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INFLUENCE OF OMEGA-3 IN STANDARDBRED HORSE: HAEMATOLOGICAL PARAMETERS

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Abstract

The aim of this study was to investigate the effect of omega-3 supplementation on some haematological parameters like red blood cells (RBC), haemoglobin (Hgb), haematocrit (Hct), white blood cells (WBC), neutrophils (Neu) and lymphocytes (Lym) that may have a direct effect on the performance of athletic horses. Ten regularly trained Standardbred horses (6 geldings and 4 females, 4–5 years old, mean body weight 500 ± 25 kg) were used for the study. They were randomly divided into two groups. The experimental group received an omega-3 dietary supplementation every day for 30 days. The control group received no supplementation. Every 10 days, horses took part in a 1660 metre harness race and blood samples were collected from each horse: one in the morning before race (pre) and one after race (post). The application of one-way analysis of variance for repeated mesures (ANOVA) showed a statistically significant difference due to the race in the two different groups. The results obtained in the present study show a discordant effect of supplementation with omega-3 on RBC, HCT and Hgb, while omega-3 supplementation has been shown to have a better effect on WBC, Neu and Lym, but further studies should be performed to better evaluate the benefits of these on the performance of the athletic horse.

Key words: horse, physical exercise, haematological parameters, polyunsaturated fatty acid

Over the past three decades, there has been substantial interest in the importance of polyunsaturated fatty acids in various biological functions. Polyunsaturated fatty acids (PUFAs) cannot be synthesized by the animal, therefore, they must necessarily be introduced with the diet. In fact, the mammals' cells are devoid of enzymatic systems that insert double bonds along the carbon chain of the fatty acids (Testino et al., 2010). Several foods, rich in omega-3 polyunsaturated fatty acids, like flaxseed oil and fish oil, exert anti-inflammatory and immunomodulatory effects and may be useful as a nutritional countermeasure to exercise-induced stress, inflamma-

tion and immune dysfunction in athletes. In particular previous studies have shown a greater validity of fish oil supplementation compared to flaxseed oil supplementation in increasing the long-chain PUFA eicosapentaenoic acid (EPA, 20:5 omega 3) and docosahexaenoic acid (DHA, 22:6 omega 3) in plasma and RBC (Hess et al., 2012; Vineyard et al., 2010). The omega-3 PUFAs such as EPA and DHA seem to have additional anti-inflammatory properties, primarily through their effects on the neutrophil and macrophage components of the inflammatory response (Park et al., 2013). Other positive effects of omega-3 are related to fertility and fetal development (Abayasekara and Wathes, 1999). EPA and DHA, derived from fish oil, for example, can cause dual inhibition of cyclo-oxygenase-2 and 5-lipoxygenase pathways for metabolism of arachidonic acid (AA). EPA is a less preferred substrate than AA for both pathways and generally through substrate competition inhibits release of AA-derived eicosanoids, thus reducing the generation of proinflammatory "tetraene" 4-series leukotrienes and 2-series prostanoids and production of cytokines from inflammatory cells (Mickleborough, 2008). The fish oil, which contains the largest quantity of PUFA compared to other foods, is the staple food of commercial supplements. Several studies have shown that administration of supplements of omega-3 has resulted in improved performance in the equine athlete (O'Connor et al., 2004; Woodward et al., 2005) and in human athlete (Brilla and Landerholm, 1990; Christensen et al., 1999). In fact, during strenuous exercise, due to the oxidation of membrane lipids, cell and tissue damage may occur (Clarkson and Thompson, 2000). An omega-3-based integration provides an intake of antioxidants that could limit the cell damage caused by free radicals and prevents the onset of fatigue (Packer, 1997). Furthermore, fish oil supplementation causes a delay in the exercise-induced decrease of EMF (erythrocyte membrane fluidity), recently identified as indirect oxidant marker (De Moffarts et al., 2007; Portier et al., 2006). The study of omega-3 supplementation can be interesting because the increase of the knowledge of their effects on haematological parameters can provide a starting point concerning a regular use of these substances to improve athletic performance.

On the basis of this knowledge and considering the changes occurring in response to exercise (Fazio et al., 2011), the purpose of this study was to evaluate the effect of omega-3 supplementation on some haematological parameters, which are directly affected by exercise and at the same time could affect athletic performance, in athletic horses subjected to regular training and competition programme.

Material and methods

Animals

Ten regularly trained Standarbred horse (6 geldings and 4 mares, 4–5 years old, mean body weight 500±25 kg) were enrolled in this study with the owner's informed consent.

