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Original Research

Omega-3 Fatty Acid Food Enrichment Influences Some Serum Acute Phase Proteins Concentration and White Blood Cell Count in Athlete Horses

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ABSTRACT

The effect of exercise and dietary supplementation with polyunsaturated fatty acids (PUFAs) on serum amyloid A (SAA), fibrinogen (Fb), and white blood cell (WBC) count was studied in eight Italian Saddlebreds jumper and eight Thoroughbred horses. Horses were divided into four groups: two experimental groups including four jumpers (A_J) and four Thoroughbreds (A_T) that received 4-week PUFAs supplementation and two control groups including four jumpers (B_J) and four Thoroughbreds (B_T). Blood samples were collected before the starting of PUFAs supplementation at rest ($T0_R$), 10 minutes ($T0_{PE10}$), and 24 hours postexercise ($T0_{PE24}$) and after PUFAs supplementation at rest ($T1_R$), 10 minutes ($T1_{PE10}$), and 24 hours postexercise ($T1_{PE24}$). All horses showed a WBC increase after exercise ($P < .0001$). Higher Fb values were found in experimental groups than control groups at $T1_R$, $T1_{PE10}$, and $T1_{PE24}$ ($P < .005$). Serum amyloid A values changed ($P < .001$) at $T0_R$ and $T0_{PE10}$ vs. $T0_{PE24}$ and at $T1_R$ and $T1_{PE10}$ vs. $T1_{PE24}$ in experimental and control groups. Higher SAA values were found in experimental groups than control groups at $T1_R$, $T1_{PE10}$, and $T1_{PE24}$ ($P < .005$). Our findings showed that SAA concentration changed 24 hours after exercise, but it remains unchanged shortly after exercise. These results suggest that the evaluation of SAA immediately after exercise is not clinically useful and demonstrate that the intense exercise is able to induce an acute phase response although it is a noninflammatory condition. The significant effect of dietary PUFAs supplementation found on Fb and SAA levels emphases the PUFAs' property of modulating inflammation in jumper and Thoroughbred horses.

1. Introduction

The acute phase response (APR) is considered to be the first reaction of the body to immunological stress [1]. This is a dynamic process, involving systemic and metabolic changes that provide an early nonspecific defense mechanism against stressful events that cause changes in body homeostasis before specific immunity is achieved [2]. The response is formulated by a number of different acute phase proteins (APPs) that vary in magnitude and type among animal species [3]. During APR, the serum APPs concentration changes and physiological, biochemical, and metabolic modifications occur within the individual [4] to reestablish homeostasis and to promote healing [5]. Acute phase proteins as serum amyloid A (SAA) and fibrinogen (Fb) have been shown to be valuable biomarkers of the APR as they tend to increase following pathological conditions

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such as inflammation, infection, neoplasia, trauma, and stressful events such as exercise and transportation [6]. Serum amyloid A, normally found complexed with lipoproteins, is one of the most conserved proteins in mammals, supporting the premise that it has a basic and key role in the innate immune system [5]. In addition, Fb, an essential protein in the coagulation cascade [7], has been recognized to be part of the APR [5]. Several studies examined the APPs after moderate or strenuous exercise both in humans [8] and equine species [9,10] because exercise is a stressful condition that can increase host's susceptibility to several diseases [11].

Over the past years, an increasing interest was paid to the use of dietary supplementation with polyunsaturated fatty acids (PUFAs) because these molecules are believed to enhance the performance of equine athletes [12]. These molecules also showed additional property of modulating inflammation both in humans [13] and animals [14,15]. Long-chain n-3 PUFAs are potentially potent anti-inflammatory agents because they decrease the production of inflammatory eicosanoids, cytokines, reactive oxygen species, and the expression of adhesion molecules [16]. A study on rats showed that omega-3-treated subjects showed lower white blood cell (WBC) count when compared with sucrose-treated rats [15]. Supplementation with PUFAs has been shown to modify the inflammatory response also in full-sized and miniature mares by reducing basal production of tumor necrosis factor-α [14].

In the present study, we aimed to assess the effect of exercise and dietary supplementation with PUFAs on SAA and Fb and on WBC count in jumper and Thoroughbred horses.

