Dietary fish oil supplementation affects serum fatty acid concentrations in horses

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J Anim Sci 2007.85:2183-2189.
doi: 10.2527/jas.2006-528 originally published online May 15, 2007;

Dietary fish oil supplementation affects serum fatty acid concentrations in horses[1]

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ABSTRACT: Thirteen horses of Thoroughbred or Standardbred breeding were used to study the effect of dietary fish oil supplementation on blood lipid characteristics. Horses were assigned to either fish oil (n = 7) or corn oil (n = 6) treatment groups for 63 d. The fish oil contained 10.8% eicosapentaenoic acid (EPA) and 8% docosahexaenoic acid (DHA). Each horse received timothy hay and a mixed-grain concentrate at rates necessary to maintain BW. Oil (corn or fish) was top-dressed on the concentrate daily at a rate of 324 mg/kg of BW. The n-6:n-3 ratio was approximately 3.6:1 for horses receiving the corn oil diet and 1.4:1 for horses receiving the fish oil diet. Horses were exercised 5 d/wk during the study. Before supplementation, there was no difference in the concentrations of any serum fatty acids between the 2 treatment groups. The mean basal concentrations of EPA and DHA on d 0 were 0.04 and 0.01 mg/mL, respectively. After 63 d, horses receiving the fish oil treatment, but not those receiving the corn oil treatment, had increased concentrations of EPA and DHA ($P < 0.05$). Fish oil supplementation for 63 d also increased the concentrations of C22:0, C22:1, and C22:5 fatty acids ($P < 0.05$). Overall, horses receiving fish oil had a decreased concentration of n-6 fatty acids ($P < 0.05$) and a greater concentration of n-3 fatty acids ($P < 0.01$), resulting in a lower n-6:n-3 fatty acid ratio after 63 d ($P < 0.05$). Serum cholesterol concentrations increased ($P < 0.05$) during the supplementation period in horses receiving the corn oil but not in horses receiving the fish oil. Compared with horses receiving corn oil, horses receiving fish oil had lower serum triglycerides at d 63 ($P < 0.05$). These results demonstrate that 63 d of fish oil supplementation at 324 mg/kg of BW was sufficient to alter the fatty acid profile and blood lipid properties of horses receiving regular exercise.

Key words: exercise, omega-3 fatty acid, fish oil, horse

J. Anim. Sci. 2007. 85:2183–2189
doi:10.2527/jas.2006-528

INTRODUCTION

Omega-3 fatty acids, particularly eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**), have been shown to have many beneficial effects in animals and humans. These effects include reducing the occurrence of lipid disorders and coronary artery disease and increasing red blood cell deformability, insulin sensitivity, and vascular compliance (Mueller and Talbert, 1988; Simopoulos, 1991). Many of these responses could influence the ability of horses to perform strenuous exercise. For the beneficial side effects to occur, EPA and DHA must be present in blood to be incorporated into other tissues. Fish oil supplementation has been shown to increase the serum concentration of EPA and DHA in proportion to the amount of fish oil fed (Ashes et al., 1992; Bjerve et al., 1993).

In addition to altering the fatty acid profile, supplementation of DHA and EPA can affect serum cholesterol and plasma triglyceride concentrations in some situations. Many studies in both rats (Surette et al., 1992; Fickova et al., 1998) and humans (Saynor and Gillott, 1992; Christensen et al., 1999) have demonstrated a decrease in circulating plasma triglycerides associated with n-3 fatty acid supplementation. Cholesterol is also sometimes lowered, often in hypercholesterolemic subjects (Saynor and Gillott, 1992). Conversely, addition of vegetable oil to horse diets has been associated with increased circulating cholesterol concentrations (Siciliano and Wood, 1993; Orme et al., 1997). It is relatively common to add vegetable oil to the diets of exercising horses, but few studies have reported the effects on plasma lipid characteristics.

[1]This paper was published as number 06-07-025 of the Kentucky Agricultural Experiment Station, and funding for this project was provided by Omega Protein (Hammond, LA) and the Kentucky Agricultural Experiment Station.

