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Circulating fatty acid profiles in response to three levels of dietary omega-3 fatty acid supplementation in horses[1]

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ABSTRACT: Fatty acids of the n-3 type confer health benefits to humans and other species. Their importance to equine physiology could include improved exercise tolerance, decreased inflammation, and improved reproductive function. The circulating fatty acid profile and the acquisition and washout of fatty acids in response to n-3 supplementation were determined for horses in the current study. A fatty acid supplement high in eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid was fed to deliver EPA plus DHA at 0 (control), 10, 20, or 40 g/d to 16 mares (n = 4/group) for 28 d. Plasma was collected at –11, 3, 7, 10, 16, 23, 30, 37, 44, 70, and 87 d relative to the beginning of supplementation. Plasma was analyzed for the presence of 35 fatty acids by gas chromatography. Plasma EPA and DHA increased ($P < 0.05$) in a dose-responsive manner by 3 d of feeding and reached peak concentrations by 7 d. Peak EPA and DHA concentrations of the 40 g/d supplement group were approximately 13× and 10× those of controls, respectively. Plasma EPA and DHA demonstrated a steep decline ($P < 0.05$) from peak values by 9 d after cessation of supplementation and were near presupplementation values by 42 d. Omega-3 supplementation also increased ($P < 0.05$) concentrations of fatty acids C14:0, C17:1n-7, C18:1*trans*-11, C18:3n-6, C18:4n-3, C20:3n-6, C20:4n-6, and C22:5n-3 and decreased ($P < 0.05$) concentrations of C18:1*cis*-9 fatty acid. Seasonal effects, apparently unrelated to supplementation and likely due to the availability of fresh forage, were also noted. Unlike ruminants, there were no detectable concentrations of CLA in equine plasma. These results indicate that the circulating fatty acid milieu in horses can be influenced through targeted supplementation. Possible implications of increased n-3 plasma and tissue concentrations on specific physiological function in the equine remain to be elucidated.

Key words: docosahexaenoic acid, eicosapentaenoic acid, horse, omega-3 fatty acid, polyunsaturated fatty acid

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INTRODUCTION

Omega-3 fatty acids (**FA**), particularly eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**), confer a number of health benefits to humans, including increased vascular compliance, antihypertensive and antiatherosclerotic effects (Robinson and Stone, 2006). Improved vascular compliance (Portier et al., 2006) from EPA/DHA might assist exercise-induced hypertension and associated pulmonary hemorrhage in the equine. Omega-3 FA supplementation lowered heart rate (O'Connor et al., 2004) in exercising horses, which may delay fatigue. In humans, n-3 FA inhibit the production of cytokines involved with chronic inflammatory and autoimmune diseases (Simopoulos, 2002). Similar effects in horses may be useful in combating the acquisition and progression of osteoarthritis associated with athletic function (Munsterman et al., 2005). Omega-3 FA supplementation increased stride length (Woodward et al., 2005) in exercising horses and decreased inflammatory markers in arthritic horses (Manhart et al., 2007). Indicators of pulmonary inflammation were also decreased (Khol-Parisini et al., 2007). Supplemental EPA and DHA have been associated with positive effects on fertility and fetal development in several species (Abayasekara and Wathes, 1999). In the stallion, n-3 FA supplementation improved seminal characteristics (Brinsko et al., 2005; Harris et al., 2005). Omega-3 balance can affect eicosanoid biochemistry and as such may have an effect on reproductive (luteal) function in the mare. We know little about the naturally occurring concentration of n-3 FA in horses, the amount of EPA and DHA required

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Table 1. Delivery (g/d) of n-6, n-3, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) at a protected fatty acid supplement volume of 681 g/d as fed

	Supplement[1]			
Item	0 g (control)	10 g	20 g	40 g
n-3 (g)	4.98	17.21	29.23	53.09
n-6 (g)	4.15	3.29	2.71	4.78
n-6:n-3	0.83:1	0.19:1	0.09:1	0.09:1
EPA (g)	0.37[2]	5.15	9.92	17.81
DHA (g)	0.43[2]	6.15	11.75	21.32

[1]Protected fatty acid supplementation was delivered from a marine-derived preparation (JBS United, Sheridan, IN) designed to deliver EPA plus DHA at a combined concentration of 0 (control), 10, 20, or 40 g/d.
[2]Reflects slight carryover from the mixer.

to influence circulating FA concentrations, or the time frame over which FA profiles are influenced. The objective of this study was to define the circulating FA profile for the horse and the acquisition and washout of FA in response to 3 levels of n-3 supplementation.

MATERIALS AND METHODS

All animal procedures were reviewed and approved by the Southern Illinois University, Carbondale, Institutional Animal Care and Use Committee.

Light horse mares (n = 16) of differing breeds (Arabian, Holsteiner, Quarter Horse, Standardbred, and Thoroughbred), 4 to 22 yr of age and 387 to 591 kg of BW, were divided among 4 groups (n = 4 each). The groups were balanced for age, BW, BCS, and metabolic rate as much as practicable, with each group containing at least 1 lighter-built, higher-metabolism horse (Arabian-type) and 2 heavier-built, slower-metabolism horses (Quarter Horse or Standardbred). Average group BW were 511 ± 81, 513 ± 125, 465 ± 57, and 499 ± 32 kg. Each group also contained a younger (≤10 yr) and older (≥15 yr) horse; most horses were 10 to 17 yr of age. All horses began the study at a BCS of ≥5 (Henneke et al., 1983) and maintained their BCS throughout the study (beginning and ending average BW was 497 ± 109 and 485 ± 112 kg, respectively). The study was conducted from February 6 to May 14, 2004. Mares were allowed ad libitum access to grass hay and water, with pasture access during the study period.

