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The effect of dietary fish oil supplementation on exercising horses

C. I. O’Connor1, L. M. Lawrence, A. C. St. Lawrence, K. M. Janicki, L. K. Warren, and S. Hayes

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ABSTRACT: Ten horses of Thoroughbred or Standardbred breeding were used to study the effects of dietary fish oil supplementation on the metabolic response to a high-intensity incremental exercise test. Horses were assigned to either a fish oil (n = 6) or corn oil (n = 4) treatment. The fish oil (Omega Protein, Hammond, LA) contained 10.6% eicosapentaenoic acid and 8% docosahexaenoic acid. Each horse received timothy hay and a textured concentrate at a rate necessary to meet its energy needs. The supplemental oil was top-dressed on the concentrate daily at a rate of 324 mg/kg BW. Horses received their assigned diet for 63 d, during which time they were exercised 5 d/wk in a round pen or on a treadmill. During wk 1, horses exercised for 10 min at a trot. After wk 1, exercise time and intensity were increased so that at wk 5, exercise time in the round pen increased to 30 min (10 min of cantering and 20 min of trotting) per day. Starting at wk 6, horses were exercised 3 d/wk in the round pen for 30 min and 2 d/wk on a treadmill for 20 min. After 63 d, all horses performed an exercise test consisting of a 5-min warm-up at 1.9 m/s, 0% grade, followed by a step test on a 10% grade at incremental speeds of 2 to 8 m/s. Blood samples were taken throughout exercise. During exercise, horses receiving fish oil had a lower heart rate (treatment × time interaction; \( P < 0.05 \)) and tended to have lower packed cell volume (treatment effect; \( P = 0.087 \)). Plasma lactate concentrations were not affected by treatment. Plasma glucose concentrations were not different between groups during exercise but were lower (treatment × time interaction; \( P < 0.01 \)) for the fish oil group during recovery. Serum insulin tended to be lower in fish oil horses throughout exercise (treatment effect; \( P = 0.064 \)). There was a tendency for glucose:insulin ratios to be higher for fish oil-treated horses throughout exercise (treatment effect; \( P = 0.065 \)). Plasma FFA were lower (treatment × time interaction; \( P < 0.01 \)) in horses receiving fish oil than in horses receiving corn oil during the initial stages of the exercise test. Serum glycerol concentrations also were lower in fish oil-treated horses \( (P < 0.05) \). Serum cholesterol concentrations were lower in horses receiving fish oil (treatment effect; \( P < 0.05 \)), but serum triglycerides were not affected by treatment \( (P = 0.55) \). These data suggest that addition of fish oil to the diet alters exercise metabolism in conditioned horses.

Key Words: Dietary Fat, Equine, Exercise, Fish Oil, Horse, Omega-3 Fatty Acids

Introduction

Because of its energy density, fat is often added to the diets of exercising horses; however, little attention has been given to the effects of dietary fatty acid (FA) composition on the metabolic responses of exercising horses. The most common fats used in horse diets are corn oil and soybean oil, although animal fat has been used. These fats are not good sources of the omega-3 FA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Conversely, fish oil is an excellent source of EPA and DHA. In other species, these dietary omega-3 FA have had beneficial effects, including decreasing blood lipid concentrations and increasing membrane fluidity and insulin sensitivity (Mueller and Talbert, 1988; Simopoulos, 1991). Many researchers have hypothesized that omega-3 FA supplementation would provide benefits during exercise. Lortet and Verger (1995) reported that exercised rats receiving fish oil had a decreased resting heart rate and mean aortic pressure compared with rats fed lard or sunflower oil. Brilla and Landerholm (1990) reported that fish oil increased maximal oxygen consumption \( (\text{VO}_{2\text{max}}) \) in men who exercised 3 h/wk for 10 wk. However, Raastad et al. (1997) found that 10.5 wk of fish oil supplementation did not affect endurance, heart rate, or \( \text{VO}_{2\text{max}} \) of professional male soccer players.

The amount of omega-3 FA given to humans has varied. Raastad et al. (1997) provided approximately 2.6 g/d, whereas Brilla and Landerholm (1990) provided 4...
g/d. Supplementation rates as high as 9 g/d have been used in patients with coronary artery disease (Vacek et al., 1989). Because no previous studies on omega-3 FA supplementation of horses had been conducted, we estimated that an appropriate daily intake for horses would be about 60 mg of omega-3 FA/kg BW. Therefore, the objective of this project was to investigate whether 9 wk of omega-3 FA supplementation at a rate of 60 mg/kg BW would affect the metabolic response of horses to exercise.