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Received August 2, 2006.
Accepted April 26, 2007.

Therefore, it was hypothesized that dietary lipid source (fish oil or corn oil) would affect serum concentrations of triglycerides, cholesterol, and individual fatty acids of horses undergoing a conditioning program.

MATERIALS AND METHODS

The experimental design and all procedures were approved by the Institutional Animal Care and Use Committee of the University of Kentucky.

Treatments

Thirteen mature geldings (11 Thoroughbreds and 2 Standardbreds), with an average BW of 542 ± 68 kg, were used in this study. Horses were individually housed in 4 × 15 m partially covered pens. The horses were acclimated to the individual pens and fed a basal diet of timothy hay and concentrate for 1 wk and then randomly assigned to either a corn oil treatment group (CO; n = 6) or a fish oil treatment group (FO; n = 7). More horses were initially allocated to the FO group in case some horses would not consume the fish oil. The treatment diets were created by top-dressing either menhaden fish oil (Omega Protein Inc., Hammond, LA) or corn oil (Hallway Feeds, Lexington, KY) on the morning concentrate at a rate of 324 mg of oil/kg of BW. No antioxidant was added to either the fish or corn oil. Horses received these treatments for 63 d. The daily intake of n-3 fatty acids was estimated to be 60 mg/kg of BW based on extrapolation from human studies (Vacek et al., 1989; Brilla and Landerholm, 1990; Raastad et al., 1997), and 324 mg of fish oil/kg of BW provided an equivalent amount of n-3 fatty acids. The amount of individual fatty acids consumed, on a milligrams per kilogram of BW basis, from each feed is listed in Table 1. The nutrient composition of the diet has been reported previously (O'Connor et al., 2004).

Before the adaptation period, horses had access to pasture and were fed the same concentrate used during the adaptation and experimental periods. The concentrate fed before the beginning of this study was 6.5% lipid. During the study, the amount of concentrate was adjusted as the workload increased. By the end of the study, horses were fed timothy hay (0700) at 1.7% of BW and concentrate at approximately 0.6% of BW (Hallway Feeds). The concentrate was divided into 2 meals per day, which were provided at 0700 and 1600. At the end of the study, the average DE intake of the horses was 28 Mcal of DE/d, with the added oil providing approximately 5.7% of the total DE. The daily DE intake was consistent with recommendations for horses at a moderate workload (NRC, 1989).

Table 1. Fatty acids supplied by each feedstuff[1] (mg of fatty acid/kg of BW)

Fatty acid	Textured feed	Timothy hay	Corn oil	Fish oil
C16:0	69.06	44.01	37.46	48.73
C16:1	0.35	0.73	0.44	32.74
C18:0	0.40	5.31	8.22	7.78
C18:1	157.84	11.14	74.0	26.43
C18:2	168.41	44.78	178.17	5.95
C18:3n-6	0.17	1.33	0.31	3.62
C18:3n-3	16.12	75.82	10.85	4.78
C20:0	2.02	6.68	1.16	1.86
C20:1	2.63	1.88	2.60	6.04
C20:2	0.31	2.70	0.31	3.51
C20:3n-6	0.12	1.01	0.12	1.56
C20:3n-3	0.11	1.88	ND[2]	3.46
C20:4	0.14	0.99	ND	1.84
C20:5	0.33	1.02	0.16	37.15
C22:0	1.16	4.71	0.57	1.34
C22:1	0.68	1.38	0.17	1.32
C22:2	0.36	2.96	<0.10	0.29
C22:4	0.18	0.74	0.88	0.97
C22:5	<0.10	2.16	0.34	5.82
C22:6	0.26	0.70	1.14	28.01
Total n-6	169.0	48.8	179.5	13.93
Total n-3	16.8	79.4	12.2	73.4
n-6:n-3 ratio	10.1	0.62	14.8	0.19

[1]Complete information on the nutrient content of the diet was reported previously (O'Connor et al., 2004).
[2]ND = not detectable.