Protected FA (**PFA**) supplementation was delivered from a marine-derived preparation (JBS United, Sheridan, IN) designed to deliver EPA plus DHA at a combined concentration of 0 (control), 10, 20, or 40 g/d (Table 1). The dry powdered supplement was mixed with molasses to improve its texture and palatability and was offered mixed with a minimal amount (≤0.5 kg) of whole oats. The supplement was fed individually at a rate of approximately 681 g/d (1.5 g/kg of BW) once daily for 28 d. The supplement was introduced during an initial acclimation period of approximately 1 wk (d –7 to d 0 of the study), wherein small volumes (<0.25 kg) of supplement were introduced once daily; after acclimation, supplement refusal was rare.

Whole blood was collected on –11, 3, 7, 10, 16, 23, 30, 37, 44, 70, and 87 d relative to commencement of full-supplement feeding (full feeding = d 1). This provided for measuring FA profile changes for approximately 60 d after the cessation of supplementation. Exactly 10 mL of blood was collected via jugular venipuncture into heparinized (0.25 mL, 1,000 IU/mL) syringes. Plasma was separated by centrifugation (2,500 × *g* for 15 min), and 2 mL of plasma was pipetted into duplicate 16 × 125-mm glass tubes and stored frozen (–20°C) until prepared for analysis.

Frozen plasma samples were lyophilized (FreeZone 6 Liter Freeze Dry System, Labconco Corp., Kansas City, MO) in 16 × 125-mm glass culture tubes, sealed with Teflon-lined caps and stored at –15°C. Before methylation, 1.0 mL of hexane containing 0.59 mg of methyl 10-nonadecenoate (Nu-Chek Prep, Elysian, MN) was added as an internal standard. Fatty acid methyl esters (**FAME**) were formed by in situ transesterification according to the methods of Dionisi et al. (1999). Briefly, 4.0 mL of 0.75 *N* methanolic HCl was added to the freeze-dried samples and incubated for 18 h at 25°C. The hexane layer containing the FAME was then dried over anhydrous Na_2SO_4 and stored at –15°C.

The analysis for FAME was performed on a GC-2010 gas chromatograph equipped with an AOC-20i automatic injector, an AOC-20s automatic sampler, a split injection port, a flame ionization detector (Shimadzu Scientific Instruments, Columbia, MD), and a 100-m SP-2560 fused silica capillary column (0.25 mm i.d. × 0.2 mm film thickness; Supelco, Bellefonte, PA). An injection volume of 1.0 mL was split 1:20, and the He carrier gas was maintained at a linear velocity of 23 cm/s throughout the analysis. The column was held at 170°C for 50 min after injection and then programmed at 5°C/min to a final temperature of 249°C for 10 min. The injector and detector were set at 255°C.

A preliminary screening analysis was performed on plasma samples from all 16 horses in the study, obtained before n-3 FA supplementation (d –11) and at 24 d of supplementation. A panel of 57 FA were screened (C10:0 through C24:1n-9), including isomers of C18:1, CLA, and C18:2n-6. The reference standard used included *trans*-6, *trans*-9, *trans*-11 C18:1, and all 4 c/t 9, 12-C18:2 isomers (Supelco). No peaks corresponding to any of these reference standards were observed in plasma except 18:1 11*trans* and 18:2n-6. This allowed the development of a streamlined analysis of only 35 FA (omitting detection for conjugated isomers undetected above) that was used for all samples.

Chromatograms were examined for the presence of the 35 FAME by comparing the peak retention times with those of a standard prepared from pure FAME (Resteck, Bellefonte, PA; Nu-Chek Prep; Supelco; Larodan Fine Chemicals, Malmo, Sweden). Fatty acids were quantified (mg) and divided by the internal standard

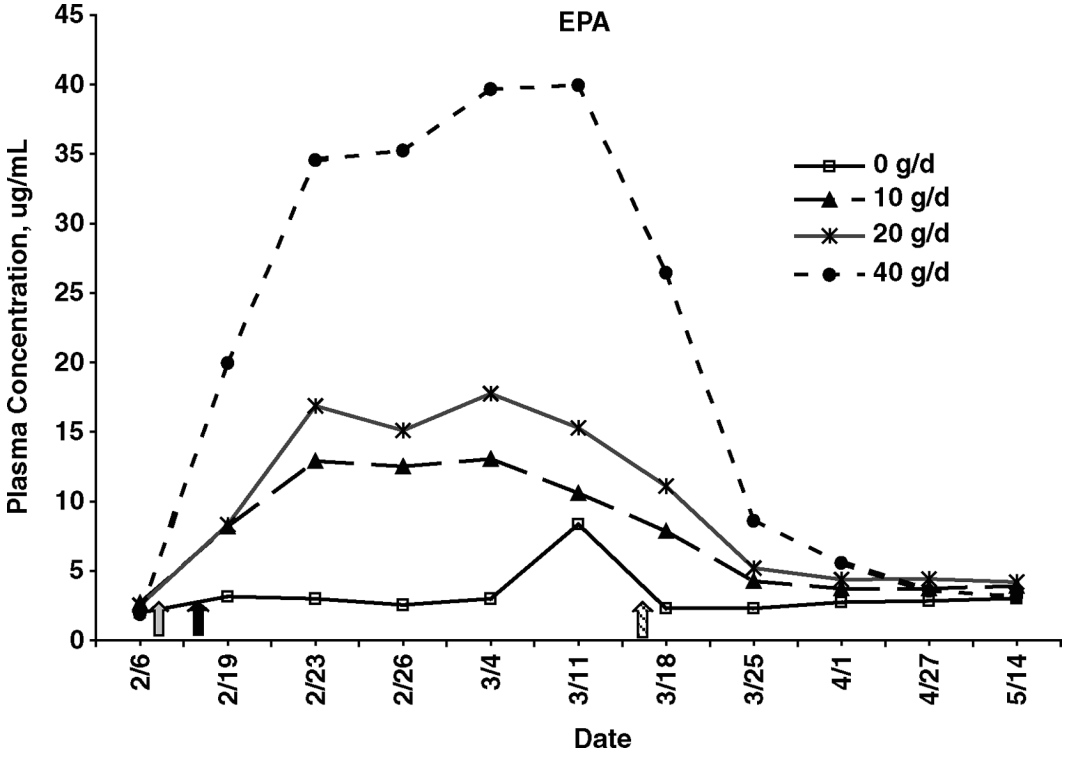

Figure 1. Plasma eicosapentaenoic acid (EPA) concentrations in response to protected fatty acid supplementation in mares. Black arrow indicates the commencement and slashed arrow the cessation of full-supplement feeding. Gray arrow indicates the period of acclimation to the diet. Concentrations of EPA in response to all levels of supplementation were different ($P < 0.05$) from those of the nonsupplemented control (0 g/d) by 7 d of supplementation (2/23) except for the 10 and 20 g/d doses on 3/11. SEM = 2.14 (0 g/d); 2.15 (10 g/d); 2.40 (20 g/d); and 2.14 (40 g/d); EPA demonstrated treatment ($P < 0.001$), day ($P < 0.001$), and day × treatment ($P < 0.001$) effects.

