Ahiflower Oil Demonstrates Improved Anti-Inflammatory Activity and Long-Chain Polyunsaturated Fatty Acid Conversion in Horses

Results of a Randomized Controlled Dietary Trial

Dr. Mary A. McNiven

Dept. of Health Management
Atlantic Veterinary College
University of Prince Edward Island
Charlottetown, PE C1A 4P3

mcniven@upei.ca

Dr. Juan Carlos Rodriguez

Dept. of Pathology & Microbiology
Atlantic Veterinary College
University of Prince Edward Island
Charlottetown, PE C1A 4P3

jrodriguez@upei.ca

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Background:

Arthritis is the most significant cause of lameness in horses and can lead to the early retirement of otherwise healthy animals. Previous research in a variety of species has shown that n-3 and certain n-6 polyunsaturated fatty acids (PUFA) have the potential to mitigate the pain and inflammation caused by arthritis, as well as possibly slowing down joint degradation. The long-chain n-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are metabolized to anti-inflammatory eicosanoids while some n-6 PUFAs (such as arachidonic acid (AA)) form the inflammatory eicosanoids. In arthritic horses, EPA and DHA supplementation from fish oil are known to result in an increased stride length and reduced inflammatory markers. Incorporation of n-3 long-chain PUFA to circulating cells and muscle tissues could potentially improve chronic inflammatory conditions in horses. Because EPA and DHA are normally only found in marine sources which can be expensive, are generally not sustainable and can be unpalatable to herbivorous horses, precursor n-3 fatty acids from plant sources should be investigated.

Ahiflower oil® is unique in that it contains the omega-3 precursor fatty acid, stearidonic acid (SDA), which human and animal studies have shown that will convert metabolically to EPA and DHA more directly than the precursor fatty acids in other oils such as canola or flax (ALA (alpha-linolenic acid)). The conversion of ALA to SDA is known as the rate-limiting step in the omega-3 metabolic pathway in the production of EPA and DHA and therefore animals may not benefit as significantly from the ALA found in oils such as flax as they may from ingestion of SDA. Research has shown that horses are only able to convert ALA and SDA to EPA and typically not through to DHA and may not benefit from n-3 oil from flax.

In addition, unlike either fish oil or flax oil, Ahiflower oil also contains gamma-linolenic acid (GLA), an n-6 fatty acid that is the equivalent fatty acid to SDA in the omega-6 pathway. GLA is metabolized to produce anti-inflammatory compounds and it helps to control the release of pro-inflammatory AA from tissues.

We investigated the conversion rate of dietary Ahiflower oil which contains the highest naturally-occurring SDA in Standardbred racehorses to EPA and DHA and compared it to the conversion rates from flax and corn oils over a ten week period.

In addition, we measured inflammatory markers in racehorses before the trial, and after ten weeks of supplementation to allow tissue turnover of fatty acids to determine if the long-chain PUFA had an effect on inflammation.

Objectives:

1) Determine if the conversion of SDA from Ahiflower (C18:4 fatty acid) to EPA and DHA is more efficient than conversion of ALA from flax (C18:3 fatty acid) in horses.
2) Determine if Ahiflower oil (omega-3) reduces inflammation in performance horses more effectively than corn oil (omega-6) or flax oil (omega-3).
3) Evaluate the palatability of the Ahiflower oil supplement for horses.

Methodology:

Thirty racing Standardbred horses were selected from four locations in Prince Edward Island and blocked according to age and location. Horses were randomly assigned to one of three treatments within block: Ahiflower oil (AF), flax oil (FL), corn oil (CO), with 10 horses assigned to each treatment. Trainers of the horses were blinded as to which treatment their horses were receiving. The horses were fed their standard rations and the oil was added to the concentrate feed twice a day at a rate of 40 mL/day (38 g/d). Horses continued in their regular routine of jogging, training, and racing. Trainers were asked to be observant of changes in feed consumption, behaviour or racing form and were given booklets for each horse for recording. Blood was collected from the jugular vein on Days 0 and 70. A complete blood cell count, chemistry, fibrinogen (indication of inflammation), and fatty acid profile of whole blood were measured.

