Epidemiologia ed Ecologia. Facoltà di Medicina Veterinaria, Università di Torino. Via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy – Tel. +39 011 6709208 – Fax: +39 011 670 9240 - Email: achille.schiavone@unito.it

Paper received January 21, 2004; accepted February 26, 2004

ABSTRACT

In sporting horses the use of dietary polyunsaturated fatty acids (PUFAs) could enhance performance because these fatty acids are very important in membrane permeability, and in particular they seem to increase the possibility of long chain fatty acids entering mitochondria to be burnt. The composition of cellular membranes and lipoprotein fatty acids composition is strictly related to dietary fat quality; percentages of polyunsaturated fatty acids and amount of antioxidants also affect tissue susceptibility to lipid peroxidation.

Six horses were used in a latin square design in which three homogeneous groups were subsequently assigned three different dietary treatments for one month each:

Control group (C): basic diet; Oil group (O): Basic diet + 200g/day oil rich in PUFAs (Crossential GLA TG20, Croda ®); Vitamin E group (O+E): basic diet + 200 g/day oil rich in PUFAs (Crossential GLA TG20, Croda ®) + 5 g/day α -tocopheryl-acetate (Egon-E, Acme ®). At the end of each experimental period blood samples were taken by jugular vein puncture. Serum oxidative status was evaluated by TBARs and d-ROMs assessment. Oxidative markers showed the highest mean values for the oil group, even if no statistically significant differences were found.

Key words: Horse, Polyunsaturated fatty acids (PUFAs), Vitamin E, Oxidative stress.

RIASSUNTO

EFFETTO DELLA SOMMINISTRAZIONE DIETETICA DI ACIDO γ -LINOLENICO E VITAMINA E SULLO STATO OSSIDATIVO SIERICO DI CAVALLI SOTTOPOSTI A LAVORO MODERATO

Nel cavallo sportivo, la somministrazione di acidi grassi poliinsaturi (PUFA) può migliorare le prestazioni, dal momento che tali acidi grassi svolgono un'importante funzione nel garantire la permeabilità delle membrane e, in particolare, agevolerebbero il transito degli acidi grassi a catena lunga all'interno dei mitocondri per essere catabolizzati a fini energetici. La composizione delle membrane cellulari e degli acidi grassi delle lipoproteine è influenzata dalla qualità dei lipidi forniti con l'alimento e dalla disponibilità di molecole ad azione antiossidante, fattori che a loro volta influenzano i fenomeni di perossidazione lipidica.

Nel presente studio sono stati utilizzati sei cavalli divisi in tre gruppi secondo uno schema a quadrato latino 3x3. A ciascun gruppo sono stati assegnati in successione i tre trattamenti alimentari della durata di un mese ciascuno: gruppo controllo (C): dieta base; gruppo olio (O): dieta base + 200g/die di olio ricco in PUFA (Crossential GLA TG20, Croda ®); gruppo olio

+ vitamina E (O+E): dieta base + 200g/die di olio ricco in PUFA (Crossential GLA TG20, Croda ®) + 5 g/die di α -tocoferil-acetato (Egon-E, Acme ®). Al termine di ciascun periodo sperimentale sono stati prelevati campioni di sangue dalla vena giugulare. Lo stato ossidativo del siero è stato valutato attraverso la misurazione di TBARs e d-ROMs, mostrando valori tendenzialmente più elevati per il gruppo olio, anche se non è stata raggiunta la significatività statistica.

Parole chiave: Cavallo, Acidi grassi poliinsaturi (PUFA), Vitamina E, Stress ossidativi.

Introduction

The oxidation and the production of free radicals and reactive oxygen species (ROS) are an integral part of life and animal metabolism both at rest and during work. In normal conditions ROS and free radicals are inactivated by the natural defenses of the organism, to prevent the cellular damage that these highly reactive species could produce (Gondim *et al.*, 2000; Papas, 1999). The unbalance between antioxidant and prooxidant agents can cause a negative situation known as oxidative stress. Several factors such as nutrition, age, exercise conditions and diseases can affect the antioxidant status of the horse (Kirschvink and Lekeux, 2002). The dietary factors involved in the prevention of the negative effects of oxidative stress are: daily intake of antioxidant and prooxidant substances, feed manufacturing and storage conditions, use of feed additives and nutritional supplements (Papas, 1999).