peak area. The mean recovery from a quantitative standard containing the 35 FAME was 100.6% (SD = 4.55%).

Individual FAME results were calculated as the concentration (mg/mL) in plasma and as a percentage of total plasma FA. Analysis of variance was conducted using the MIXED procedure (SAS Inst. Inc., Cary, NC) for a completely randomized design with repeated measures. Data collected presupplementation were found to be statistically different between horses, and therefore, the covariance analysis was used. The model contained the effects of covariance, treatment, day, and treatment × day interaction. Least squares means were used to compare treatment means. Additionally, preplanned linear and quadratic effects were tested. Significance was declared at $P \leq 0.05$.

RESULTS

One-third (11 of 35) of the FA analyzed demonstrated a response to treatment. Nearly 2/3 (22 of 35) of the FA analyzed demonstrated a time-related effect. Only 2 FA, 14:1n-5 *cis* and 16:1n-5 *trans,* showed no response at all (data for these not shown).

A preliminary FA profile analysis indicated that CLA isomers (C18:2 *cis*-9, *trans*-11; *trans*-8, *cis*-10; *cis*-11, *trans*-13; *trans*-10, *cis*-12; *cis*-8, *cis*-10; n-9 *cis*-11; *cis*-9, *cis*-11; *cis*-10, *cis*-12; *cis*-11, *cis*-13; *trans*-11, *trans*-13) were not observed in the plasma of horses in any treatment group (results not shown).

The greatest plasma concentration change was detected in the targeted n-3 FA, EPA (C20:5n-3) and DHA (C22:6n-3). Supplemented mares displayed an increase ($P < 0.05$) in circulating EPA (Figure 1) and DHA (Figure 2) by 3 d of supplementation and progressed to a sustained and dose-related peak level by 7 d of n-3 FA feeding. The pattern of EPA and DHA increase displayed a significant ($P < 0.0004$) linear effect. When horses were supplemented at a rate of 40 g of EPA and DHA per day, peak plasma EPA concentrations reached over 13× control values, and peak DHA concentrations were approximately 10× control values. Plasma EPA and DHA demonstrated a steep (~4-fold) decline from peak values by 9 d after cessation of feeding and were not different ($P > 0.05$) from presupplementation levels by 42 d postsupplementation.

Marine-derived n-3 FA supplementation increased the plasma concentrations of other FA of the n-3 type (Figures 3 and 4). These were C22:5n-3 (docosapentaenoic acid) and C18:4n-3 (stearidonic acid). Two other n-3 FA, 18:3n-3 and 20:3n-3, did not respond to treatment. Six additional FA, C14:0, C17:1n-7, C18:1*trans*-11 (transvaccenic acid), C18:3n-6, C20:3n-6, and