**Materials and Methods**

**Treatments**

Ten mature geldings (one Standardbred and nine Thoroughbreds) with a BW of 560 ± 63 kg and previous treadmill experience were used in this study. All horses remained clinically healthy and sound for the duration of the study. Horses were individually housed in 4 × 15-m partially covered pens, except in the week before exercise testing when they were individually housed in box stalls. The experimental design and all procedures were approved by the Institutional Animal Care and Use Committee of the University of Kentucky. Before the start of the experiment, horses were acclimated to a basal diet of timothy hay and concentrate for 1 wk, and then randomly assigned either to a corn oil (n = 6) or fish oil (n = 4) treatment group. More horses were initially allocated to the fish oil group in case some horses would not consume the fish oil. Horses were fed timothy hay (0700) at 1.7% BW (as-fed) and textured concentrate (barley, corn, soybean meal, molasses, oats, and supplement pellet) at approximately 0.6% BW (0700 and 1630; Hallway Feeds, Lexington, KY). During the study, the amount of concentrate was adjusted as workload increased. The concentrate was divided into two meals per day at 0700 and 1600. At the end of the study, the diet supplied, on average, 28 Mcal of DE/d, two meals per day at 0700 and 1630; Hallway Feeds, Lexington, KY). During wk 6, 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, 3-min canter at 6.5 m/s, 5-min trot at 4.5 m/s, 2-min canter at 6.5 m/s, and a 5-min trot at 4.5 m/s. On days the horses were not exercised, they were turned out in pairs for no more than 30 min in a 20- × 40-m dirt paddock.

**Exercise Testing Procedures**

At the end of wk 8, horses were moved to the exercise testing facility. Horses were housed in box stalls and exercised only on a high-speed treadmill (Sato I, Equine Dynamics, Lexington, KY). Three days per week, the horses completed a 30-min workout consisting of a 5-min walk at 1.9 m/s on a 0% grade followed by a 5-min trot at 4.5 m/s, 3-min canter at 6.5 m/s, 5 min at 4.5 m/s, 2 min at 6.5 m/s, 5 min at 4.5 m/s, and then 5 min at 1.9 m/s. Two days per week, the horses completed a 23-min workout consisting of 5 min at 1.9 m/s on a 0% grade followed by a 4-min, 4-m/s trot on a 10% grade, 2 min at 5 m/s, 2 min at 6 m/s, and then, while at a walk, the treadmill was lowered to a 0% grade, and the workout was finished with 4 min at 4 m/s and 5 min at 1.9 m/s. During wk 10, each horse performed a standard

**Conditioning Program**

Before the start of the study the horses had been housed on pasture with no forced exercise for at least 3 mo. Once horses were assigned to dietary treatments, they began a conditioning program. During the first 5 wk, horses were exercised 5 d/wk in a 14-m diameter round pen with sand footing. During wk 1, horses exercised at a trot for 10 min/d. During the following weeks exercise time and intensity was increased so that at wk 5, exercise time in the round pen increased to 30 min/

<table>
<thead>
<tr>
<th>Itema</th>
<th>Textured feedb</th>
<th>Timothy hay</th>
<th>Corn oilb</th>
<th>Fish oilc</th>
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<tbody>
<tr>
<td>DM</td>
<td>89.1%</td>
<td>91.2%</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ether extract</td>
<td>6.5%</td>
<td>1.7%</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CP</td>
<td>14.8%</td>
<td>9.3%</td>
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<td>—</td>
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<tr>
<td>DE, Mcal/kg</td>
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<td>2.02</td>
<td>8.98</td>
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<tr>
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<td>17.8%</td>
<td>58%</td>
<td>—</td>
<td>—</td>
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<tr>
<td>ADP</td>
<td>9.4%</td>
<td>23.5%</td>
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<td>Ca</td>
<td>0.93%</td>
<td>0.49%</td>
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</tr>
<tr>
<td>P</td>
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<td>0.26%</td>
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</tr>
<tr>
<td>K</td>
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<td>1.83%</td>
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<tr>
<td>Na</td>
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<td>0.002%</td>
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<td>0.091</td>
<td>1.034</td>
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<td>3.224</td>
<td>1.365</td>
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<tr>
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<td>0.344</td>
<td>0.532</td>
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<td>0.950</td>
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<td>0.356</td>
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<td>0.760</td>
<td>0.100</td>
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<tr>
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<td>0.060</td>
<td>0.247</td>
<td>0.339</td>
<td>8.008</td>
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</tbody>
</table>

*aPatty acids are listed as a percentage of the lipid in the sample.
bObtained from Hallway Feeds, Lexington, KY.
cObtained from Omega Protein, Inc., Hammond, LA.