Conditioning Program

Before the beginning of the study, the horses had been housed on pasture with no forced exercise for at least 3 mo. Once the horses were assigned to the dietary treatments, they began a conditioning program. During the first 5 wk, the horses were exercised 5 d/wk in a 14-m diam. round pen with sand footing. The horses were exercised in the round pen in pairs consisting of 1 horse from each treatment. During wk 1, the horses exercised at a trot for 10 min/d. During the subsequent weeks, the exercise time and intensity were increased so that by wk 5, the exercise time in the round pen increased to 30 min (10 min of cantering and 20 min of trotting) per day. Beginning during wk 6 and continuing until the end of the study, the horses were exercised 3 d/wk in the round pen for 30 min and 2 d/wk on a treadmill for 20 min. The treadmill workout consisted of a 5-min trot at 4.5 m/s, a 3-min canter at 6.5 m/s, a 5-min trot at 4.5 m/s, a 2-min canter at 6.5 m/s, and a 5-min trot at 4.5 m/s. On days on which the horses were not exercised, they were turned out in pairs for no more than 30 min in a 20 × 40 m dirt paddock.

Sample Collections

Blood samples were collected from the horses on 3 occasions: d 0 (before supplementation), 28, and 63. All blood samples were collected at 0700, before the horses were fed their morning meal, and there were at least 17 h between their last exercise bout and the blood sampling. Blood was analyzed for cholesterol, NEFA, triglycerides, white blood cell number, partial prothrombin time, prothrombin time, hemoglobin, platelet number, packed cell volume, and fatty acid profile.

Laboratory Analyses

Serum triglyceride concentrations were determined using a commercially available kit (Sigma Diagnostics, St. Louis, MO). Serum cholesterol and plasma NEFA concentrations were also determined with commercial kits (Cholesterol CII and NEFA C, Wako Chemicals, Richmond, VA).

To obtain the fatty acid profile, total lipids were extracted from 0.5 mL of serum or 0.5 g of ground feedstuff with 7.2 mL of hexane:isopropanol (J. T. Baker, Phillipsburg, NJ, 3:2, vol/vol, with 0.05% butyl hydroxytoluene), 3.6 mL of Na_2SO_4 solution (J. T. Baker, 1 g/15 mL of distilled water), and 0.05 mL of internal standard (Nu-Chek Prep, Elysian, MN, C15:0). Lipid extracts were then transmethylated with 14% boron trifluoride in methanol (Sigma-Aldrich, St. Louis, MO) to form fatty acid methyl esters using a slight variation of the procedure described by Morrison and Smith (1964). The methyl esters were concentrated by evaporation under N and resuspended in 200 μL of hexane in a gas chromatography vial. The sample vials were stored at –80°C until analysis.

The total lipid extracts were analyzed with a gas chromatograph (Shimadzu, Columbia, MD) equipped with a flame ionization detector and a column (i.d. 30 m × 0.25 mm, 0.22-μm film). Nitrogen was used as the carrier gas, with the flow rate at 1 mL/min and pressure at 79 mmHg. The injector core temperature was 250°C, and the detector temperature was 260°C. The column temperature was programmed to begin at 140°C and then increase to 240°C at a rate of 4°C/min and remain at 240°C for 20 min. Chromatograms were obtained and analyzed using chromatography software (Shimadzu). A standard (Nu-Chek Prep) containing fatty acids ranging from C14:0 to C24:1 was analyzed and used to calibrate the machine before running samples each day. Additionally, a quality control sample of pooled serum was analyzed with each batch of samples. The quality control had an interassay CV of 10% and an intraassay CV of 5%. Total n-3 and total n-6 fatty acids were calculated by summing the concentrations of the respective fatty acids. The fatty acids summed to obtain total n-3 fatty acids are as follows: 18:3n-3, 20:3n-3, 20:5, and 22:6. The fatty acids summed to obtain total n-6 fatty acids are as follows: 18:2, 18:3n-6, 20:3n-6, 20:4, and 22:4.