The dietary supplementation of oil rich in polyunsaturated fatty acids (PUFAs) has received a great deal of interest also in horse nutrition (Bergero *et al.*, 2002), because these molecules are believed to enhance performance. These fatty acids are very important in maintaining membrane permeability (Gibney and Bolton-Smith, 1988), thus providing better exchange possibilities for the substrates involved in energetic metabolism. In particular PUFAs seem to increase the possibility of long chain fatty acids entering mitochondria to be burnt. In addition, n-3 PUFAs have anti-inflammatory properties (Higgins and Less, 1984); in athlete horses many anti-inflammatory drugs are often used to control joint, skeletal and muscle injuries and inflammations induced by severe exercise. The use of dietary PUFAs without appropriate antioxidant supple-

mentation, however, could unbalance the antioxidant/prooxidant ratio of the organism and could consequently cause damages to the cellular membranes. In fact, it is well known that the composition of cellular membranes and also lipoprotein fatty acids reflects the dietary fat composition, and it is frequently assumed that there is a relationship between dietary fat quality and tissue susceptibility to lipid peroxidation induced by oxidative stress. The extent of the oxidation of different unsaturated fatty acids is related to the total number of double bonds and to the fatty acid chain length (Liu *et al.*, 1997).

Vitamin E (α -tocopherol) plays a central role in protecting PUFAs from oxidative damage: it is present in membranes in the approximate ratio of 1 molecule to 1000 lipid molecules and its phytyl tail gives it the unique ability to position itself in the membrane bilayer with the active chroman ring close to the surface (Papas, 1999). Dietary supplementation of vitamin E has been shown to reduce lipid peroxidation (as determined by thiobarbituric acid reactive substances assay - TBARs) in horse muscle (Pettersson, 1991). During exercise, the oxygen uptake and the production of free radicals increases and can more easily lead to oxidative stress with deleterious effects on the cellular structures involved in physical activity (White *et al.*, 2001). Increased levels of indicators of oxidative status, such as TBARs, were observed in horses both after a race at maximum speed and after endurance competition (Gondim *et al.*, 2000; White *et al.*, 2001). However, it is not well known if a diet rich in polyunsaturated fatty acids could alter the blood serum oxidative status of horses performing very light exercise.

The objective of this investigation was to compare the effects of three different diets on the serum oxidative status of horses performing very light exercise.

Table 1. Experimental design (30 days for each period plus 15 days delay between periods for adaptation)

	First period	Second period	Third period
Group 1	C	O	O+E
Group 2	O	O+E	C
Group 3	O+E	C	O

C = basic diet; O = basic diet + 200g/day oil rich in PUFAs;

O+E = basic diet + 200 g/day oil rich in PUFAs + 5 g/day α -tocopheryl-acetate

Material and methods

Animals and diets

Six horses were used in a Latin square design in which three homogeneous groups were subsequently assigned three different dietary treatments lasting one month each plus a fifteen day delay between periods for adaptation (Table 1):

Control group (CG): basic diet;

Oil group (O): Basic diet + 200g/day oil rich in PUFAs (Crossential GLA TG20, Croda ®);

Vitamin E group (O+E): basic diet + 200 g/day oil rich in PUFAs (Crossential GLA TG20, Croda ®) + 5 g/day α -tocopheryl-acetate (Egon-E, Acme ®). The composition of the dietary oil is shown in Table 2.

The basic diet was composed of first cut meadow hay (7.2% crude protein and 34% crude fiber on a dry matter basis) and barley (10.0% crude protein and 5.4% crude fiber on dry matter basis) at a feeding level near one (maintenance). The forage/concentrate ratio was approximately 75/25.

During experimental periods animals performed very light exercise (a 20 minute walk and 10 minute trot per day at lungeing rein).

Samples collection

At the end of each experimental period blood samples were taken from the jugular vein of each horse. After serum separation by centrifugation, aliquots of each sample were frozen and stored for TBARs (Turpeinen *et al.*, 1995) assessment, after addition of butyl hydroxyl toluene (BHT; 1 mmol/l), and d-ROMs measurement (Diacron s.r.l. – Grosseto, Italy ®).

Determination of ROMs

Reactive oxygen metabolites (ROMs) were

determined by spectrophotometer and a reagent kit (DIACRON s.r.l., Grosseto, Italy). The method is based on the production of a stable colored organic radical cation at low pH (4.8) with maximum absorption at 505 nm. The ROM's method allows dosing of all hydroperoxide present in a biological sample, generated from compounds such as proteins, lipids, nucleic acids and aminoacids. Results are expressed in Carr units (1 Carr unit corresponds to 0.024 mmol/l of H₂O₂).

