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## Serum lipid profile modification related to polyunsaturated fatty acid supplementation in thoroughbred horses

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### ABSTRACT

The importance of polyunsaturated fatty acids (PUFAs) within the biological functions of animals has been widely recognized. In this study, exercise and PUFAs' supplementation effects on serum triglycerides (Try), total cholesterol (Chol), and nonesterified fatty acids (NEFAs) concentration were evaluated in athletic horses. Ten regularly trained thoroughbred horses were randomly divided in two groups, control group (CG;  $n=5$ ) and experimental group (EG;  $n=5$ ). EG received a 4-week PUFA supplementation; CG received no dietary supplement. Blood samples were collected from the animals every 10 days before (PreD10, PreD20, PreD30) and after (PostD10, PostD20, PostD30) 1700 metres of race and were tested for selected parameters. Two-way analysis of variance for repeated measures showed lower Chol levels at PreD10, PreD20, and PreD30 in EG with respect to control CG ( $P < .05$ ). EG showed lower NEFA levels than CG at PreD20 and PostD30 ( $P < .05$ ). Increase of Try levels was found in EG at PreD20 and PostD30 ( $P < .05$ ). The results of this study highlighted both exercise and PUFA supplementation diet's effect on selected lipid parameters in thoroughbred horses. In addition PUFA supplementation diet seems to improve metabolic adaptation to physical exercise probably by increasing animal capacity for fatty acids' utilization in muscle.

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Horse; lipid metabolism; exercise; polyunsaturated fatty acid

## 1. Introduction

Lipids represent one of the most important substances involved in many metabolic pathways in mammals (Pagan 1998). They play multiple roles and are an essential element in the composition of cellular membrane, in the transmission of cellular signals and, furthermore, they provide an important metabolic intermediate in the skeletal muscle metabolism representing the most important energy stock in the body (Hinchcliff et al. 2004). Concerning equine athlete, lipid metabolism is important in aerobic and in anaerobic exercise (Hodgson & Rose 1994; Piccione et al. 2009). Physical exercise induces various stress responses and metabolic adaptations. Similarly to others stressors, including delivery, transport, and environmental conditions (Budzynska 2014; Casella et al. 2012; Piccione et al. 2015), physical exercise needs adequate response to re-establish homeostatic equilibrium (Arfuso et al. 2016). Exercise has great influences on lipid metabolism through its action on some particular hormones (Pösö & Hyyppä 1999). In particular, catecholamines (norepinephrine and epinephrine) influence respiratory and cardiovascular functions and enhance the possibility of the body to react to exercise, optimizing and increasing the use of energy substrates through their action on metabolism in adipose, muscular, and hepatic tissue. Catecholamines determine an increase of the plasma-free fatty acids through their action on the hormone-responsive lipases of adipose tissue (Hinchcliff et al. 2004; Pösö et al. 1989; Sen & Packer 2000).

The influence of physical exercise on oxidative stress, inflammatory response, and on haematological and biochemical parameters makes dietary supplementation with polyunsaturated fatty acids (PUFAs) (6–8) a popular matter in athletic horses' nutrition studies (O'Connor et al. 2004; Orme et al. 1997). The most biologically active PUFAs are Omega-3 and Omega-6 fatty acids. They have shown positive effects on both physiological and pathologic conditions (Holub & Holub 2004; Piccione et al. 2014). Fatty acids could promote the athletic performance, allowing the utilization of energetic substrates and improving the stability and permeability of cellular membranes (Gibney & Bolton-Smith 1988; Piccione et al. 2013). Furthermore, they show an important influence on aerobic and anaerobic metabolism, muscle glycogen synthesis and use, and peripheral oxygen transport (Harking et al. 1992; Watson et al. 1993). A fatty acid supplementation in horse diet must include omega-3 and omega-6 fatty acids both in a proper ratio (1:1, 1:3, 1:6) depending on the diet. In fact, natural diets, thanks to pasture grass, possess significant amounts of omega-3 fatty acids, while the ratio of omega-3 to omega-6 fatty acids shows important changes in animals fed with traditional equine diet without pasture grass, which causes a decrease in the supply of omega-3 fatty acids and exceed in omega-6 fatty acids. Fatty acids represent an important source of energy and their metabolic effects on lipids were already taken into consideration in athletic horses (O'Connor et al. 2004; O'Connor et al. 2007). The aim of this study was to