Table 1. Fatty acid profiles of the oil supplements (dosage 40 mL/d or 37 g/d).

	Ahiflower oil	Corn oil	Flax oil
C16:0	4.68	11.9	5.24
C18:0	1.77	1.62	3.28
C18:1n9	8.90	28.3	18.6
C18:1n7	0.65	0.78	0.82
C18:2n6	12.1	55.5	15.6
C18:3n6	5.13	-	-
C18:3n3	43.1	0.89	55.9
C18:4n3	21.9	-	-
C20:0	0.25	0.42	0.12
C20:1n9	0.94	0.34	0.19
C20:2n6	0.11	-	-

Results:

All oils were readily consumed by the horses and there were no reports of palatability issues or other ill effects. Day 0 blood parameters were similar for all horses regardless of treatment assignment. Table 1 shows the fatty acid profile of the oil supplements.

The complete fatty acid profiles of blood collected on Day 70 by treatment are shown in Table 2. The main effects of dietary treatment appeared to be with the omega-3 fatty acids. For total omega-3 fatty acids, the corn oil treatment had significantly lower levels than the flax or the Ahiflower treatments.

Blood ALA (C18:3n3) levels were significantly higher for the flax oil-fed horses compared with the horses fed Ahiflower or corn oils. This and the fact that the long chain omega-3 fatty acids were significantly higher for Ahiflower oil-fed horses demonstrate that the conversion of C18:3n3 to C18:4n3 is the rate-limiting step in horses. The higher levels of C18:4n3 in the Ahiflower oil were more readily converted to the longer chain omega-3 fatty acids.

Blood Gamma-linolenic acid (GLA) (C18:3n6) accumulated in the horses fed Ahiflower oil and the level of Dihomo-gamma-linolenic acid (DGLA) (C20:3n6) was also significantly higher for the Ahiflower oil-fed horses. This is the fatty acid thought to be responsible for the anti-inflammatory effects of C18:3n6.

Table 2. Fatty acid profiles of whole blood from horses supplemented for 70 days with Ahiflower, corn or flax oil.

% of FA	Ahiflower oil	Corn oil	Flax oil	SEM
14:0	0.27	0.25	0.23	0.023
16:0	13.7	13.2	13.0	0.32
18:0	15.0 b	15.1 b	15.7 a	0.23
20:0	0.37	0.38	0.39	0.018
22:0	0.25	0.28	0.28	0.016
24:0	0.51 b	0.59 a	0.55 ab	0.029
16:1n7	0.59	0.52	0.53	0.036
18:1n9	14.8	15.1	14.2	0.45
18:1n7	1.04 a	0.97 b	1.01 ab	0.022
20:1n9	0.44	0.43	0.42	0.024
22:1n9	0.12	0.13	0.13	0.009
24:1n9	0.23	0.26	0.25	0.019
18:2n6	47.7	48.7	48.4	0.64
20:2n6	0.34	0.38	0.36	0.018
20:4n6	1.13	1.18	1.10	0.062
22:4n6	0.079 b	0.103 a	0.078 b	0.0085
22:5n6	0.028	0.033	0.029	0.0038
18:3n6	0.27 a	0.06 b	0.05 b	0.02
20:3n6	0.37 a	0.30 b	0.29 b	0.016
18:3n3	1.2 b	0.85 c	1.71 a	0.099
18:4n3	0.17 a	0 b	0 b	0.029
20:3n3	0.07 b	0.05 c	0.09 a	0.005
20:4n3	0.11 a	0.007 b	0.004 b	0.009
20:5n3	0.06 a	0 c	0.02 b	0.005
22:5n3	1.14	1.0	1.12	0.09
22:6n3	0.049	0.037	0.047	0.006

ab – means with different superscripts are significantly different $P<0.05$.

Table 3. Summary of fatty acids and differences in n3 fatty acids between Day 70 and Day 0.