Statistical Analysis

Differences in serum triglycerides, NEFA, cholesterol, blood variables, and fatty acids between the FO and CO groups were analyzed using the mixed covariant test (mixed COVTEST) procedure for repeated measures (SAS Inst. Inc., Cary, NC). The main effects in the model included treatment, horse, and time. Treatment and time were considered fixed effects, and horse was considered a random effect. When the time × treatment interaction was significant, differences between treatments at individual time points and differences within treatments at time points were compared using the PDIFF of the least squares means with the slices option.

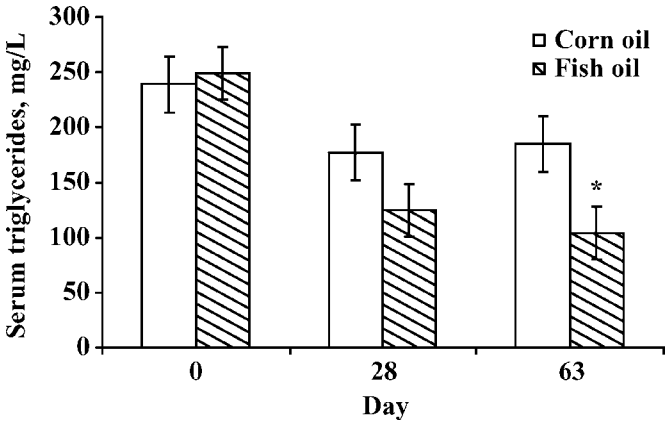

Figure 1. Serum triglyceride concentrations before treatments were imposed (d 0) and during the supplementation period when horses received the corn oil (n = 6) or fish oil (n = 7) treatment and regular exercise (treatment × time interaction, $P < 0.05$; *fish oil vs. corn oil, $P < 0.05$).

RESULTS

There was no difference between treatment groups on d 0 or 28; however, serum triglyceride concentrations were lower in the FO group than the CO group on d 63 (treatment × time, $P = 0.015$; Figure 1). In response to supplementation, plasma NEFA decreased in horses receiving FO but not in horses receiving CO (treatment × time, $P = 0.046$; Figure 2). However, plasma NEFA concentrations were not different among treatments on d 0, 28, or 63. Serum cholesterol concentrations were not different between treatment groups on d 0 (Figure 3). During the supplementation period, there was a time × treatment interaction ($P < 0.05$) on serum cholesterol concentration. Serum cholesterol concentration increased in horses receiving the CO treatment, whereas cholesterol concentration did not change in horses receiving the FO treatment. On d 28, CO-treated horses had greater serum cholesterol concentrations than horses receiving FO ($P < 0.05$).

Mean values for red and white blood cell number, platelet number, hemoglobin, packed cell volume, prothrombin time, partial prothrombin time, and percentage of lymphocytes are shown in Table 2. No treatment or treatment × time interactions were observed at any of the sampling times for any of the blood variables. Platelet number, prothrombin and partial prothrombin time, red blood cell number, and lymphocyte percentage did respond during the 63-d study (time effect; $P < 0.05$).

Before supplementation (d 0), there was no difference in individual serum fatty acid concentrations between

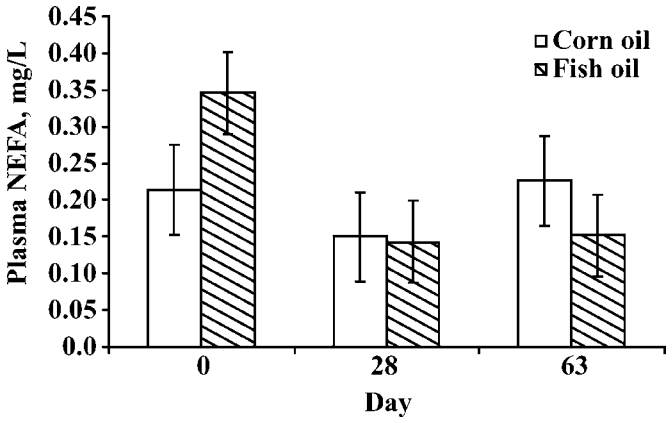

Figure 2. Plasma NEFA before treatments were imposed (d 0) and during the supplementation period when horses received the corn oil (n = 6) or fish oil (n = 7) treatment and regular exercise (treatment × time interaction, $P < 0.05$).