Before starting the study, horses were subjected to clinical examination, routine haematology and biochemistry analyses at rest conditions, and only healthy sub-

jects were used. All animals were housed in individual boxes (3.50×3.50 m) under natural winter photoperiod (sunrise at 7:28, sunset at 17:38) and 18–21ºC indoor temperatures. Horses were in a standard training programme on a 1,000-m track at "La Favorita" race track (38°09'07'' N, 13°20'43'' E) Palermo, Italy. Weekly training programme is presented in Table 1.

Table 1. Weekly training programme protocol for all horses

Gait	Duration (minutes)	Speed (m/minutes)
First, second, fourth and fifth day		
walk	10	100
slow trot	25	350
walk	10	100
Third day		
walk	15	100
trot	6	670
walk	15	100
Sixth day		
walk	15	100
trot	Simulation race	1660 m
walk	15	100

The horses were fed twice a day a diet consisting of a total mixed ration (Unimix Sprint Horses, Bernunzio Feeds Factory, Enna, Italy) composed as follows: 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 total of 5±1 kg/day distributed in two meals (at 7:00, 19:00). Horses also received about 6±1 kg/horse/day of hay (first cut meadow hay, sun cured, late cut with 6.9% crude protein on average). Water was available *ad libitum*. Horses were divided into two groups: Experimental group (EG) and Control group (CG). The two groups were divided so as not to interfere with the proper conduct of racing of La Favorita race track. EG and CG groups competed on two separate days, Tuesday and Friday. Every horse, before the race, was controlled by the official veterinarian of the racecourse, and they could run only after the positive medical examination and each horse ran ten days after the last race. EG received 70 ml of Omega Horse once a day for 30 days. Composition of food supplement is shown as percentage in Table 2.

Blood sampling, experiment design and analysis

Blood samples were collected by means of jugular venipuncture in vacutainer tubes containing EDTA (Terumo Corporation, Japan), before (pre) and after (post) 1660 metre harness race (D0). Blood samples were collected 10 minutes before horses were warmed up for the race and within 30 minutes after the race in the horses box, the day 1 before the start of omega-3 supplementation (D0) and every 10 days (D10-D20-D30) for all experimental period.

Whole blood samples were placed on ice pending analyses that were performed within 2 h after collection, by means of Heco Vet automatic analyser (SEAC, Florence, Italy) for the assessment of red blood cells (RBC 10^6/μL), hematocrit (Hct %),

haemoglobin (Hgb g/dL) and white blood cells (WBC 10^3/μL), neutrophils (Neu cells/μL) and lymphocytes (Lym cells/μL).

Table 2. Nutritional composition of supplementation: fatty acids as a percentage of fat

Active Principle	Content %
a-myristic acid (C12:0)	7.0
Palmitic acid (C16:0)	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
α-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 Unsatured Fatty Acids	5.8
Vitamin E	0.2
Butylhydroxytoluene, BHT	0.015

Statistical analysis

A Bartlett test was applied to verify the homoscedasticity of data and all passed the test. $P<0.05$ was considered statistically significant, with an alpha level of 95%. One-way analysis of variance for repeated measures (ANOVA) and Bonferroni's post hoc were used to assess the statistical significance differences due to the race in the two different groups. The data were analysed with Stats package of R (Core Team (2013).

Results

Mean values ± standard error of mean SEM of RBC, Hct, Hgb, WBC, Neu and Lym are shown in Table 2, Figures 1–2.

The application of one-way analysis for repeated measures ANOVA showed a statistically significant effect of the time (post race vs pre race) on RBC, Hgb, Hct and WBC in all experimental conditions in both groups ($P<0.05$).

In particular, multiple comparisons post hoc showed significantly higher values of RBC at Post D0, Post D10, Post D20, Post D30 in CG with respect to Pre race ($P<0.05$), in EG higher RBC values were found in Post D0, Post D10, Post D20 with respect to Pre race ($P<0.05$), and a significantly lower value at Post D20 with respect to Post D10 and Post D30 versus Post D0 in CG and EG, respectively. ANOVA showed significantly higher values of Hct at Post D0, Post D10, Post D20, Post D30 with respect to Pre race ($P<0.05$) in both groups and a significantly lower value at Post D20 and Post D30 versus Post D10 in CG. A significantly higher value of Hgb

was observed at Post D0, Post D10, Post D20 and Post D30 ($P<0.05$) in both groups with respect to Pre race, and a significantly lower value at Post D20 versus Post D10 in CG. A significantly higher value of WBC was observed at Post D0, Post D10 and Post D30 ($P<0.05$) in CG with respect to Pre race, in EG higher values were observed at Post D0, Post D10 and Post D20 ($P<0.05$) with respect to Pre race. Neu showed significantly higher values in CG at Post D10, Post D20 and Post D30 ($P<0.05$) with respect to Pre race and Post D0, Post D10, Post D20 higher values of Neu were observed in EG with respect to Pre race. ($P<0.05$). Lym showed significantly higher values in CG at Post D10 ($P<0.05$), at Post D20 and Post D30 higher values were observed in EG with respect to Pre race.

