Effect of dietary supplementation with omega 3 on clotting time, fibrinogen concentration and platelet aggregation in the athletic horse

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ABSTRACT

Twenty clinically healthy and regularly trained horses, 10 Sella Italiana (Jumper) and 10 Thoroughbreds, were randomly divided into two subgroups. The first subgroup received a dietary supplement Omega Horses 70 ml/day for 30 days (experimental group); the second subgroup received no dietary supplement (control group). All horses were subjected to a simulated race to test their performance levels. The same race was performed on both groups at the end of experimental period. On blood samples, collected before and after the first test (T0–T0pe), every 7 days for a month (T1–T2–T3–T4) and after the second test (T4pe), Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), Fibrinogen Concentration (Fb) and platelet aggregation were assessed. The application of two-way repeated measures analysis of variance (ANOVA) identified a significant effect of time (4 weeks monitoring) on PT only in Jumpers, on APTT and Fb both in Jumpers and Thoroughbred. A statistically significant effect of treatment was observed during the 4 weeks of monitoring on PT, APTT and Fb while no significant change was observed on platelet aggregation. Both experimental groups showed higher PT and APTT values and lower Fb values than control groups. Moreover, a statistically significant effect of treatment was observed on PT and Fb in T4–T4pe periods in both breeds. This study highlights the effects of omega 3 dietary supplementation on horse’s clotting parameters providing useful information to improve athletic horse’s management.

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1. Introduction

Horses, along with all other mammalian species, have a dietary requirement for the cis-polyunsaturated 18-carbon (eg, C18:3 and C18:2) fatty acids (FA), which comprise the essential fatty acids (EFAs). The most common sources of supplemental omega-3 FA used in equine industry are flaxseed and fish oil. The main omega-3 FA contained in flaxseed is α-linolenic acid (ALA; 18:3 omega-3), whereas the main omega-3 FA contained in fish oil are eicosapentaenoic acid (EPA; 20:5 omega-3) and docosahexaenoic acid (DHA; 22:6 omega-3). Horse capability to bioconvert ALA to EPA and DHA has not been determined, but in humans, the bioconversion rate of ALA to EPA is less than 10% and that of ALA to DHA is less than 0.10% (Williams and Burdge, 2006). Feeding horses with fish oil markedly increased the concentrations of circulating EPA and DHA in plasma (Hall et al., 2004; King et al., 2008; O’Connor et al., 2007). Optimal levels of omega-3 fatty acids have been shown to reduce inflammatory responses, support immune function, and enhance fertility rate. Continuing research is revealing more information about the benefits of supplementing horses with omega-3 fatty acid to achieve a...
more nutritionally sound balance. Dietary supplementation with flaxseed oil has been shown to decrease the leukocyte response to inflammation (Morris et al., 1991, 1989) in horse. Flaxseed oil and marine fatty acids supplementation decrease platelet aggregation in different species (Allman et al., 1995; Casali et al., 1986). This has been attributed to the competition between EPA and arachidonic acid (AA) since both oils are substrate for the enzyme cyclooxygenase. The predominant products of AA in platelets are thromboxane A₂ and prostaglandin I₂. They both have opposite functions compared with thromboxane A₃ and prostaglandin I₃, that derive from EPA and represent potent vasodilators and platelet inhibitors (Hendra and Betteridge, 1989).

Swine fed cod liver oil showed a reduction in serum thromboxane B₂ levels and an increase in platelet fatty acid deposition of EPA. Fish oil supplements increased levels of tissue plasminogen activator (TPA) and decreased concentrations of plasminogen activator inhibitor, both enhancers of fibrinolysis (Barcelli et al., 1985).

On the basis of this knowledge, the aim of this study was to investigate the influence of omega-3 supplementation on clotting parameters and platelet aggregation in Jumper and Thoroughbred horses.