Table 2. Fatty acid composition of dietary oil (% of fatty acid methyl esters)

Fatty acid	Relative percentage
C16:0	10.31
C16:1	0.37
C18:0	3.03
C18:1	18.22
C18:2 n6	38.91
C18:3 n6	22.41
C18:4 n3	0.79
C20:1 n9	3.37
C22:1 n11	1.77
C22:5 n3	0.80
SFA	13.34
MUFA	23.73
PUFA	62.91
SFA/PUFA	0.15

SFA = saturated fatty acid;

MUFA = monounsaturated fatty acid;

PUFA = polyunsaturated fatty acid;

UFA = unsaturated fatty acid

Table 3. Blood serum oxidative bio-markers values in the three experimental periods (mean \pm SD)

	First period	Second period	Third period
TBARs ($\mu\text{mol MDA/l}$)	0.50 \pm 0.16	0.44 \pm 0.09	0.44 \pm 0.25
d-ROMs (Carr units)	85.76 \pm 9.03	87.01 \pm 12.95	91.03 \pm 6.39

Table 4. Blood serum oxidative bio-markers for the different dietary treatments (mean \pm SD)

	Control	Oil	Oil + Vit. E
TBARs ($\mu\text{mol MDA/l}$)	0.45 \pm 0.20	0.53 \pm 0.15	0.40 \pm 0.17
d-ROMs (Carr units)	84.59 \pm 7.64	93.94 \pm 10.01	85.27 \pm 9.19

Determination of TBARs

In this method malondialdehyde (MDA, a product of lipid peroxidation) is expressed as thiobarbituric acid reactive substances (TBARs) and spectrophotometrically detected in protein-free serum as described by Turpeinen *et al.* (1995). 2 ml of TBA (thiobarbituric acid) solution (0.67% glacial acetic acid/water, 50:50, vol/vol), 0.28 ml of BHT (1.0 mM final concentration) and 0.07 ml FeCl_3 (0.25 mM final concentration) were added to 0.5 ml of protein-free serum. Samples were heated for 60 min. at 100°C, cooled to 4 °C and centrifuged at 12000 rpm for 15 min. MDA was detected at 532 nm. MDA-bis-(diethylacetal) standards (from 0.49 to 3.95 mmol/l) were prepared daily.

Statistical analysis

Data were processed by multifactorial analysis of variance (MANOVA) procedure, including interaction in the model, using the dietary treatment and the period as factors, to assess the serum oxidative status (SPSS, 1997).

Results and discussion

The results for the determinations of TBARs and d-ROMs in the different periods and for the different dietary treatments are shown in Table 3 and 4; no significant differences were recorded for

both factors studied although oil supplementation determined a mean increase in both TBARs and d-ROMs values. The interaction between the two variables is near the upper significance level for d-ROMs ($p=0.09$). The supplementation with vitamin E induced the mean values of TBARs and d-ROMs near to those observed in the control group although the supplementation of oil was the same as in the oil group.

Several studies investigated the role of antioxidant supplementation such as vitamin E and C to prevent lipid peroxidation after exercise. It was observed that the TBARs value remained almost unchanged after an intense exercise in vitamin E (Pettersson *et al.*, 1991) or vitamin C (White *et al.*, 2001) supplemented horses, while in control groups increasing levels were observed. Serum biomarkers of lipid peroxidation usually increase during exercise, even in horses (Chiaradia *et al.*, 1998). The level of the oil addition and the light work performed by the horses in the present trial did not influence the oxidative status, although a clear trend for the alteration of the oxidative status induced by the only use of oil rich in PUFAs can be detected by the mean increase of both indicators status if a correct supplementation of antioxidant is not supplied together. We expected larger differences among groups; a masking effect could be ascribed to the relatively short "washing

out" period between the experimental periods. For this reason new data obtained using larger wash out windows could be useful.

Otherwise our results are in accordance with some Authors whose findings showed a negligible effect of vitamin E at rest or after a low intense exercise (Lovlin *et al.*, 1987). Other Authors underline the variability in the response of vitamin E supplementation that in some cases did not show any antioxidant effect on serum oxidative bio-markers (Sacheck and Blumberg, 2001).