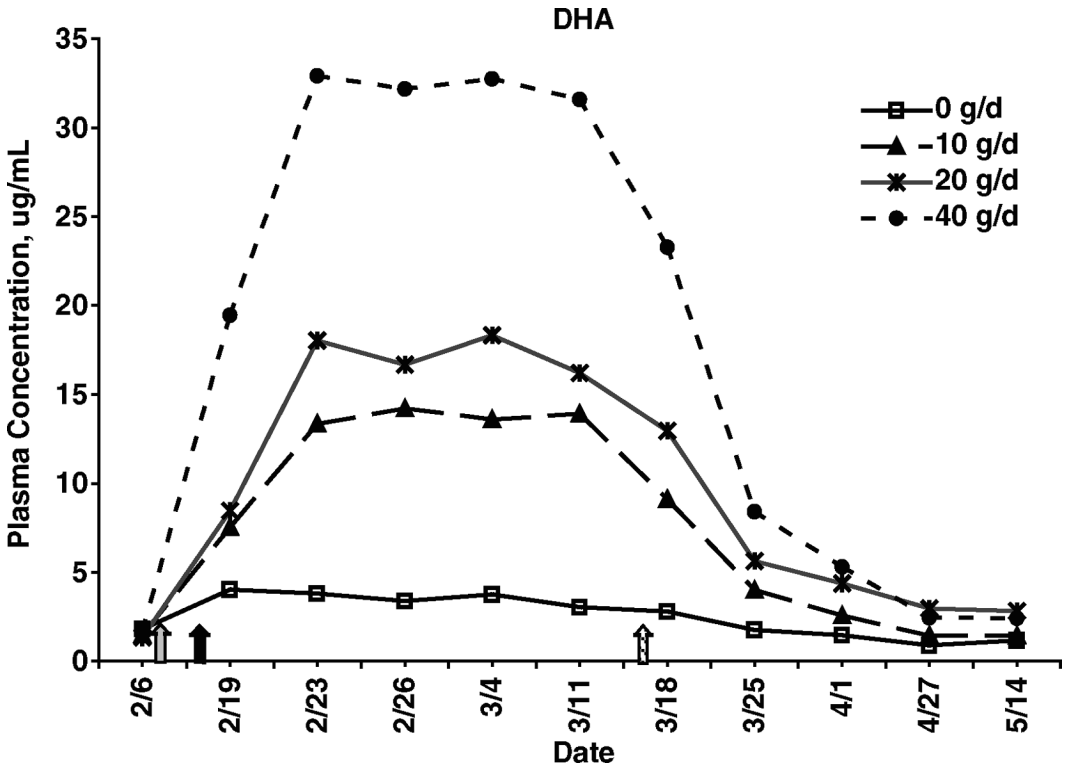

Figure 2. Plasma docosahexaenoic acid (DHA) concentrations in response to protected fatty acid supplementation in mares. Black arrow indicates the commencement and slashed arrow the cessation of full-supplement feeding. Gray arrow indicates the period of acclimation to the diet. Concentrations of DHA in response to all levels of supplementation were different ($P < 0.05$) from those of the nonsupplemented control (0 g/d) during and to 9 d after (3/25) the supplementation period. SEM = 1.99 (0 g/d); 1.94 (10 g/d); 2.17 (20 g/d); and 1.94 (40 g/d); DHA demonstrated treatment ($P < 0.0001$), day ($P < 0.0001$), and day × treatment ($P < 0.0001$) effects.

C20:4n-6 (arachidonic acid) increased ($P < 0.05$) in response to treatment, and 1, C18:1*cis*-9, decreased ($P < 0.05$; Table 2). Significant ($P < 0.01$) linear effects were noted for all of these FA except C14:0 and C17:1n-7, each of which displayed quadratic effects ($P < 0.02$).

Horses consuming a typical (control) diet had greater plasma concentrations ($P < 0.05$) of n-6 FA compared with n-3 FA (mean percentage of total FA = 47.18% n-6 vs. 6.34 n-3 FA, Table 3). Twenty-four days of n-3 FA supplementation increased ($P < 0.05$) the overall n-3 FA concentrations (μg/mL) in the plasma and reduced the ratio of n-6:n-3 FA from a mean of 8.0:1 in control mares to 6.4:1 in mares fed 10 g/d and 20 g/d and to 4.5:1 in mares fed 40 g/d (Table 3).

Twenty FA demonstrated a significant time effect only, and an additional 2 displayed a time effect as well as a time × treatment interaction (Table 4). These 22 FA appeared to respond to seasonal influences. The plasma concentrations of many of these FA increased or decreased across all treatment groups, including controls, beginning around March 11 and continued to change after the period of supplementation was discontinued on March 16.

DISCUSSION

This research indicates that dietary supplementation of a specific marine-derived PFA source of EPA and DHA can rapidly and significantly increase the circulating concentrations of these n-3 FA as well as other FA in horses. Circulating concentrations of EPA and DHA were proportional to the level of supplementation. Similar results were reported in sheep (Ashes et al., 1992). Plasma concentrations of these FA are labile, however. Plasma concentrations of up to a 13-fold increase of EPA and DHA equilibrated at their peak levels by 7 d after commencement of supplementation and declined by up to 75% by 9 d after supplement cessation. Others (Harris et al., 2005) reported a longer time (30 d) to attain peak n-3 FA plasma concentrations in a small population of stallions after supplementation of a similar compound. The current study indicates that the rate of plasma accretion of EPA and DHA in horses appears to be a more rapid response than in humans (Arterburn et al., 2006). Elevation of EPA and DHA in plasma, particularly at greater supplementation levels, persisted for up to 2 wk past supplement withdrawal but was not different from presupplement levels by 6 wk. This rate of plasma clearance generally agrees with results of fish oil supplementation of yearling horses (Vineyard et al., 2007), wherein plasma DHA but not EPA remained elevated beyond baseline through 5 wk, but not 8 wk, postsupplementation.

Other n-3-type FA that responded to PFA supplementation were likely the result of ingestion rather than

