The Professional Animal Scientist 34:284–292
https://doi.org/10.15232/pas.2017-01700

Effect of n-3 polyunsaturated fatty acids on markers of inflammation in young horses in training

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ABSTRACT

To determine the effects of n-3 PUFA supplementation on markers of inflammation in young horses in training, 16 Quarter Horses (2 to 4 yr) were used in a randomized complete block design for a 140-d trial. Treatments consisted of a control diet (n = 8) fed at 1% BW or a treatment diet (n = 8) of concentrate fed at 0.75% BW and 700 g of a marine n-3 supplement formulated to provide 15 g of eicosapentaenoic acid and 20 g of docosahexaenoic acid. Exercise protocol was divided into 2 phases: phase 1 (d 0 to 110) consisted of early training and phase 2 (d 111 to 140) consisted of advance maneuvers. Synovial fluid was obtained from the carpal joint every 28 d and analyzed for white blood cell count, total protein, and specific gravity. Blood samples were also collected at 28-d intervals for fatty acid analysis by gas chromatography, and concentrations of carboxypeptide type II collagen (CPII) and chondroitin sulfate-846 (CS-846) were determined by ELISA. Data were analyzed using the MIXED procedure of SAS. Plasma eicosapentaenoic acid and docosahexaenoic acid increased ($P \leq 0.01$) in response to supplementation. However, diet did not affect serum CPII or CS-846 nor synovial white blood cell count, total protein, and specific gravity. Levels of CS-846 tended to increase over time ($P = 0.09$) and CPII concentration increased ($P < 0.01$) in response to changes in exercise. These results indicate further studies are needed to determine the efficacy of n-3 supplementation as a preventative measure against development of osteoarthritis.

Key words: equine, exercise, inflammation, n-3 fatty acid

INTRODUCTION

Inflammatory joint disorders are considered to be a common cause of lameness in horses as well as the leading cause for early retirement of athletic horses (Jeffcoat et al., 1982). Young horses have the greatest rate of cartilage formation and can easily repair and replace damaged joint tissue (Brama et al., 2000). However, during early training and exercise, horses are subjected to large mechanical forces that can potentially lead to an imbalance between the synthesis and degradation of articular cartilage. The enzymatic breakdown of articular cartilage results in the release of inflammatory mediators including cytokines and eicosanoids that are synthesized through the cyclooxygenase pathway.

The n-3 PUFA such as eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**) compete with n-6 PUFA during metabolism to produce less potent inflammatory compounds that are associated with degenerative joint disease. A previous study using arthritic horses demonstrated the effectiveness of n-3 supplementation to mitigate inflammation (Manhart et al., 2009). However, little information exists concerning supplementation to juvenile horses in training.

Determining the efficacy of n-3 supplementation as a preventative measure against the development of joint disease in young horses poses great difficulty. Often, performance characteristics are difficult to measure objectively and may not be sensitive enough to detect the early stages of joint damage. However, markers of cartilage metabolism may provide insight to the effectiveness of n-3 supplementation. Carboxypeptide of type II collagen (**CPII**) and chondroitin sulfate 846 (**CS-846**) concentrations can be determined as synthesis of type II collagen and proteoglycans. In an attempt to repair cartilage, these biomarkers are released at increased concentrations into synovial fluid and blood (Frisbie et al., 1999). Synovial fluid concentrations of white blood cells (**WBC**), specific gravity (**SG**) and total protein (**TP**) have also been used as markers of inflammation and increase in response to articular cartilage damage (Bertone et al., 2001). Therefore, the objective of this study was to determine the effect of n-3 fatty acid supplementation on markers of joint inflammation in young horses in training.

MATERIALS AND METHODS

Horses and Dietary Treatments

Sixteen American Quarter horses (mares and geldings) from the Texas A&M University Horse Center herd were used in a randomized complete block design for a 140-d

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trial. Horses ranged from 2 to 4 yr and weighed between 357 and 439 kg, and they were initially blocked by BW, age, and sex. All care and sampling of horses was approved by the Texas A&M University Institutional Animal Care and Use Committee.