Discussion

The results obtained in the present study showed a significant effect of harness race on the studied haematological parameters. The significant increase post race of haematological parameters in CG and EG, according to bibliography (Kearns et al., 2002; McGowan and Hodgson, 2014; Piccione et al., 2010), are due to the exercise that pushes the body to find a dynamic equilibrium through adaptive changes. These adaptive changes can also affect the composition of body fluids, primarily the blood. The changes in blood composition included an increase in RBC number, Hct and Hgb values and a change in WBC count, Neu and l Lym (Kingstone, 2008; Shaskey and Green, 2000) (Figures 1 and 2).

These changes are due to particular adaptation to exercise, including splenic contraction caused by catecholamines. RBC, Hct and Hgb are closely related to each other and together with WBC could be used as indices of performance. The red cell pool is under the direct influence of catecholamine concentrations, so exercise has a variable effect on erythrogram parameters, depending on the speed and duration of the exercise bout (McKeever and Hinchliff, 1995; Person and Lydin, 1973) and it can be seen as effect of spleen contraction. The results concerning the effects of administration of omega-3 on haematological profile in literature are quite discordant, probably due to the different animal species used in the various scientific researches (Buckley et al., 2009; O'Connor et al., 2004; Piccione et al., 2010). In our study, after 30 days of omega-3 supplementation in EG we did not observe an increase of RBC after exercise, and this was also statistically significant with respect to D0 post exercise (Figure 1).

Omega-3, inserted between the phospholipids membrane, determines important changes in the flexibility of the cells, improving their deformability (Ernst et al., 1990). This effect is very important for the blood cells such as erythrocytes, in fact, they acquire the ability to pass more easily and quickly through capillaries (Testino et al., 2010), thus ensuring a better oxygen and blood supply to tissues. In horse, in particular, an increase was observed in endothelium-activated releasing factor which causes an improvement of vascular compliance through the decrease in arterial vasoconstriction and, consequently, reduces the exercise-induced hypertension which is the basis of pulmonary stress in horses with this pathology (King et al., 2008).

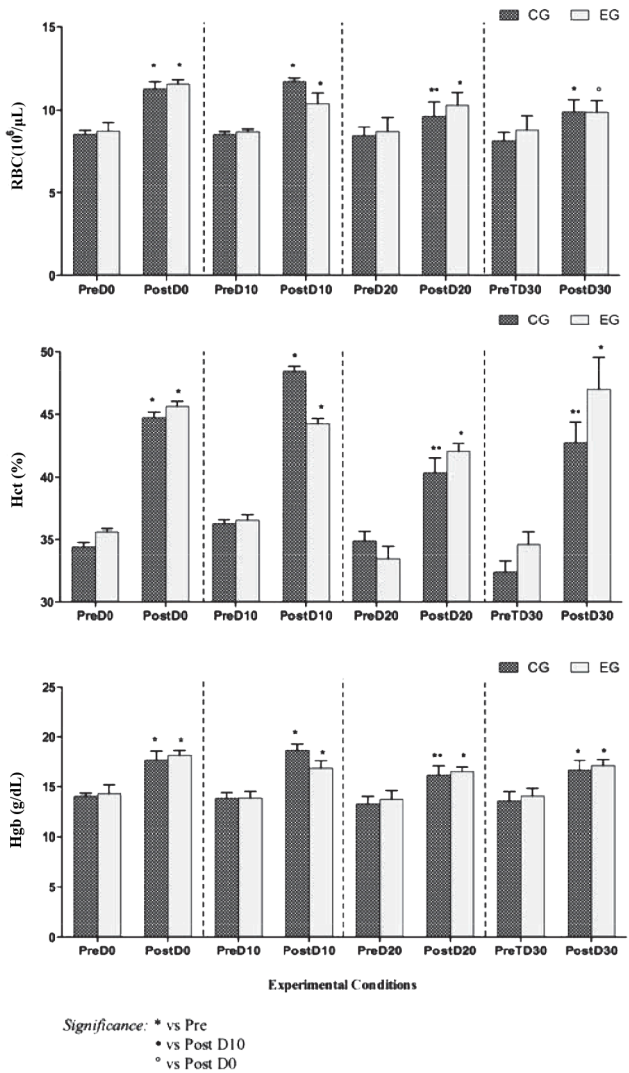

Figure 1. Patterns of RBC (10^6/µL), Hct (%) and Hgb (g/dL) ± Standard error of mean in ten regularly trained Standardbred horses during experimental trial