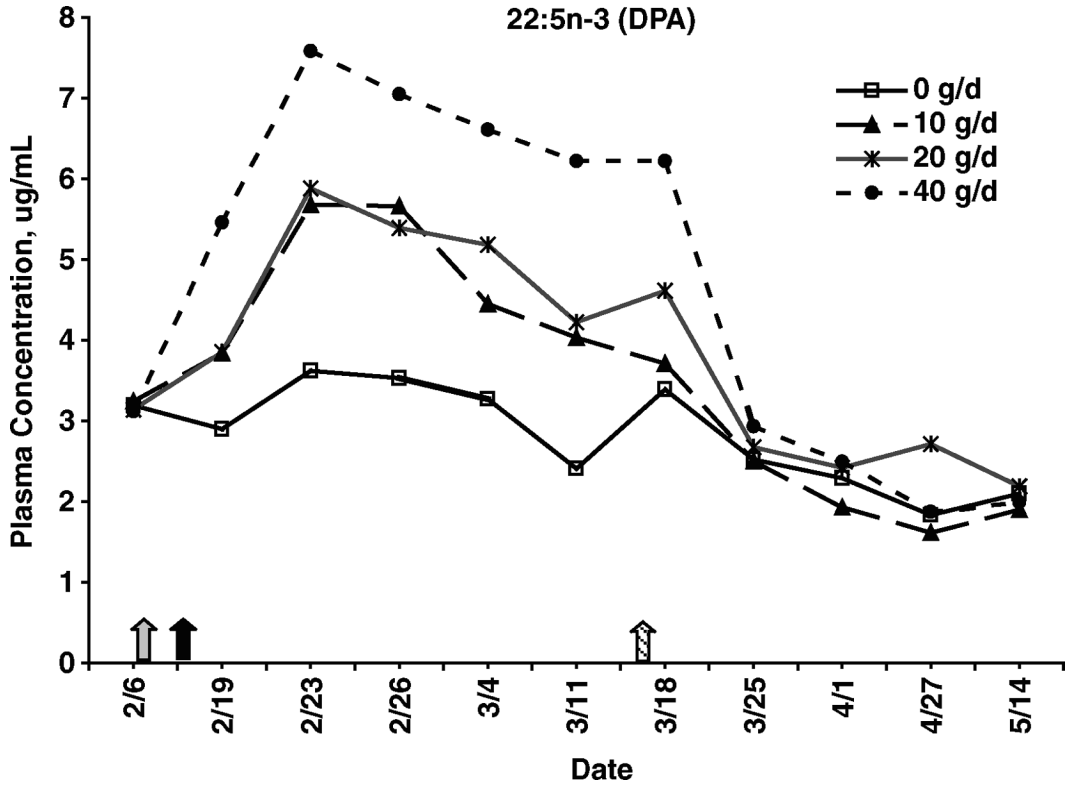

Figure 3. Plasma docosapentaenoic acid (DPA) concentrations in response to protected fatty acid supplementation in mares. Black arrow indicates the commencement and slashed arrow the cessation of full-supplement feeding. Gray arrow indicates the period of acclimation to the diet. Concentrations of DPA in response to 20 and 40 g/d supplementation were different ($P < 0.05$) from the nonsupplemented control (0 g/d) after 7 d of supplementation (2/23). The 10 g/d dose differed from the control on all treatment days, except 3/4 and 3/11. SEM = 0.4 (0 g/d); 0.41 (10 g/d); 0.45 (20 g/d); and 0.41 (40 g/d); DPA demonstrated treatment ($P < 0.004$), day ($P < 0.001$), and day × treatment ($P < 0.001$) effects.

conversion from other FA. For example, C18:4n-3 and C22:5n-3 were present in greater concentration in the treatment supplements compared with the control (Table 5). The opposite situation was also true in the case of the 2 FA of the n-3 type, 18:3n-3 (linolenic acid) and 20:3n-3. The concentration of linolenic acid was much greater in the control diet than in the PFA supplements, and 20:3n-3 was not detectable in the treatment or control supplements (Table 5). This may explain the lack of response to PFA supplementation for these 2 n-3 FA (Table 4).

There is a complex interaction between FA of the n-3 group and the n-6 group. One of these relationships is a competition for enzymes controlling metabolic pathways between these 2 groups of PUFA. An overabundance of n-6 FA is associated with negative cardiovascular, inflammatory, and other effects in humans and other species (Hayes, 2001; Robinson and Stone, 2006). The n-3 group of FA, and EPA and DHA in particular, are associated with the opposite, beneficial effects on cardiovascular, inflammatory, neurological, reproductive, and other functions (Newton, 2001). In general, increased concentrations of circulating n-3 FA pull the FA enzyme pathways toward a healthier mix of metabolites. A 5:1 ratio of n-6:n-3 FA is considered optimal for humans (Newton, 2001), particularly if the n-3 FA are supplied in the form of EPA and DHA.

A recommended n-6:n-3 FA ratio for horses has not been reported, although data from humans and other species (Newton, 2001) indicate that dietary supplementation to increase the concentration of n-3 FA could have beneficial health effects for the equine. Nonsupplemented horses in the present experiment consumed a diet considered to be typical for average management practices in the United States (fresh or cured grass forage plus <0.5 kg of whole oats), which resulted in a plasma n-6:n-3 FA ratio of approximately 7.5:1 during the winter when horses were consuming grass hay and approximately 6:1 during the spring when horses were consuming a greater amount of fresh spring grass. The n-6:n-3 ratio was decreased with specific n-3 supplementation. The supplement containing the greatest concentrations (40 g/d) of EPA and DHA and the lowest n-6:n-3 ratio increased circulating n-3 FA concentrations sufficiently to shift the n-6:n-3 ratio to 4.5:1. If we apply the human recommendations of a 5:1 n-6:n-3 ratio to the equine, the present experiment indicates that a PFA supplementation level of 30 to 35 g/d of EPA

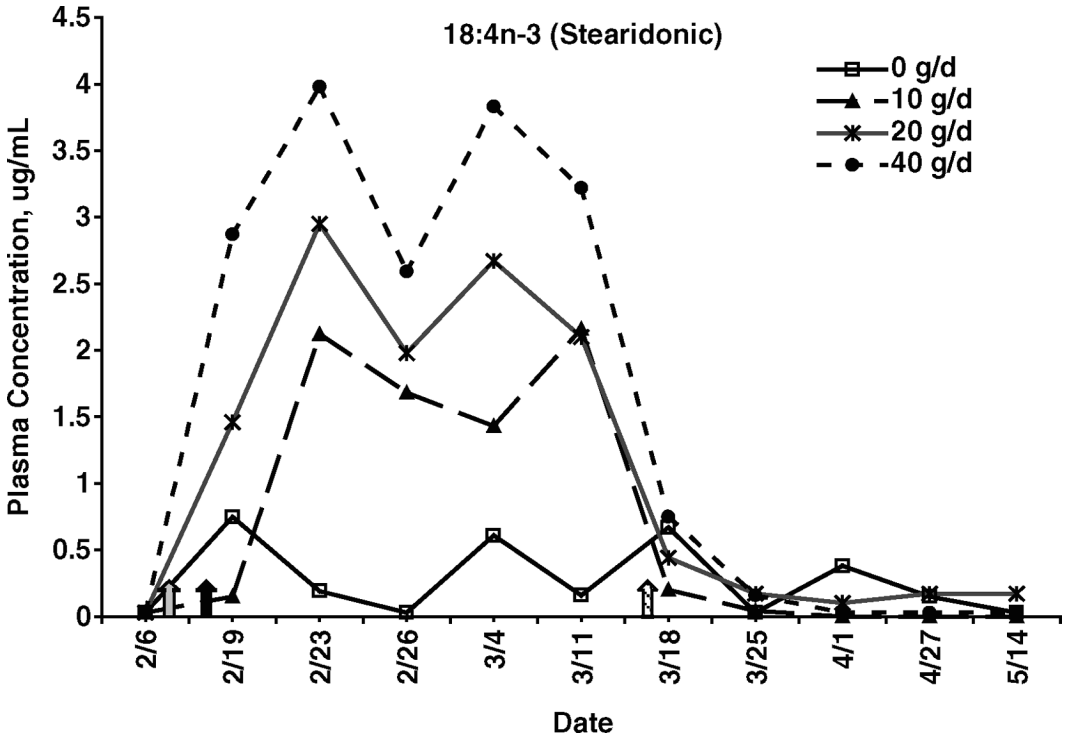

Figure 4. Plasma stearidonic concentrations in response to protected fatty acid supplementation in mares. Black arrow indicates the commencement and slashed arrow the cessation of full-supplement feeding. Gray arrow indicates the period of acclimation to the diet. Stearidonic concentrations in response to 20 and 40 g/d of supplementation were different ($P < 0.05$) from those of the nonsupplemented control (0 g/d) after 7 d of supplementation (2/23). The 10 g/d dose differed from the control ($P < 0.05$) on all treatment days, except 2/26 and 3/4. SEM = 0.38 (0 g/d); 0.39 (10 g/d); 0.44 (20 g/d); and 0.38 (40 g/d); C18:4n-3 demonstrated treatment ($P < 0.003$), day ($P < 0.001$), and day × treatment ($P < 0.001$) effects.

plus DHA would be required. This represents a very high level of n-3 supplementation.