2. Material and method

2.1. Animals

Ten clinically healthy and regularly trained Sella Italiana horses (7–10 years old, with a mean body weight 500 ± 30 kg) from the same horse training center, and ten clinically healthy and regularly trained Thoroughbred horses (2–5 years old, with a mean body weight 390 ± 15 kg) from the same horse training center were used. All animals were housed in individual boxes (3.50 × 3.50 m) under natural spring photoperiod (sunrise at 06:00 h, sunset at 18:00 h), at an average ambient temperature of 18–21 °C.

All animals were fed standard rations, consisting of hay (first cut meadow hay, sun cured, late cut, average 8 kg/horse/day, 6.9% crude protein on average) and a mixture of cereals (oats and barley, 50% each, about 3.5 kg/horse/day) provided three times a day (at 7:00, 12:00 and 19:00). The composition of the cereal mixture was (dry matter basis) 13.0% crude protein, 20.7% crude fiber and 3.4% ether extracts, with a calculated net energy content of 0.80 UFC (Unité Fouragire Cheval).

Two groups of horses were randomly divided into two subgroups; for the first subgroup Omega Horse 70 ml/day (N.F.B. lanes, Milano, Italy) was added to the ration for 30 days (experimental group); for the second group no dietary supplement was added (control group). Water was available ad libitum. Omega Horse was easily miscible with food and appetizing, so all horses consumed the exact amount of supplement (70 ml/day).

2.2. Protocol

Before starting of Omega horse supplementation (T0) all horses were subjected to a simulated race to test their performance levels. All Sella Italiana horses performed a standardized obstacle course 350 m in length and with 14 obstacles (1.20 m height). All Thoroughbred horses took part in an official 1700 m race (average speed 800 m/min). Both control and experimental groups performed the same test at the end of Omega Horse supplementation (T4).

2.3. Sample collection

Blood samples were collected by jugular venipuncture before and after the first test (T0–T0pe), every 7 days for a month (T1–T2–T3–T4) and after the second test (T4pe) in vacutainer tubes containing 3.8% sodium citrate (1 part citrate: 9 parts blood) to assess Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), Fibrinogen Concentration (Fb) and platelet aggregation. All samples were stored at 4 °C pending analyses that were performed within 2 h from blood collection. PT, APTT and Fb were assessed on citrated plasma by means of a standard kit made especially for the SEAC Clot 2 coagulometer (SEAC, Florence, Italy). To determine platelet aggregation, platelet-rich plasma (PRP) was obtained by centrifugation of blood samples at 300 g for 20 min at room temperature. The upper 2/3 of the PRP was carefully removed, using a plastic transfer pipette, and was transferred into plastic containers. Successively, the platelet-poor plasma (PPP) was prepared by further centrifugation of the remaining blood at 3000 g for 10 min. After the addition of ADP to PRP, platelet aggregation was recorded for at least 4 min using an aggregometer (CLOT2, SEAC, Florence, Italy). The maximum degree of aggregation was expressed as a percent of the maximum possible change in light transmission. The initial velocity of aggregation (slope) was determined by drawing a line tangent through the steepest linear part of the aggregation tracing, and determining the slope from one point along the curve. The slope of this tangent was expressed as percent per minute.

2.4. Statistical analysis

All the results were expressed as means ± SD. Data were normally distributed (p > 0.05, Kolmogorov-Smirnov test). Two-way repeated measures analysis of variance (ANOVA) was used to determine statistically significant effect of time and Omega Horse supplementation on parameters studied during the four weeks of monitoring and during the exercise tests in Jumper and Thoroughbred horses.

A p value < 0.05 was considered statistically significant. Bonferroni’s multiple comparison test was applied for post hoc comparison. The data were analyzed using the STATISTICA 8 software (StatSoft, Inc.).

3. Results

The trends of the clotting parameters recorded during 4 weeks of monitoring in Jumpers and Thoroughbred groups are shown in Figs. 1 and 2.

The application of two-way ANOVA identified a significant effect of time (4 weeks monitoring) on PT (Jumpers – F(4,32) = 6.08; p = 0.0004), on APTT (Jumpers – F(4,32) = 4.95; p = 0.03; Thoroughbred – F(4,32) = 21.40; p < 0.0001) and on Fb (Jumpers – F(4,32) = 5.93; p < 0.01; Thoroughbred –
Fig. 1. The trends (Mean ± SD) of the clotting parameters recorded at rest during 4 weeks of monitoring in Jumper groups.