the treatment groups (Table 3). The mean basal concentrations of EPA (C20:5) and DHA (C22:6) on d 0 were 0.04 and 0.01 mg/mL, respectively. After 63 d of supplementation, horses receiving the FO displayed increased EPA and DHA ($P < 0.05$). The horses receiving CO did not exhibit any change in the concentrations of EPA and DHA in serum after 63 d of supplementation (P = 0.21 and 0.89, respectively). After the 63-d supplementation period, the FO-treated horses had increased concentrations of C20:3n-3 ($P < 0.10$), C22:0, C22:1, C22:4, and C22:5 ($P < 0.05$). Horses receiving CO had increased serum concentrations of C18:0 (P = 0.08) and C18:2 ($P < 0.05$) and decreased concentrations of C20:4 and C20:3n-6 ($P < 0.05$). The CO horses also tended to have decreased concentrations of C22:5 ($P < 0.10$). Horses receiving FO had increased amounts of total n-3 fatty acids (0.24 on d 0 to 0.34 mg/mL on d 63; P = 0.014) in the serum, whereas horses receiving CO had increased amounts of total n-6 fatty acids (2.3 mg/mL on d 0 to 2.6 mg/mL on d 63; P = 0.05). The serum ratio of n-6 to n-3 fatty acids was not different between groups on d 0, but after 63 d of supplementation, the ratio increased in the CO horses from 9.3 ± 1.2 to 15.2 ± 1.2 ($P < 0.05$) and decreased in the FO horses from 9.4 ± 1.2 to 5.9 ± 1.1 ($P < 0.05$).

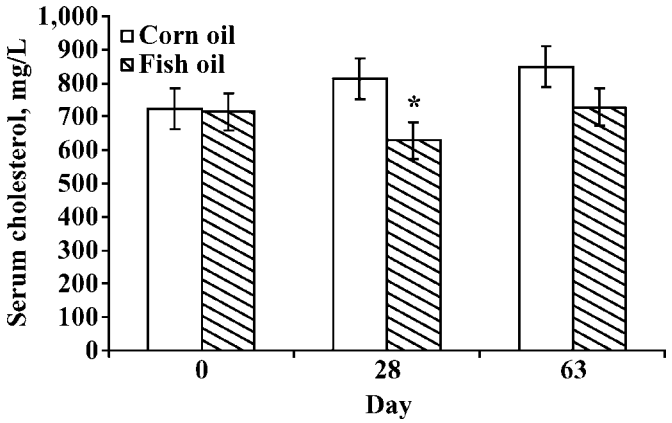

Figure 3. Serum cholesterol concentrations before treatments were imposed (d 0) and during the supplementation period when horses received the corn oil (n = 6) or fish oil (n = 7) treatment and regular exercise (treatment × time interaction, $P < 0.05$; *fish oil vs. corn oil, $P < 0.05$).

DISCUSSION

Red blood cell count and lymphocytes did exhibit a time effect; however, the values were not outside the reference ranges for equine blood (Lassen and Swardson, 1995). Partial prothrombin time, prothrombin time, and platelet count also exhibited time effects. It has been shown that racehorse training may increase red blood cell numbers, hemoglobin, and packed cell volume (Allen and Powell, 1983), but similar changes have not been observed with endurance training (Rose and Hodgson, 1982). It is likely that these changes over time resulted from an effect of training.