The control diet in this study was forage-based and similar to that consumed by free-roaming horses; it follows that the relatively high n-6:n-3 ratio achieved in control animals indicates that the diet to which horses are naturally adapted delivers significantly greater n-6 FA compared with n-3 types. Considering their naturally low intake of EPA and DHA, horses may be able to attain beneficial health effects from lower n-3 supplementation levels and with a greater n-6:n-3 ratio than recommended for humans. Further research is required to determine the exact health benefits associated with EPA and DHA supplementation in the equine and to determine the optimum n-6:n-3 FA ratio in the equine diet to support these health benefits.

The n-6:n-3 ratio decreased between the presupplement sampling period and 42 d postsupplement (Table 3), when the plasma concentrations of n-3 FA under the influence of supplementation had largely dissipated. The change in this ratio can be largely attributed to a decline in the percentage of n-6 FA making up the total circulating FA pool (mean = 47.2% presupplement vs. 40.7% postsupplement) rather than a substantial increase in n-3 FA. Although n-3 FA increased in proportion to n-6 FA during the time of supplementation, they returned to presupplement concentrations after the washout period (6.3% presupplement vs. 6.6% postsupplement). Changes between presupplement and postsupplement ratios are likely due to the difference in FA content between the grass hay consumed by horses during the presupplement period and fresh spring grass serving as the forage source during the latter phase of the study. This seasonal shift from cured to fresh forage was also reflected in the concentrations of a number of other FA whose concentrations changed as a function of time rather than EPA and DHA supplementation.

The predominant FA identified in a variety of grass and legume species was α-linolenic acid, accounting for over 50% of the total FA content of forages tested (Dewhurst et al., 2001; Clapham et al., 2005). Linoleic and palmitic (C16:0) acids, combined with α-linolenic, make up over 95% of the total FA in fresh forage (Clapham et al., 2005). Circulating concentrations of all 3 of these FA demonstrated a very strong time effect in the current study. Circulating concentrations of α-linolenic acid in particular appeared to follow the grass growth pattern for our region; concentrations increased during early March and declined again by late April and May.

Other studies in horses have indicated changes in FA concentrations in equine plasma after supplementation

Table 2. Plasma concentration of non n-3 fatty acids (μg/mL) affected by protected fatty acid supplementation in mares

Fatty acid	Treatment,[2] g/d	Presupplement	Supplement[1]			Postsupplement			
		Feb 6	Feb 19	Feb 23	Mar 11	Mar 25	Apr 1	Apr 27	SEM
14:0[3]	0	6.50	4.50	5.91	6.14	6.49	7.59	6.99	0.95
	10	6.54	5.31	7.20	7.15	4.19	3.94	4.72	0.96
	20	5.98	5.16	8.77	6.94	3.33	3.84	4.63	0.96
	40	6.14	6.88	9.07	8.88	4.67	4.92	4.32	0.96
17:1n-7[3]	0	1.09	0.92	1.25	1.37	0.94	0.96	0.84	0.17
	10	1.08	1.28	1.58	1.58	1.15	0.94	1.06	0.16
	20	1.08	1.21	1.43	1.67	1.12	0.97	1.00	0.19
	40	1.08	1.31	1.36	1.43	1.11	1.08	0.99	0.16
18:1*trans*-11[3,4]	0	0.39	0.49	0.76	0.29	0.02	1.47	0.16	0.16
	10	0.50	0.89	1.16	1.16	0.34	0.16	0.00	0.50
	20	0.32	0.89	1.44	1.37	0.48	0.28	0.01	0.33
	40	0.51	1.33	1.90	2.15	0.38	0.29	0.00	0.16
18:1*cis*-9[3]	0	144.6	84.1	114.4	147.5	132.6	189.7	239.5	14.4
	10	134.8	97.2	113.6	98.9	99.8	115.2	184.1	14.4
	20	126.6	90.5	108.8	99.1	85.1	111.6	194.1	16.6
	40	137.6	90.8	92.7	88.6	116.2	149.4	172.8	14.4
18:3n-6[3,4]	0	1.30	0.47	1.15	1.00	1.06	1.28	1.92	0.24
	10	1.20	1.07	1.77	2.33	1.23	1.16	1.45	0.24
	20	1.27	0.58	2.02	1.70	1.08	1.08	1.95	0.27
	40	1.25	1.95	2.63	2.72	1.31	1.28	0.98	0.24
20:3n-6[3,4]	0	3.48	3.19	3.57	3.46	3.22	3.66	4.05	0.29
	10	3.34	3.34	4.18	4.03	3.31	3.19	4.08	0.29
	20	3.31	3.60	4.40	4.18	3.41	3.31	4.32	0.33
	40	3.37	3.83	5.01	4.85	4.20	3.93	3.87	0.29
20:4n-6[3,4]	0	10.57	9.25	10.54	10.51	8.75	8.99	7.75	0.78
	10	10.39	11.52	13.62	13.04	10.13	8.92	8.40	0.78
	20	10.47	11.85	14.35	14.03	10.41	10.04	8.54	0.87
	40	10.49	12.77	15.82	18.05	12.28	10.91	7.49	0.78