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evaluate the effect of PUFAs' dietary supplementation on fat metabolism in thoroughbred horses in order to improve the knowledge of the effect of PUFAs on metabolic processes occurring during exercise in horses undergoing specific training programmes and give useful information for better management of athletic horses.

## 2. Materials and methods

### 2.1. Animals

Ten regularly trained thoroughbred horses (6 geldings and 4 mares, 4–5 years old, mean body weight  $500 \pm 25$  Kg) were enrolled in this study with the owner's informed consent. Before starting the study, the horses were subjected to clinical examination, routine haematology, and haematochemical analyses at rest, and only healthy subjects were used. All animals were housed in individual boxes ( $3.50 \times 3.50$  m) under natural winter photoperiod (sunrise at 07:28 am, sunset at 17:38 pm) and 18–21°C indoor temperatures. The horses were fed twice a day a diet consisting of hay (first cut meadow hay, sun cured, and late cut;  $7 \pm 1$  kg/d) and total mixed ration (Bernunzo Feeds Factory, Enna, Italy) composed of the following: crude protein 16%, ash 10.09%, crude fat 6%, crude fibre 7.35%, sodium 0.46%, lysine 0.85%, methionine 0.35%, omega-3 0.65%, at a total of  $10 \pm 2$  kg/day distributed in two meals (at 7:00, 19:00). Water was available ad libitum. All horses were randomly divided into two groups: Control group (CG) and experimental group (EG). EG received 70 ml of Omega Horse orally by means of a syringe. The composition of the supplement was: crude fat 99.4% (total omega 3: 22.5 g, Eicosapentaenoic acid (EPA) 11.5 g, Docosaenoic acid (DHA) 7.7 g, VitE: 2 g), crude protein 0.09%, crude fibre 0.09%, crude ash 0.04% (NBF Lanes, Milan, Italy) (Table 1), once a day for 30 days.

### 2.2. Blood sampling and analysis

Blood samples were collected by means of jugular venipuncture in tubes without anticoagulant agent (Terumo Co., Tokyo, Japan) every 10 days before (PreD10, PreD20, PreD30) and within 10 min after (PostD10, PostD20, PostD30) 1700 metres' harness race performed at the 'La Favorita' race track (Table 2) Palermo, Italy. Blood samples were placed on ice and transferred to the laboratory within 2 h after collection. After

**Table 1.** Nutritional composition of supplementation: fatty acids as a percentage of fat.

Active principle	Content %
$\alpha$ -Myristic acid (C12:0)	7.0
Palmitic acid (C13:0)	18.0
Palmitoleic acid (C16:1)	9.5
Stearidonic acid (C18:0)	4.5
Oleic acid (C18:1)	19.0
Linoleic acid (C18:2)	1.5
$\alpha$ -linolenic acid (C18:3)	0.5
Eicosapentaenoic acid, EPA (C20:5)	18.0
Docosapentaenoic acid (C22:5)	4.0
Docosahexaenoic acid, DHA (C22:6)	12.0
Other unsaturated fatty acids	5.8
Vitamin E	0.2
Butylhydroxytoluene, BHT	0.015

standing at room temperature for 20 min, the tubes were centrifuged at  $1300 \times g$  for 10 min and the obtained serum was stored at  $-25^\circ\text{C}$  until analysis. Only sera, not lipemic or haemolysed, were analysed to test triglycerides (Try), total cholesterol (Chol), and nonesterified fatty acids (NEFAs) concentration with commercially available kits (Biosystems, Reagents and Instruments, Barcelona, Spain; Gesse Diagnostics, Rome, Italy; Randox, Crumlin, UK) by means of an automated analyzer ultraviolet-visible spectrophotometer (SEAC, Florence, Italy).