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exercise test. All exercise tests were conducted in a temperature-controlled testing facility (20°C) on a high-speed treadmill between 0700 and 1100 each testing day. Each exercise test began with a 5-min warm-up walk (1.9 m/s), with the treadmill surface in a horizontal position. During the fifth minute, the treadmill was elevated to a 10% (6°) incline. The treadmill speed was increased to 2 m/s for the start of the exercise test. Every 2 min thereafter, the treadmill speed was increased 1 m/s until 8 m/s (a moderate gallop) was reached. Upon completion of 2 min at 8 m/s, the treadmill speed was decreased to 1.9 m/s and the treadmill surface was returned to horizontal. The horse then completed a 10-min cool-down walk at 1.9 m/s. The horse was taken off the treadmill and hand-walked for an additional 20 min.

To ensure that all horses would be tested in a similar feeding state, all feed was removed 12 h before the exercise test. Additionally, horses were not fed their morning ration on the day of testing. On the morning of testing, an indwelling catheter was placed in the left jugular vein of each horse under local anesthesia for blood sampling. Blood samples were taken before the exercise test (Pre) and during the last 20 s of the warm-up and each exercise speed. Additional blood samples were taken every 2 min for the first 10 min of cool-down, and then every 5 min for the last 20 min of hand walking. Blood samples were also taken 1, 3, 6, 8, and 24 h after the completion of the 8.0-m/s portion of the exercise test. Before exercise testing, horses were also fitted with an on-board heart rate monitor (Equistat HR-SAE, EQB, Unionville, PA). Heart rate was recorded during the last 5 s of each speed during the exercise test and every 2 min during the first 10 min of the cool down.

**Laboratory Analyses**

Plasma was analyzed simultaneously for glucose and lactate with a glucose/lactate autoanalyzer (YSI 2300 STAT PLUS, Yellow Springs Instrument Co., Yellow Springs, OH). Serum triglyceride, glycerol, creatine kinase (CK), and aspartate amino transferase (AST) concentrations were determined using commercially available kits (Sigma Diagnostics, St. Louis, MO). The triglyceride assay is based on the measurement of glycerol, thus all triglyceride values were corrected for glycerol present in each sample. Serum cholesterol and plasma FFA concentrations were also determined through the use of commercial kits (Cholesterol CII and NEFA C, Wako Chemicals, Richmond, VA). Serum insulin concentrations were determined using a solid phase 125I RIA (Coat-A-Count, Diagnostic Products Co., Los Angeles, CA). Packed cell volume was determined with whole blood that was drawn into a microcapillary tube, centrifuged, and read using a microcapillary reader. All laboratory procedures were performed in duplicate except packed cell volume, which was performed in triplicate. Interassay variation was <5% for all assays except glycerol, which had an interassay variation of <10%.

**Statistical Analyses**

Differences in plasma and serum variables between the corn and fish oil groups were analyzed using the SAS (SAS Inst., Inc., Cary, NC) mixed covariance test (mixed COVTEST) procedure with repeated measures. The mixed covariance test was used because of unequal treatment sizes. The class variables were defined as horse, treatment, and time. Time was the repeated variable and the covariance structure was defined as compound symmetry. Data were separated into exercise and recovery samples and analyzed separately. Significance was declared at $P < 0.05$, and because of the small number of horses in each treatment, trends were recognized at $0.05 < P < 0.10$. Differences between treatments at individual time points were examined whenever treatment × time interactions had $P < 0.10$. Data were summarized as least squares means ± SEM.

**Results**

In both treatments, heart rate increased as treadmill speed increased (time effect; $P < 0.01$; Figure 1). Resting heart rates were the same for both treatment groups, but horses receiving the fish oil treatment had lower heart rates throughout the exercise test (treatment × time interaction; $P < 0.05$). By 2 min after exercise, the heart rates for both treatment groups were similar and remained the same throughout recovery. Packed cell volume increased throughout the exercise test (time
effect; $P < 0.01$) in both treatment groups (Figure 2). There was a trend for horses receiving the fish oil treatment to have lower packed cell volume during exercise (treatment effect; $P = 0.087$). Plasma lactate concentration (Figure 3) increased during exercise resulting in peak concentrations of over 12.0 mmol/L at 2 min after exercise in both groups (time effect; $P < 0.01$). Fish oil supplementation did not affect plasma lactate concentration during exercise ($P = 0.62$). During recovery, lactate concentrations decreased similarly in both groups (time effect; $P < 0.01$).