Studies on goats showed a reduction of WBC number corresponding to an increase of the amount of fatty acids administered with diet. A similar effect was observed even on rats in which supplementation with omega-3, causing a decrease of WBC number, has been associated with an anti-inflammatory effect due to the decrease of leukocyte function (Brenner et al., 1999; Murphy et al., 2007; Qadir et al., 2009). Immediately after exercise, changes in WBC are likely due to catecholamine release, splenic contraction and by bone marrow release, even if the last one is extremely low in number. The spleen releases not only stored erythrocytes but also

the leukocytes, especially lymphocytes, into the peripheral circulation. These facts cause an increase in WBC count (Iversen et al., 1994; Rossdale et al., 1982; Simon, 1991; Snow et al., 1983). Leucocytosis has been reported previously for Standardbred trotters undergoing repeated episodes of training on a racetrack (Korhonen et al., 2000). Our results showed a statistically significant increase of WBC after race in both groups under all experimental conditions except in EG during the D30. In the same way of WBC, Neu and Lym trends show a neutrophilia and lymphocytosis typical of physical exercise. Neutrophilia disappears after 20 days of omega-3 supplementation in EG. Also, the EG had lower values of both Lym that Neu (Figure 2). The mechanisms through which dietary fatty acids can modulate circulating WBC numbers are poorly understood, probably the decrease is due to a greater induction in endothelial cells of adhesion molecules for circulating leukocytes (Ley et al., 2007).

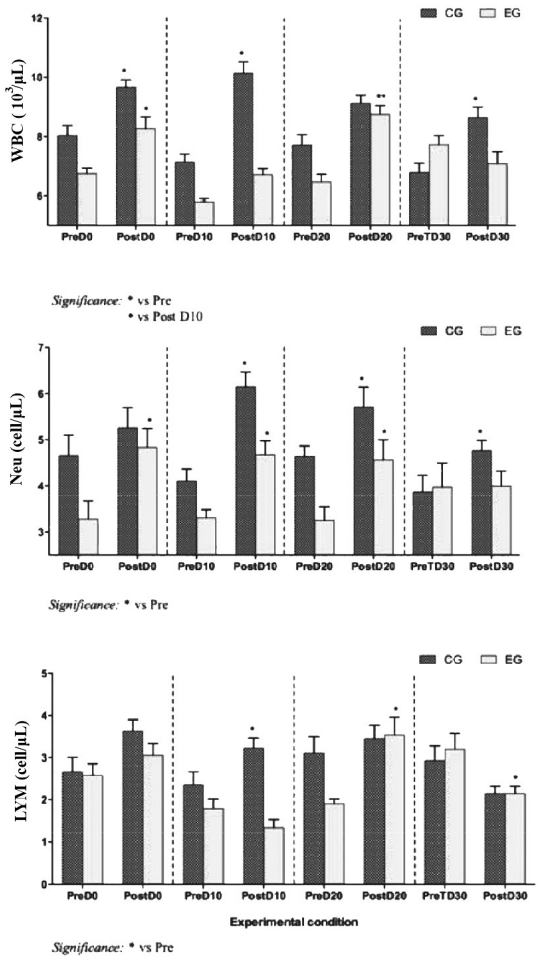

Figure 2. Patterns of WBC (10^3/µL), Neu (cells/µL) and Lym (cells/µL) ± Standard error of mean in ten regularly trained Standarbred horses during experimental trial

Ernst et al. (1990) had demonstrated, in human athlete, that EPA and 1.05 g/day DHA for 3 weeks attenuates the rise in acute-phase proteins occurring after exercise and stops postexercise immunosuppression of the immunoglobulin M-plaque forming response (Benquet et al., 1994). Tartibian et al. (2009) assessed the effect of 324 mg/day EPA and 216 mg/day DHA administered over 30 days before and during 48 h after step training.

In conclusion, the results obtained in the present study show a discordant effect of supplementation with omega-3 on RBC, Hct and Hgb, while omega-3 supplementation has been shown to have a better effect on WBC, Neu and Lym. We can hypothesize a reduction in splenic contraction at D30 in EG, indicating a reduced stress response to exercise. However, further studies should be performed to better evaluate the benefits of these on the performance of the athletic horse.

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