Fig. 2. The trends (Mean ± SD) of the clotting parameters recorded at rest during 4 weeks of monitoring in Thoroughbred groups.

$F_{(4,32)}=9.67; p < 0.0001$. A statistically significant effect of treatment was observed during the 4 weeks of monitoring on PT (Jumpers - $F_{(4,32)}=25.38; p < 0.001$; Thoroughbred - $F_{(4,32)}=12.76; p < 0.05$). APTT (Jumpers - $F_{(4,32)}=5.79; p = 0.04$; Thoroughbred - $F_{(4,32)}=8.53; p < 0.01$) and on Fb (Jumpers - $F_{(4,32)}=5.93; p = 0.04$; Thoroughbred - $F_{(4,32)}=2.96; p = 0.03$). Moreover, a statistically significant effect of treatment was observed on PT (Jumpers - $F_{(4,32)}=21.22; p < 0.001$; Thoroughbred - $F_{(4,32)}=17.27; p < 0.05$) and Fb in T4 - T4pe periods in both breeds (Jumpers - $F_{(4,32)}=56.91; p < 0.0001$; Thoroughbred - $F_{(4,32)}=5.54; p < 0.05$) (Table 1).

In Jumper, the experimental group showed higher PT values respect to control group. This gap was mainly observed at T1 (control $= 9.36 ± 0.18$ s; experimental $= 10.46 ± 0.38$ s), T2 (control $= 9.75 ± 0.25$ s; experimental $= 10.70 ± 0.32$ s) and T4 (control $= 9.13 ± 0.23$ s; experimental $= 10.50 ± 0.16$ s). APTT gradually increased from T0 (39.32 ± 3.38 s) to T4 (46.42 ± 2.23 s) in experimental group, contrary to the control group that showed no linear trend. Fb showed a significant decrease in experimental group respect to control in T3 (control $= 167.10 ± 5.84$ mg/dL; experimental $= 144.70 ± 9.13$ mg/dL) and T4 (control $= 162.10 ± 12.00$ mg/dL; experimental $= 130.60 ± 6.58$ mg/dL).

In Thoroughbred, PT increased from T0 (10.30 ± 0.88 s) to T4 (11.4 ± 0.61 s) in experimental group respect to control group that had no linear trend. APTT differ between two groups at T3 (control $= 44.02 ± 3.25$ s; experimental $= 46.48 ± 2.07$ s). Fb gradually decreased from 156.80 ± 11.21 mg/dL at T0 to 123.30 ± 17.62 mg/dL at T4 in experimental group.

The application of two-way ANOVA showed a significant effect of exercise on aggregation during T0/T0pe and T4/T4pe in Jumper ($F_{(1,8)}=5.00; p < 0.05$; $F_{(1,8)}=22.23; p < 0.001$) and Thoroughbred ($F_{(1,8)}=10.70; p < 0.01$; $F_{(1,8)}=10.44; p < 0.01$); a significant effect of exercise on slope was also observed at during T0/T0pe and T4/T4pe in Jumpers ($F_{(1,8)}=7.13; p < 0.05$; $F_{(1,8)}=11.28; p < 0.001$) (Table 2).

4. Discussion

Our results showed a significant effect of time (T0, T1, T2, T3, T4) on PT, APTT and Fb. As previously reported by several authors (Abbate et al., 1993; Dischinger et al., 1980; Heinrich et al., 1990; Kluft et al., 1988; Van Den Burg et al., 2000) the bleeding time and fibrinogen concentration are particularly influenced by different factors such as age, feeding, daily and seasonal variations.