Horses were randomly assigned within block to 1 of 2 dietary treatments. Dietary treatments were formulated to be isocaloric and isonitrogenous with a control diet (n = 8) that consisted of a 12% CP pelleted concentrate (Producer's Cooperative Association, Bryan, TX) at 1% BW as fed per day. The treatment diet (n = 8) consisted of the pelleted concentrate fed at 0.75% BW (as fed) with an additional 700 g/d of a commercial marine-based n-3 supplement (JBS United Feeds Inc., Sheridan, IN) mixed with the concentrate immediately before feeding. The supplement was formulated based on previous studies (Ross et al., 2007; Manhart et al., 2009) to provide 15 g of EPA and 20 g of DHA per day. All horses received approximately 1% BW (as fed) coastal bermudagrass hay (*Cynodon dactylon*) per day with ad libitum access to water.

All horses were housed individually in 3 × 3 m stalls and fed their respective dietary treatments individually at 12-h intervals, with intakes and refusals measured daily. Horses consumed an average of 3.9 kg/d of concentrate and approximately 8.0 kg/d of hay. Refusal of the treatment diet was negligible and limited primarily to the first 4 d of the trial. Body weight and BCS were also recorded every 2 wk, and concentrate intake was adjusted accordingly. Individual BCS were determined every other week using the 1 to 9 scale described by Henneke et al. (1983). Grain, hay, and supplement were sampled throughout the trial, and a composted sample was analyzed for nutrient content, including GE (Table 1) and fatty acid concentrations (Table 2).

Table 1. Gross energy content (Mcal/kg) of diet components

Dietary component	GE
Concentrate[1]	3.85
Coastal bermudagrass hay	3.97
Supplement[2]	5.10

[1]12% CP pelleted concentrate (Producer's Cooperative Association, Bryan, TX).

[2]Commercial marine-based n-3 product (JBS United Feeds Inc., Sheridan, IN).

Exercise Protocol

Phase 1: Ground Work and Early Training. Exercise was conducted in conjunction with an equine behavior and training class. All horses were randomly assigned to students in the class (n = 13) or to graduate students (n = 3) affiliated with the project. Horses were exercised 5 d/wk for a total of 30 to 40 min in accordance with the course requirements.

During the first 9 wk of the trial, exercise activities consisted of longing at 10- to 20-min intervals with only initial saddling and mounting for 10 to 20 min. Riding activities during this time consisted of walking and steady jogging. As the semester progressed, longing time decreased (10 to 15 min) and more emphasis was placed on increasing duration and intensity of riding (20 to 25 min). Riding throughout the semester was conducted as a group, which consisted primarily of walking, trotting, and loping circles in both directions as well as some straight-line work.

Table 2. Unsaturated fatty acid profile (mg/g) of concentrate, coastal bermudagrass hay, and marine-based n-3 supplement

Fatty acid[1]	Concentrate[2]	Coastal bermudagrass hay	Supplement[3]
C16:1 (PA)	0.01	17.2	0.44
C18:1 (OA)	6.71	18.21	0.81
C18:2n-6 (LA)	16.08	8.46	1.47
C18:3n-3 (ALA)	1.37	3.23	1.70
C20:1 (ESA)	0.18	1.87	0.01
C20:4n-6 (ARA)	0.01	1.98	0.01
C20:3n-6 (DGLA)	0.01	0.25	0.01
C20:5n-3 (EPA)	0.01	0.50	21.78
C22:6n-3 (DHA)	0.01	0.48	24.70

[1]PA = palmitoleic acid, OA = oleic acid, LA = linoleic acid, ALA = α-linolenic acid, ESA = eicosenoic acid, ARA = arachidonic acid, DGLA = dihomo-γ-linolenic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid.

[2]12% CP pelleted concentration (Producer's Cooperative Association, Bryan, TX).

[3]Commercial marine-based n-3 product (JBS United Feeds Inc., Sheridan, IN).