### 2.3. Statistical analysis

A Barlett test was applied to verify the homoscedasticity of data and all passed the test.  $P < .05$  was considered statistically significant, with an alpha level of 90%. Two-way analysis of variance (ANOVA) for repeated measures and Bonferroni's *post hoc* were used to assess the statistical significant effect of exercise and PUFA supplementation diet at different time points on serum Try, Chol, and NEFAs. The data were analysed with Stats package of R: R Core Team (2013) (R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, 2013, URL: <http://www.R-project.org/>).

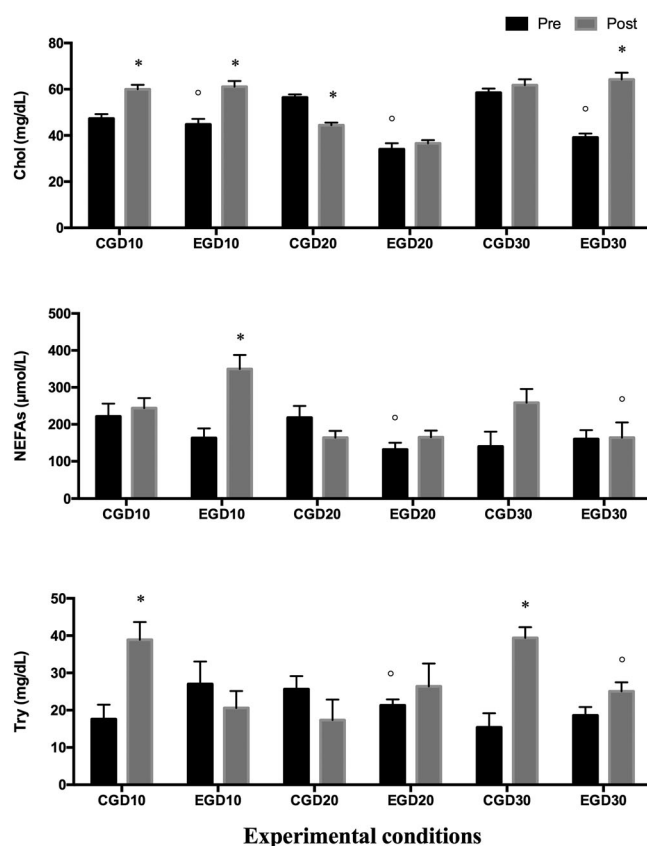
## 3. Result and discussion

Two-way ANOVA showed significant effect of exercise (Pre-race respect to Post-race) in all studied parameters ( $P < .05$ ). Moreover, a significant effect of PUFA supplementation (CG with respect to EG) was found on Chol, Try, and NEFAs values ( $P < .05$ ). During the experimental period of this study, Chol and Try showed normal mean levels within the physiological range for the horses (Kaneko 1997). Since other researchers studied the effect of omega 3 supplementation in horses (Bowen et al. 2013; De Moffarts et al. 2007; Orme et al. 1997) we actually have limited knowledge about the normal ranges of NEFAs in physiological conditions in horses; however the values obtained in this research were in accordance with the values already observed in Piccione et al. (2009). The analysis of our results showed a significant effect of exercise on selected parameters. In fact, significantly higher values of Chol, NEFAs, and Try were found in the Post-race. In particular, Chol showed higher levels at PostD10 with respect to PreD10 in both groups and at PostD30 with respect to PreD30 in EG; whereas lower Chol levels were found at PostD20 compared to PreD20 in CG. NEFAs showed a statistically significant increase at D10 in EG and Try levels' increase at D10 and D30 in CG ( $P < .05$ ). The trend of these parameters represents a possible metabolic and hormonal response of horses to physical exercise. During physical exercise an increased sympathetic