In contrast to O’Connor et al., 2007, who did not find variations in bleeding time in horses supplemented with fish oil, our results indicate that omega 3 supplement has significant effects on PT and APTT values. The exact mechanism by which omega 3 increase PT level remain undetermined. Probably, PUFA influences the extrinsic pathway of coagulation (measured by PT) and in particular tissue factor (TF) activity. TF is the cell surface receptor for the serine protease factor VIIa. The complex of TF with factor VIIa catalyzes the conversion of the inactive pro tease factor X into the active protease factor Xa. Factor Xa
activates prothrombin (factor II) to thrombin (factor IIa); thrombin, in turn, converts fibrinogen to fibrin.

APTT is a performance indicator measuring the efficacy of both the "intrinsic" and the common coagulation pathways and the vitamin K-dependent (prothrombin, factors VII, X) and vitamin K-independent (factor V) coagulation factors. An attractive hypothesis is that fish oil modulates an hepatic transcriptional or translational process, which controls the production or release of coagulation factors. From studies with rats, it has been suggested that dietary controls the production or release of coagulation factors. 

In this study, platelet aggregation showed a significant decrease after exercise (T0 vs. T0pe e T4 vs. T4pe) in both breeds. It is known that exercise has variable effects on equine blood parameters (Piccione et al., 2008) and causes modifications of platelet function (El Sayed et al., 2000; Sakita et al., 1997), although the exact mechanisms and the regulatory pathways involved in the effects of exercise on platelet function are not completely understood. Also in humans studies on the effect of physical exercise on platelet function are not completely understood. Also in humans studies on the effect of physical exercise on platelet function are not completely understood. Also in humans studies on the effect of physical exercise on platelet function are not completely understood. Also in humans studies on the effect of physical exercise on platelet function are not completely understood. Also in humans studies on the effect of physical exercise on platelet function are not completely understood. Also in humans studies on the effect of physical exercise on platelet function are not completely understood. Also in humans studies on the effect of physical exercise on platelet function are not completely understood. Also in humans studies on the effect of physical exercise on platelet function are not completely understood. Also in humans studies on the effect of physical exercise on platelet function are not completely understood. Also in humans studies on the effect of physical exercise on platelet function are not completely understood. Also in humans studies on the effect of physical exercise on platelet function are not completely understood. Also in humans studies on the effect of physical exercise on platelet function are not completely understood. Also in humans studies on the effect of physical exercise on platelet function are not completely understood. Also in humans studies on the effect of physical exercise on platelet function are not completely understood. Also in humans studies on the effect of physical exercise on platelet function are not completely understood. Also in humans studies on the effect of physical exercise on platelet function are not completely understood. Also in humans studies on the effect of physical exercise on platelet function are not completely understood. Also in humans studies on the effect of physical exercise on platelet function are not completely understood. Also in humans studies on the effect of physical exercise on platelet function are not completely understood. Also in humans studies on the effect of physical exercise on platelet function are not completely understood. Also in humans studies on the effect of physical exercise on platelet function are not completely understood. Also in humans studies on the effect of physical exercise on platelet function are not completely understood.
by physical exercise counter-regulate the negative effects of adrenaline on platelet aggregation. In the first case increased adrenaline levels result in decreased aggregation to adrenaline, probably due to adrenergic receptor down-regulation (Kjeldsen et al., 1995). In the second case, noradrenaline is able to stimulate endothelial cells to release prostacyclin and nitric oxide, both of which are known to be potent inhibitors of platelet aggregation (Jones et al., 1993); in fact the increased prostacyclin production and plasma nitric oxide metabolites may suppress platelet reactivity (de Graaf et al., 1992).

Omega Horse treatment showed no effect on percentage of aggregation and slope of aggregation. It was reported that platelets response to polyunsaturated fatty acids is species-specific (Hansen et al., 2002). In human and dog, changes in platelet aggregation are linked to the high levels of omega 3 in diet, while in rat platelet aggregation is strongly linked to omega 6/omega 3 ratio.

Our results about omega 3 supplementation in athletic horses are in agreement with others (O’Connor et al., 2004, Woodward et al., 2005). This study highlights the effects of omega 3 dietary supplementation on horse’s clotting parameters providing useful information to improve athletic horse’s management.

Conflict of interest

None.

References