At the onset of exercise, plasma glucose concentrations were not different from horses receiving the corn oil treatment ($P = 0.95$). Plasma glucose concentration increased slightly throughout exercise and continued to increase during early recovery in both treatment groups (time effect; $P < 0.01$; Figure 4). During recovery, plasma glucose concentration of the corn oil-treated horses continued to rise until 10 min after exercise, whereas the plasma glucose concentration of the fish oil-treated horses leveled out at 4 min after exercise. Horses fed the fish oil treatment had lower glucose concentrations from 10 min after exercise to the end of recovery at 30 min after exercise (treatment × time interaction; $P < 0.01$).

Serum insulin (Figure 5) decreased throughout exercise in both groups (time effect; $P < 0.01$), and there was a trend for insulin concentrations to be lower in fish oil-supplemented horses (treatment effect; $P = 0.064$). Plasma glucose to serum insulin ratios were calculated (data not shown) and found to increase throughout exercise in both groups (time effect; $P < 0.01$). There was a trend for glucose:insulin ratios to be higher for the fish oil-treated horses throughout exercise (treatment effect; $P = 0.065$). Plasma FFA concentrations (Figure 6) decreased throughout exercise in both treatments (treatment × time interaction; $P < 0.01$) and were lower for fish oil-supplemented horses at rest, 1.9, 2, 3 ($P < 0.05$), 4, and 5 m/s ($P < 0.10$) than for the corn oil-supplemented horses. Serum glycerol concentrations (Figure 7) increased throughout exercise for both treatments (time effect; $P < 0.01$) and were lower in horses fed the fish oil treatment (treatment effect; $P < 0.05$).

Serum cholesterol was lower (treatment effect; $P < 0.05$) for fish oil-supplemented horses (Figure 8). Serum triglyceride concentrations (Figure 9) increased during...
Figure 5. Serum insulin response before (Pre), during the exercise test, and following the completion of the exercise test (Post) when horses received either the corn oil (n = 4) or fish oil (n = 6) treatment. Insulin concentrations decreased throughout exercise in both treatments (time effect; \( P < 0.01 \)). There was a trend for fish oil-treated horses to have lower insulin concentrations (treatment effect; \( P = 0.064 \)).

Exercise (time effect; \( P < 0.01 \)), but there were no effects of fish oil supplementation. The AST and CK activities increased in both treatments (time effect; \( P < 0.01 \)) until approximately 6 h after exercise, and then began to decrease (data not shown). Neither variable increased above normal levels, nor were they affected by fish oil treatment.

Discussion

The effects of fish oil supplementation have been studied extensively in a variety of animals, but not horses. Because previous studies in horses did not exist, both the level of fish oil supplementation and the length of the supplementation period were extrapolated from human studies. Based on the FA composition of the fish oil (Table 1) used in this experiment, the omega-3 FA intake for a 500-kg horse in this study was 30 g/d. Small amounts of EPA and DHA were found in the textured feed, timothy hay, and corn oil. The fish oil contained 10.6% EPA and 8.0% DHA. Consequently, fish oil was the only significant source of EPA and DHA in this study. The approximate ratio of n-6 to n-3 FA in the total diet was 3.6:1 for the corn oil treatment and 1.4:1 for the fish oil treatment.

During the exercise test, horses in the fish oil treatment had lower heart rates throughout the exercise
Figure 9. Serum triglyceride concentration before (Pre), during the exercise test, and following the completion (Post) of the exercise test when horses received either the corn oil (n = 4) or fish oil (n = 6) treatment. Fish oil supplementation did not affect on serum triglyceride concentrations during exercise.

Lortet and Verger (1995) observed that rats receiving dietary fish oil and regular exercise had decreased resting heart rates and blood pressure. They suggested that increased production of endothelium-derived relaxing factor and/or a decrease in blood viscosity could explain their results. Demaion et al. (2000) found that rats consuming fish oil had reduced heart rate, aortic flow, and cardiac output due to a decrease in vascular resistance. During the exercise test, fish oil-supplemented horses tended to have lower packed cell volumes. Blood viscosity was not measured, but horses with a lower packed cell volume should also display a lower blood viscosity, which might have resulted in the lower heart rates. Also, there is accumulating evidence in humans that fish oil has a cardioprotective effect (decreased susceptibility to arrhythmias and fibrillation), which may be mediated by changes in ion currents in the myocardium as a result of altered FA composition (Carroll and Roth, 2002). There is also some evidence that fish oil may alter the activity of catecholamines (Mori et al., 2000), which are important for regulation of heart rate during exercise.