The major objective of this study was to compare the effects of 63 d of fish oil and corn oil supplementation on blood lipid properties of regularly exercised horses. Decreased serum triglycerides are the most consistently reported result of fish oil supplementation in humans (Saynor and Gillott, 1992; Christensen et al., 1999) and rats (Fickova et al., 1998). In this study, after 63 d of supplementation, FO-treated horses had reduced serum triglycerides compared with CO-treated horses. The exact mechanism responsible for this decrease has not yet been elucidated; however, many studies have shown that n-3 fatty acids downregulate enzymes associated with triglyceride synthesis (Marsh et al., 1987; Surette et al., 1992). Other researchers have also reported decreased triglyceride concentrations when horses were fed soybean oil-supplemented diets (Orme et al., 1997; Geelen et al., 1999). This decrease was attributed to increased lipoprotein lipase activity and a possible increase in fatty acid oxidation. In horses, very low density lipoprotein (**VLDL**) particles are the main transporters of triglycerides and contain 57% triglycerides and 15% phospholipids compared with low-density lipoprotein particles, which contain 5.5% triglycerides and 22% phospholipids (Watson et al., 1993). Increasing the activity of lipoprotein lipase might increase the clearance of the triglyceride-rich VLDL particles from the bloodstream, resulting in decreased circulating triglycerides. However, lipoprotein lipase activity was not measured in this study, so it is unknown as to whether triglyceride concentrations were regulated by the activity of lipoprotein lipase or by decreased triglyceride synthesis.

In contrast to the decrease in serum triglyceride concentration, serum cholesterol concentrations did not change over time in the FO treatment but increased over time in the CO-fed horses. Omega-3 fatty acids

Table 2. Hematological response of exercising horses supplemented with fish oil (FO; n = 7) or corn oil (CO; n = 6) treatments

Item	Treatment	d 0	d 28	d 63	SEM
Packed cell volume, %	FO	36.0	35.9	38.0	0.91
	CO	36.5	37.0	40.5	0.99
Hemoglobin, g/dL	FO	12.6	12.5	13.1	0.35
	CO	12.9	13.0	14.0	0.38
Platelet number, No./μL	FO	107,000	98,600	85,400	9,300
	CO	116,000	95,800	84,000	8,600
Prothrombin time,[1] s	FO	9.57	12.2	8.9	0.25
	CO	9.83	12.0	8.4	0.27
Partial prothrombin time,[1] s	FO	50.7	54.0	41.3	1.9
	CO	48.0	50.5	37.8	2.0
Red blood cells,[1] million/μL	FO	7.24	7.31	7.78	0.26
	CO	6.99	7.15	7.91	0.28
White blood cells, No./μL	FO	6,400	6,800	6,640	410
	CO	6,820	6,770	6,500	440
Lymphocytes,[1] %	FO	43.3	31.4	42.6	3.5
	CO	39.2	35.5	40.5	3.8

[1]Variable responded during the 63-d study; time effect, $P < 0.05$.

Table 3. Serum fatty acid profiles of exercising horses before and after 63 d of supplementation with corn oil (CO; n = 6) or fish oil (FO; n = 7)

	d 0[1]		d 63		
Fatty acid	CO, mg/mL	FO, mg/mL	CO, mg/mL	FO, mg/mL	SEM
C14:0	0.041	0.040	0.033	0.029	0.0034
C14:1	0.0027	0.0025	0.0018	0.0027	0.0008
C16:0	0.67	0.61	0.61	0.47	0.039
C16:1	0.042	0.044	0.024	0.036	0.0043
C18:0	0.64[a]	0.60	0.72[b,x]	0.56[y]	0.043
C18:1	0.56	0.51	0.42	0.25	0.032
C18:2	2.2[c]	2.1	2.6[d,x]	1.9[y]	0.19
C18:3n-6	0.017	0.015	0.016	0.015	0.0014
C18:3n-3	0.14	0.14	0.092	0.048	0.0140
C20:0	0.018	0.016	0.018	0.016	0.0020
C20:1	0.017	0.020	0.010	0.0087	0.0023
C20:2	0.022	0.022	0.071	0.017	0.0221
C20:3n-6	0.020[c]	0.017	0.016[d,x]	0.020[y]	0.0013
C20:3n-3	0.054	0.055[a]	0.048[x]	0.063[b,y]	0.0049
C20:4	0.029[c]	0.027	0.0091[d]	0.027	0.0123
C20:5	0.050	0.028[c]	0.027[x]	0.14[d,y]	0.0132
C22:0	0.038	0.016[c]	0.015[x]	0.055[d,y]	0.0128
C22:1	0.276	0.218	0.286	0.316	0.0747
C22:2	0.012	0.0076	0.040	0.015	0.0146
C22:4	0.011	0.0070[c]	0.0075	0.012[d]	0.0026
C22:5	0.017[a]	0.012[c]	0.010[b,x]	0.022[d,y]	0.0030
C24:0	0.015	0.021	0.021	0.025	0.0103
C22:6	0.0092	0.019[c]	0.0073[x]	0.091[d,y]	0.0074
Total n-6	2.3[c]	2.1	2.6[d,x]	1.9[y]	0.20
Total n-3	0.25	0.24[c]	0.18[x]	0.34[d,y]	0.025
n-6:n-3	9.3[c]	9.4[c]	15.2[d,x]	5.9[d,y]	1.17