**Table 2.** 'La Favorita' horse race track characteristics.

Track facts	
Main track	Oval
Track material	Dirt
Track length	1.400 m
Radius of curvature	85–95 m
Banked turn straight	2–3%
Banked turn curve	5–8%

adrenal activity and a reduction in insulin concentration are likely to occur. The increased beta-adrenergic sensitivity of the adipose tissue induces lipomobilization, enhancing the use of fatty acids as an energy source. The fatty acids used in muscular metabolism are provided not only by the adipose tissue, but also by circulating lipoproteins and by the triglycerides stored in the muscle cells themselves. The aerobic metabolism leads to a solicitation and to an activation of type I and IIA muscle fibres (Assenza et al. 2012), enhancing lipolysis and increasing the ability to use lipids as energy substrate increases. However, because of the known low ability of thoroughbred horses to use lipids as energy substrate, the released lipids cannot be used in a proper way, as the ability to burn lipids by the muscle fibres is still low (Assenza et al. 2012). As a consequence, the levels of NEFAs increased and the surplus of unused NEFAs returned to the adipose tissue to be re-esterified to triglycerides (Magkos 2009; Pösö et al. 1989). In agreement with Pösö et al. (1989) our results showed higher Try and Chol levels in CG with respect to EG (Figure 1). The higher Try and Chol values in CG showed the effect of PUFA supplementation in athletic horses during exercise. In this study after 30 days, NEFAs reach levels lower than first days probably for a metabolic response to the stress exercise-induced in fact, it is note that a proper training could improve the recovery capacity and the energetic substrates use through NEFAs mobilization



Significances: \* vs Pre ( $P < 0.05$ )  
° vs CG ( $P < 0.05$ )

**Figure 1.** Mean  $\pm$  standard deviation (SD) with related statistical significances of serum total cholesterol (Chol) triglyceride (Try) and nonesterified fatty acid (NEFAs) values measured before exercise (Pred10, pred20, pred30) and after exercise (Postd10, postd20, postd30) conditions in control (CG) and experimental (EG) thoroughbred horses.

(Christensen et al. 1999; Piccione et al. 2009). The results obtained in the present study suggest that this peculiar metabolic response could be stimulated due to PUFA supplementation in athletic horse diet. In particular, following omega-3 supplementation in the EG it was possible to observe a significant decrease in Try and NEFA levels compared to the CGs during rest and after the race. Several studies have shown a significant decrease in serum triglycerides due to fish oil supplementation in human, horses, and rats (Christensen et al. 1999; Fickova et al. 1998; Hinchcliff et al. 2004). This event could be attributed to an increasing of lipoprotein lipase action and in fatty acid oxidation (Geleen et al. 1999; Orme et al. 1997) or to a down-regulation effect on triglyceride synthesis enzymes performed by fatty acids (Surette et al. 1992). Triglycerides in horses are transported mainly by very low density lipoprotein (VLDL). In fact, this lipoprotein contains about 57% of triglycerides while, for example, in low density lipoprotein (LDL) they are transported at a rate of 5.5% (Watson et al. 1993). If the lipoprotein lipase activity and the clearance of LDL increase it could result in a decrease of triglycerides circulating in bloodstream. The influence of fish oil supplementation on the enzymes involved in triglyceride catabolism could suggest an increase of fatty acid utilization in muscular tissue. The EG showed a significant decrease in NEFA concentration. NEFA values express the ratio between fatty acid mobilization from adipose tissue and fatty acid utilization in muscular tissue. The lower serum NEFA values found in the EG with respect to the CG could be due to an increase of free fatty acid utilization in order to produce energy or to decrease the fatty acid mobilization (O'Connor et al. 2004).

#### 4. Conclusion

The interaction and the importance of PUFAs within functions and biological systems of animals have been widely recognized. The results obtained in the present study highlighted the effect both of exercise and PUFA supplementation diet on selected lipid parameters in thoroughbred horses. In addition, PUFA supplementation diet seems to improve metabolic adaptation to physical exercise by athletic horses, probably by increasing animal capacity for fatty acid utilization in muscle. However, further studies should be conducted to better understand the effect and the benefic of omega-3 supplementation in athletic horses.

#### Disclosure statement

No potential conflict of interest was reported by the author(s).

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