In this study, lower heart rates in the fish oil treatment were combined with similar plasma lactate concentrations, suggesting that the differences in heart rate did not affect the availability of oxygen to the working muscles. It has been shown that during treadmill exercise, time to fatigue is directly related to the intensity of exercise, and that higher heart rates are associated with a faster time to fatigue (Hodgson et al., 1990).

In humans, extensive research has been conducted on factors affecting insulin sensitivity. Omega-3 FA supplementation is one method that has been shown to increase insulin sensitivity in a variety of animals. Miniature pigs fed 9.5 g/d of omega-3 FA were found to have increased insulin sensitivity in response to a glucose tolerance test (Behme, 1996). Similarly, rats fed 7% of their diet as fish oil exhibited increased insulin sensitivity in response to a glucose tolerance test (Chicco et al., 1996). Liu et al. (1994) supplemented rats with 3.3% of their diet as omega-3 FA for 6 wk and observed an increase in the amount of omega-3 FA in the skeletal muscle sarcolemma and a 14-fold increase in insulin binding. These researchers suggested that increasing the amount of omega-3 FA in the cell membrane might alter the action of insulin and insulin receptors resulting in increased insulin sensitivity. In the current study, the fish oil-treated horses had higher glucose to insulin ratios throughout exercise. This may indicate that the fish oil-treated horses had an increase in insulin sensitivity.

Increasing insulin sensitivity alters glucose uptake and storage. Several theories have been examined to explain this: alterations in the FA composition of membranes, changes in the quantity of insulin receptors in muscle cell membranes, a variation in insulin-stimulated glycogen synthesis in muscle cells, and changes in the type or quantity of second messengers generated by the cell (Borkman et al., 1993). Field et al. (1990) found that the amount and rate of glucose transported per binding of insulin was greater in rats fed diets high in polyunsaturated FA, resulting in a subsequent increase in glucose oxidation. Increased insulin sensitivity has been shown to decrease fasting blood FFA and triglyceride levels in humans (McCarty, 1998). In this study, the fish oil-treated horses had lower FFA concentrations than did the corn oil-treated horses following a 12-h fast. Circulating FFA concentrations represent the balance between mobilization from adipose tissue and use by muscle. The lower level of circulating FFA could be due either to decreased FA mobilization or to an increased use of the FFA for energy production. However, horses in the fish oil treatment group also had lower serum glycerol concentrations after the 12-h fast. Glycerol can be used as a marker of FA mobilization, and low serum glycerol concentrations are associated with decreased FA mobilization. Taken together the glycerol and FFA responses suggest that fish oil decreased FA mobilization.

Circulating triglyceride levels were no lower in fish oil-treated horses than in corn oil-treated horses. In humans, the triglyceride-lowering effect of fish oil in appears to be most pronounced in individuals with hypertriglyceridemia. The lack of a triglyceride-lowering effect of fish oil in horses might simply be a result of their normally low plasma triglycerides compared with humans. However, serum cholesterol concentrations were decreased by fish oil supplementation in this study, which is consistent with many reports in humans. Although there is no known clinical significance to lowered cholesterol concentrations in exercising horses, the difference between treatments suggests that the amount and duration of fish oil supplementation was sufficient to produce metabolic effects.

Because omega-3 FA have been shown to increase membrane fluidity in muscle cells, it was hypothesized...
that omega-3 FA supplementation might alter susceptibility to exercise induced muscle damage. Creatine kinase and AST are intracellular enzymes in muscle cells not typically present in serum. Large increases of these enzymes in the serum following exercise are indicative of muscle damage. Hock et al. (1987) found a significant decrease in the amount of CK released from myocardial cells following ischemic damage to the heart in rats fed 5% of their diet as fish oil for 4 wk. Studies performed in rodents suggest that EPA may be able to prevent muscle damage through inhibition of the cycloxygenase pathway (Jackson et al., 1988; Tisdale, 1996). However, in these studies, muscle damage was induced through artificial methods, and therefore their results may not be transferable to exercise-induced muscle damage. In the horse, a 20- to 200-fold increase in CK is indicative of ultrastructural damage to muscle cells (Valberg, 1996). In the current study, both CK and AST activities increased in the serum post exercise; however, the values were still within the normal range. It is possible that a longer, lower-intensity exercise test would be a better model for evaluating the effect of fish oil on susceptibility of horses to muscle damage (Siciliano, et al. 1995).

Literature Cited