[1]Plasma fatty acid concentrations during the supplementation period. Supplementation began February 17 and ended March 16; Feb 6 = 11 d before supplementation; Feb 19 = 3 d of supplementation; Feb 23 = 7 d of supplementation; Mar 11 = 24 d of supplementation; Mar 25 = 9 d postsupplementation; Apr 1 = 16 d postsupplementation; Apr 27 = 42 d postsupplementation.
[2]Treatments were protected fatty acid supplementation from a marine-derived preparation (JBS United, Sheridan, IN) designed to deliver eicosapentaenoic acid plus docosahexaenoic acid at a combined concentration of 0 (control), 10, 20, or 40 g/d.
[3]Time effect ($P < 0.05$).
[4]Time × treatment effect ($P < 0.05$).

with lower concentrations of marine-derived lipids, fish oil, or flaxseed oil preparations. Accretion or clearance times were not studied, however. O'Connor et al. (2001) reported significant increases in 12 FA compounds as well as a shift in the n-6:n-3 ratio in serum after 63 d of supplementation with fish oil containing approximately 10% EPA and 8% DHA as a percentage of total fat, or approximately 30 g/d of total n-3 FA (O'Connor et al., 2004). The FA profile reported by O'Connor et al. (2001) was not as extensive or specific as in the current study; however, there was a similar trend for increased circulating concentrations of FA of the 18:1 type as well as 20:3n-6, EPA, docosapentaenoic acid, and DHA. The use of seal blubber oil as a source of n-3 FA increased

Table 3. Plasma concentrations and ratios of n-6 vs. n-3 fatty acids (percentage of total fatty acids) in response to protected fatty acid supplementation in mares

Treatment[1]	Presupplement			24-d supplement[2]			42-d postsupplement[3]		
	n-6	n-3	n-6:n-3	n-6	n-3	n-6:n-3	n-6	n-3	n-6:n-3
0 g/d	47.13	6.30	7.5:1	48.27	6.00	8.0:1	40.23	6.48	6.2:1
10 g/d	47.37	6.22	7.6:1	48.36	7.51	6.4:1	40.98	6.63	6.2:1
20 g/d	47.50	6.12	7.8:1	47.61	7.48	6.4:1	40.75	6.78	6.0:1
40 g/d	46.74	6.71	7.1:1	44.18	9.87	4.5:1	40.95	6.67	6.1:1

[1]Treatments were protected fatty acid supplementation from a marine-derived preparation (JBS United, Sheridan, IN) designed to deliver eicosapentaenoic acid plus docosahexaenoic acid (DHA) at a combined concentration of 0 (control), 10, 20, or 40 g/d.
[2]Plasma fatty acid concentrations and ratios during the supplementation period.
[3]Eicosapentaenoic acid/DHA plasma concentrations were all near the presupplement values, and the horses were grazing spring grass.

Table 4. Plasma concentrations of fatty acids (μg/mL ± SEM) as affected by time but not protected fatty acid supplementation in mares

	Presupplement	Supplement	Postsupplement[1]		
Fatty acid	Feb 6	Mar 11	Mar 18	Apr 1	May 14
14:1n-5 *trans*	1.23 (0.1)	1.38 (0.1)	1.24 (0.1)	1.31 (0.1)	1.55 (0.1)
15:0	2.54 (0.1)	2.45 (0.1)	2.64 (0.1)	1.89 (0.1)	2.22 (0.1)
15:1n-5 *cis*[2]	1.06 (0.1)	1.37 (0.1)	1.39 (0.1)	1.26 (0.05)	1.74 (0.1)
16:0	161.9 (7.9)	153.7 (7.9)	183.2 (8.2)	167.7 (8.2)	217.0 (8.2)
16:1n-5 *cis*[2]	17.48 (1.3)	16.67 (1.3)	21.79 (1.4)	11.39 (1.4)	13.61 (1.4)
17:0[3]	6.27 (0.2)	5.87 (0.2)	5.59 (0.2)	4.83 (0.2)	5.48 (0.2)
18:0[4]	197.5 (6.7)	163.4 (6.7)	192.6 (7.0)	176.1 (7.0)	217.5 (7.0)
18:1*trans*-9[4]	0.49 (0.07)	0.32 (0.1)	0.68 (0.1)	0.51 (0.1)	0.79 (0.1)
18:1*trans*-12[4]	0.58 (0.1)	0.43 (0.1)	0.82 (0.1)	0.43 (0.1)	0.60 (0.1)
18:1*cis*-11[4]	14.31 (0.5)	14.09 (0.5)	16.07 (0.6)	9.51 (0.6)	10.27 (0.6)
18:2n-6[4]	555.5 (151.5)	535.7 (151.5)	552.9 (157.1)	485.6 (157.1)	556.8 (157.1)
18:3n-3	75.20 (5.4)	41.71 (5.4)	71.95 (5.7)	103.3 (5.7)	71.55 (5.7)
19:0	1.96 (0.1)	2.01 (0.1)	2.19 (0.1)	1.74 (0.1)	2.17 (0.1)
20:0	3.85 (0.21)	3.31 (0.2)	3.45 (0.2)	3.95 (0.2)	5.45 (0.2)
20:1n-9	3.22 (0.2)	2.68 (0.2)	2.08 (0.2)	2.86 (0.2)	4.76 (0.2)
20:2n-6	2.66 (0.1)	2.68 (0.1)	2.88 (0.1)	2.64 (0.1)	3.25 (0.1)
20:3n-3	3.54 (0.2)	2.21 (0.2)	3.34 (0.2)	4.27 (0.2)	3.14 (0.2)
22:0	0.15 (0.1)	0.04 (0.1)	0.73 (0.1)	0.18 (0.1)	0.91 (0.1)
22:4n-6	0.00 (0.1)	0.00 (0.1)	0.21 (0.1)	0.03 (0.1)	0.34 (0.1)
23:0	0.44 (0.1)	0.00 (0.1)	0.40 (0.1)	0.35 (0.1)	0.85 (0.1)
24:0[3]	0.19 (0.1)	0.00 (0.1)	0.31 (0.1)	0.29 (0.1)	0.66 (0.1)
24:1n-9	0.60 (0.1)	0.46 (0.1)	0.52 (0.1)	0.35 (0.1)	0.60 (0.1)