Conclusions

Oxidative stress is a damaging imbalance in the oxidative-antioxidative system of cells. The development of the oxidative stress is related to muscle damage, hydration status and the welfare of the animal. As pointed out in relevant literature, it is interesting to underline the variability in the response to dietary antioxidants supplementation in horses showing, in some cases, no modifications in blood serum bio-markers of lipid peroxidation.

Our results indicate that the concentration of serum oxidative bio-markers measures remains almost steady during the trial suggesting the repeatability of these evaluations. The dietary supplementation of 200 g oil rich in PUFAs does not significantly alter the serum antioxidant status in horses performing very light exercise. In this case the dietary supplementation of vitamin E can be advisable, but does not seem to be necessary. More data are required, in particular using longer experimental periods and blank windows.

Paper presented as abstract at the 53rd Annual Meeting of the European Association for Animal Production (EAAP), Cairo, Egypt, 2002.

Acknowledgments are due to Acme (Cavriago, RE, Italy) that supplied the Vitamin E (Egon-E) and Croda Italiana Spa (Mortara, PV, Italy) that supplied the PUFAs rich oil (Crossential GLA TG20).

REFERENCES

- Bergero, D., Miraglia, N., Polidori, M., Ziino, M., Gagliardi, D., 2002. Blood serum and skin fatty acid levels in horses and the use of dietary polyunsaturated fatty acids. *Anim. Res.* 51:157-163.
- Chiaradia, E., Avellini, L., Rueca, F., Spaterna, A., Porcello, F., Antonioni, M.T., Gaiti, A., 1998. Physical exercise, oxidative stress and muscle damage in racehorses. *Comp. Biochem. Phys. B.* 119:833-836.
- Gibney, M.J., Bolton-Smith, C., 1988. The effect of dietary supplement of n-3 polyunsaturated fat on platelet lipid composition, platelet function and platelet plasma membrane fluidity in healthy volunteers. *Brit. J. Nutr.* 60:5-12.
- Gondim, F., Silveira, L.R., Pereira da Silva, L., Macero, D.V., 2000. Oxidative stress and antioxidant system in horses. In: A. Lindner (ed.) *Proc. Conf. on Equine Sports Medicine and Science*, Essen, Germany, pp 131-135.
- Higgins, A.J., Less, P., 1984. The acute inflammatory process, arachidonic acid metabolism and the mode of action of anti-inflammatory drugs. *Equine Vet. J.* 16:163-175.
- Kirschvink, N., Lekeux, P., 2002. Oxidative Stress in exercising horses: fact or fiction? pp 87-91 in *Proc. 1st Europ. Congr. Equine Health and Nutr.*, Antwerp, Belgium.
- Liu, J., Yeo, H.C., Doniger, S.J., Ames, B.N., 1997. Assay of aldehydes from lipid peroxidation: gas chromatography-mass spectrometry compared to thiobarbituric acid. *Anal. Biochem.* 14:161-166.
- Lovlin, R., Cottle, W., Pyke, I., Kavanagh, M., Belcastro, A., 1987. Are indices of free radicals damage related to exercise intensity? *Eur. J. Appl. Physiol.* 45:927-934.
- Papas, A.M., 1999. Antioxidant status, diet, nutrition and health. Ed. CRC Press, New York, USA.
- Pettersson, K.H., Hintz, H.F., Schryver, H.F., Combs, G.F., 1991. The effect of vitamin E on membrane integrity during submaximal exercise. *Equine Ex. Physiol.* 3:315-322.
- Sacheck, J.M., Blumberg, J.B., 2001. Role of vitamin E and oxidative stress in exercise. *Nutrition.* 17:809-814.
- SPSS, 1997. 7.5 per Windows®, Chicago, Illinois, U.S.A.
- Turpeinen, A.M., Alftthn, G., Valsta, L., Hietanen, E., Salonen, J.T., Schunk, H., Nyysönen, K., Mutanen, M., 1995. Plasma and lipoprotein lipid peroxidation in humans on sunflower and rapeseed oil diet. *Lipids.* 30:485-492.
- White, A., Estrada, M., Walker, K., Wisnia, P., Filgueira, G., Valdes, F., Arandeda, O., Behn, C., Martinez, R., 2001. Role of exercise and ascorbate on plasma antioxidant capacity in thoroughbred race horses. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.* 128:99-104.