2. Materials and Methods

Sixteen healthy horses, eight Italian Saddlebred jumpers (8–11 years old, six geldings and two males, mean body weight 480 ± 20 kg) and eight Thoroughbreds (3–6 years old, three geldings and five males, mean body weight 370 ± 15 kg), were enrolled in this study with the informed consent of the owners. Before starting the study, horses were subjected to clinical examination and routine hematology and biochemistry at rest conditions, to ensure of their healthy status. All animals were housed in individual boxes (3.50 × 3.50 m) under natural spring photoperiod (sunrise at 05:30 hours and sunset at 18:30 hours) and 19°C to 22°C indoor temperature. Horses were fed three times daily (06.00 AM; 12.00 AM; and 06.00 PM), the food intake was about 2.8% of horse body weight, the forage-to-concentrate ratio was 60:40 [17], and water was available ad libitum. Forage was dried grass hay with crude protein 9%, crude fiber 35%, Ca 0.4%, and P 0.23%. Commercially available concentrates had crude protein 14%, crude fat 4.8%, crude fiber 9%, ash 9.6%, Ca 1.2%, P 0.6%, vitamins, and trace elements (per kg concentrate: Vit A 26,000IU; Vit D3 3,200; Vit E 170 mg; Vit K 2.5 mg; Vit B1 12 mg; Vit B2 18 mg; Vit B12 0.03 mg; PP 180 mg; Fe 115 mg; Cu 42 mg; Zn 170 mg; and Se 0.6 mg). Both jumpers and Thoroughbreds were randomly divided into two equal groups of four subjects each. The experimental groups A_J (jumpers) and A_T (Thoroughbreds) received the PUFAs supplementation (Omega Horse, NBF Lanes, Milan, Italy) 70 mL/d (total n-3 PUFAs 22.5 g; eicosapentaenoic acid [EPA]: 11.5 g; docosahexaenoic acid [DHA] 7.7 g), for 30 days (Table 1). Supplementation was appetizing, easily miscible with cereals, and each horse assumed the exact amount of supplement provided. The control groups B_J (jumpers) and B_T (Thoroughbreds) received no dietary supplement during the experimental period (30 days). Before starting the dietary supplement administration (T0) and at the end of the experimental period (T1), horses were subjected to simulated events: Jumpers performed a show jumping course of 700 m length with 13 fences (six verticals of 1.20–1.30 m in height; three oxers of 1.25 m in height and 1 m in spread; one triple combination of verticals of 1.25 m in height and oxer of 1.20 m in height and 1 m in spread); Thoroughbreds performed a 2,100-m race (average speed 700 m/minute). During the experimental period, animals continued their specific training programs for jumpers and Thoroughbreds, respectively [18]. From each horse, blood samples were collected by jugular puncture at rest conditions ($T0_{REST}$ and $T1_{REST}$), within 10 minutes from the end of exercise ($T0_{PE10}$ and $T1_{PE10}$) and after 24 hours from the end of exercise ($T0_{PE24h}$ and $T1_{PE24h}$). Blood samples were collected in three different vacutainer test tubes: a test tube containing ethylenediaminetetraacetic acid (EDTA) (Terumo Corporation, Tokyo, Japan) was used to assess WBC, red blood cell (RBC), and hematocrit (Hct); a test tube containing 3.8% sodium citrate (Terumo Corporation, Tokyo, Japan) was used to assess Fb; a test tube containing clot activators (Terumo Corporation, Tokyo, Japan) was used to assess serum total proteins (TPs) and SAA. White blood cell, RBC, and Hct were assessed from whole blood by using automatic counter (HeCo Vet C, SEAC, Florence, Italy).

Fibrinogen concentration was assessed on citrated plasma using a standard kit for coagulometer (Clot 2 coagulometer, SEAC, Florence, Italy). Serum TP concentration was determined by the biuret method using an Ultraviolet-Visible Spectrophotometer analyzer (SEAC, Slim, Florence, Italy), whereas SAA concentration was determined using enzyme-linked immunosorbent assay (ELISA) kit (Multispecies SAA ELISA kit, Tridelta PhaseTM range, Maynooth, Ireland) by means of a microtiter plate reader (EZ Read 400 ELISA, Biochrom, Cambridge, UK). All calibrators and samples were run in duplicate. Samples exhibited parallel

Table 1
Polyunsaturated fatty acids supplement Omega Horse composition.

Active Principle	Content %
α-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
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 unsaturated fatty acids	5.8
Vitamin E	0.2
Butylhydroxytoluene (BHT)	0.015