[1]Plasma fatty acid concentrations after cessation of supplementation. Supplementation began on February 17 and ended March 16; Feb 6 = 11 d before supplementation; Mar 11 = 24 d of supplementation; Mar 18 = 2 d postsupplementation; Apr 1 = 16 d postsupplementation; Apr 27 = 42 d postsupplementation.
[2]Treatment × time effect in addition to treatment effect.
[3]Quadratic effect.
[4]Linear effect.

plasma EPA and DHA well above values achieved from feeding sunflower oil (Khol-Parisini et al., 2007). Hansen et al. (2002) reported increases in plasma linolenic and linoleic acids as well as EPA, but not DHA, after 16 wk of supplementation with 10% flaxseed oil. The fact that these results do not agree with those generated from the current study, with the exception of EPA, may be attributable to the significant quantity of n-6 FA contained in flaxseed oil in addition to its n-3 FA content. The supplement used in the current study contained very low concentrations of linolenic and linoleic acids. In fact, the concentration of these FA decreased

Table 5. Fatty acid composition of 3 protected fatty acid supplements measured as a percentage of total fat

	Supplement[1]			
Fatty acid	0 g of EPA/DHA	10 g of EPA/DHA	20 g of EPA/DHA	40 g of EPA/DHA
14:0	4.28	8.33	9.86	10.08
16:0	24.58	17.21	17.00	15.95
17:1	ND[2]	1.46	1.51	1.58
18:0	4.11	3.70	3.74	3.58
18:2n-6	18.13	5.31	3.24	2.00
18:3n-3	15.53	5.00	2.57	1.76
18:4n-3	1.15	2.96	3.11	3.33
20:3n-3	ND	ND	ND	ND
20:4n-6	ND	0.73	0.75	0.80
20:4n-3	ND	ND	1.43	1.61
20:5n-3 (EPA)	2.50	9.44	10.05	11.19
22:5n-3	ND	1.72	1.89	2.13
22:6n-3 (DHA)	2.65	10.98	12.16	13.73

[1]Protected fatty acid supplementation was delivered from a marine-derived preparation (JBS United, Sheridan, IN) designed to deliver eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) at a combined concentration of 0 (control), 10, 20, or 40 g/d.
[2]ND = not detected or at <0.01.

with increasing concentrations of EPA and DHA in the supplement.

Several of the beneficial effects of increased n-3 FA concentration can be attributed to their competition for enzymes involved with the production of eicosanoids, particularly the prostaglandins, thromboxanes, and leukotrienes that regulate both negative and positive cardiovascular, inflammatory, and reproductive effects. Arachidonic acid (C20:4n-6) forms the precursor to these compounds. Eicosapentaenoic acid is a competitive substrate for the enzymes of the arachidonic acid cascade, and its abundance can shift arachidonic acid products from the destructive subtypes of eicosanoids in favor of the more of the protective subtypes (Robinson and Stone, 2006). Supplementation of EPA and DHA has been reported to be associated with a decrease in plasma arachidonic acid concentrations in humans (Arterburn et al., 2006). In the current study, arachidonic acid concentrations increased in a dose-related response to EPA and DHA supplementation despite only modest increases in arachidonic acid content in the supplement. This response was similar to that noted for humans and agrees with results of other equine studies (Hall et al., 2004; Khol-Parisini et al., 2007; Vineyard et al., 2007). However, it appears that arachidonic acid response to n-3 supplementation is variable in the horse and may depend upon the balance and concentration of individual FA contained in the supplement. For example, O'Connor et al. (2001) reported a decrease in arachidonic acid concentrations in horses after 63 d of fish oil supplementation, whereas Kruglik et al. (2005) reported no treatment effect for arachidonic acid in pregnant and lactating mares after PFA supplementation at approximately 18 g/d of EPA + DHA. Further research is required to understand the action and consequences of EPA and DHA supplementation on arachidonic acid and eicosanoid biochemistry in the horse.

Conjugated linoleic acid products are of current scientific interest because of their health-promoting effects when consumed by humans. No detectable CLA product was found in the plasma of any of the 16 animals on this study. The absence of CLA in our equine samples is in contrast to results reported by Park and Pariza (1998), in which a significant amount of 18:2 *cis*-9, *trans*-11 conjugated isomer was detected in 3 anonymous horse sera sources and the 18:2 *trans*-10, *cis*-12 isomer was detected in 1 sample. Information on the type, sex, diet, and history of horses from which the serum was collected was not available to these researchers. Therefore, it is impossible to compare the diet, metabolic condition, and living conditions of the horses contributing the anonymous samples with the conditions in the current study. In our study, all experimental subjects were nonpregnant females that had been long-term residents on our farm. Diets were forage-based with no supplementation aside from salt and trace minerals.

Our data indicate a species difference in the metabolism and circulatory availability of certain FA isomers between ruminants and horses. The biohydrogenation of linoleic acid is accomplished in the rumen of cattle and is influenced by the microbial mileau in that organ (Bauchart et al., 1984). Horses perform microbial digestion in the hindgut; it is possible that the location of fermentation, or the microbial environment in the equine hindgut, is responsible for the observed lack of CLA production in the horse. It is also possible that CLA production does occur in the equine hindgut, but the CLA products are not absorbed into the general circulation from this site. Incorporation of CLA into the tissues or milk of food animals is an area of interest due to the possibility of conferring health benefits to the end-consumers of these animal products (Ward et al., 2003). Because horses are not raised specifically for food production, their lack of CLA production is not of commercial effect.

We have shown that supplementation of specific PUFA into the diet of the horse can rapidly influence the milieu of circulating FA in the plasma, but these changes in FA concentrations were short-lived after discontinuation of supplementation. Assimilation of specific FA into tissue cell membranes may provide a longer-lasting source of n-3 FA.

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