[a,b]Within-treatment difference d 0 vs. d 63 ($P < 0.10$).
[c,d]Within-treatment difference d 0 vs. d 63 ($P < 0.05$).
[x,y]Between-treatment difference on d 63 ($P < 0.05$).
[1]Treatment means on d 0 did not differ ($P > 0.10$).

have been shown to have a cholesterol-reducing effect in hypercholesterolemic animals, most likely through reduction in liver synthesis of cholesterol or increased clearance of triglyceride-rich VLDL cholesterol (Warner et al., 1989). Saynor and Gillott (1992) supplemented hyperlipidemic humans with 1.8 g of EPA/d and showed that cholesterol concentrations were decreased in hypercholesterolemic subjects after 3 mo. However, cholesterol concentrations were unchanged after 3 mo of fish oil supplementation in subjects that began the study with normal cholesterol. Similarly, men with normal cholesterol levels receiving 4 g of n-3 fatty acids/d for 10 wk did not display any decrease in cholesterol levels (Brilla and Landerholm, 1990). Serum cholesterol concentrations are normally low in horses but are increased when vegetable oil is added to the diet. Siciliano and Wood (1993) reported that serum cholesterol increased in horses fed a soybean oil-supplemented diet. Orme et al. (1997) reported an increase in serum cholesterol associated with soybean oil supplementation, which was attributed to an increase in hepatic cholesterol synthesis. The cholesterol response of the corn oil-fed horses in this study is consistent with the findings of Orme et al. (1997).

In the current study, there was no difference in concentrations of individual serum fatty acids between the 2 treatment groups on d 0. After 63 d of treatment, horses receiving the FO treatment exhibited an increase in concentration of EPA and DHA, whereas horses receiving CO did not exhibit any change in the concentrations of EPA and DHA in their serum. In sheep, fish oil supplementation has been shown to increase the serum concentration of EPA and DHA in proportion to the amount of fish oil fed (Ashes et al., 1992). The NEFA profile of equine blood will also vary according to the fatty acid composition of the diet (Orme et al., 1994). In the current study, the serum fatty acids profile of both treatment groups changed with the dietary fatty acid profile, which concurs with previous research. Overall, the horses receiving FO had a lower concentration of n-6 fatty acids and a greater concentration of n-3 fatty acids, resulting in a lower n-6:n-3 fatty acid ratio. An optimal ratio of n-6:n-3 has yet to be determined for horses and may vary depending on developmental stage and health status. Suggested ratios range from 4 to 10 for humans (Albertazzi and Coupland, 2002). In 1995, Japan added a recommended n-6:n-3 ratio of 4 to their dietary allowances (Sugano, 1996). The control diet in this study had a n-6:n-3 ratio of 3.6, whereas the fish oil diet had a ratio of 1.4.

This study demonstrated that 63 d of fish oil supplementation at a rate of 324 mg/kg of BW per day was sufficient to alter serum triglyceride concentration and the fatty acid profile of exercising horses. Additionally, dietary fatty acids had an effect on the serum fatty acid profile, which indicates that it may also affect fatty acid content of other tissues. More research is needed to identify potential benefits of fish oil supplementation for horses. As further n-3 research is conducted in horses, these observations may be helpful in determining an optimum level of fish oil supplementation.

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