% of FA *	Ahiflower oil	Corn oil	Flax oil	SEM
SFA	30.1	29.9	30.2	0.18
MUFA	17.3	17.4	16.5	0.47
PUFA	52.7	52.7	53.3	0.57
n3FA	2.78 a	1.95 b	3.00 a	0.179
n6FA	49.9	50.8	50.3	0.62
LCn3	1.41 a	1.09 b	1.28 ab	0.09
LCn6	1.95	1.99	1.86	0.077
n6/n3	18.8 b	26.6 a	17.4 b	1.53
Difference Day 70-Day 0				
20:2n6	-0.01	0.04	0.02	0.015
20:3n6	0.08 a	-0.02 b	0.00 b	0.018
18:3n3	0.21 ab	0.02 b	0.65 a	0.168
18:4n3	0.17 a	-0.01 b	0.02 b	0.027
20:3n3	0.007	0.008	0.042	0.0113
20:4n3	0.10 a	0.004 b	0.002 b	0.010
20:5n3	0.044 a	-0.009 b	0.005 b	0.007
22:5n3	0.64	0.34	0.34	0.149
22:6n3	0.003	-0.007	0.0002	0.010
Total n3 (does not include 18:3n3)	0.96 a	0.33 b	0.41 b	0.16

*FA – fatty acids, S – saturated, MU – monounsaturated, PU – polyunsaturated, n3 – omega-3, n6 – omega-6, LC – long-chain (>C18).

ab – means with different superscripts are significantly different P<0.05.

Although there were no treatment differences in fatty acid levels at Day 0, the Ahiflower oil treatment showed quite dramatic increases in omega-3 fatty acids during the 70 day experimental period (Table 3). Tenfold increases in DHA and EPA were found for the Ahiflower oil-fed horses compared with flax oil-fed horses with increases in the intermediaries as well (C20:4n3, C22:5n3). This reinforces the statement that the rate-limiting step for conversion of precursor omega-3s to long-chain omega-3 fatty acids is between C18:3n3 and C18:4n3 and that feeding Ahiflower oil is a method to supply a usable precursor omega-3 fatty acids to allow for conversion In horses.

Table 4. Indications of inflammation from blood samples from horses fed ahiflower, corn or flax oil.

	Ahiflower oil	Corn oil	Flax oil	SEM
Albumin:Globulin	0.81	0.83	0.78	0.04
Neutrophil:Lymphocyte	1.56	1.71	1.88	0.19
SDH	1.56	1.89	1.22	0.38
Inflammation Index (II)	38.1	43.1	48.2	5.28
II (Day 70- Day 0)	-10.3	1.8	-0.003	7.1
AA/EPA+DHA	12.2 c	28.8 a	18.1 b	1.98

ab – means with different superscripts are significantly different $P<0.05$.

The ratio of arachidonic acid over the sum of EPA and DHA in the blood (Table 4) is an indicator of inflammatory status. This index was significantly lower for the Ahiflower oil-fed horses compared with either the flax or the corn oil-fed horses. The other inflammatory index (total blood protein –fibrinogen)/fibrinogen was numerically lower for the Ahiflower oil-fed horses but did not achieve significant differences. The difference between that inflammatory index at Day 70 and Day 0 was much greater for Ahiflower oil-fed horses than the other treatments, almost a 25% decrease in index, compared to no change for the other treatments. The other non-specific indicators of inflammation shown in Table 4 did not show any significant treatment differences and were within normal ranges.

Conclusions:

Ahiflower horses had a higher conversion rate to long chain omega-3 fatty acids compared with flax or corn oil-fed horses. C18:3n3 accumulated in the flax-fed horses. The presence of anti-inflammatory fatty acids in the blood of Ahiflower oil-fed horses may have beneficial effects on joint and overall health. Higher levels of oil or greater numbers of horses per treatment may have resulted in statistically significant differences between treatments in blood metabolic effects of oil consumption.

Suggestions for a follow-up trial

- Several levels of Ahiflower oil, higher than the present trial
- Separate plasma and red blood cells and analyze separately to investigate short and longer term changes in fatty acid profiles within the body
- Drop the expensive metabolic profile analysis and focus on inflammatory markers e.g. ELIZA kits for acute phase proteins such as interleukin 6 or serum amyloid A

Figure 1. Differences in total omega-3 fatty acids between the start and finish of the trial (Day 70 - Day 0 results).

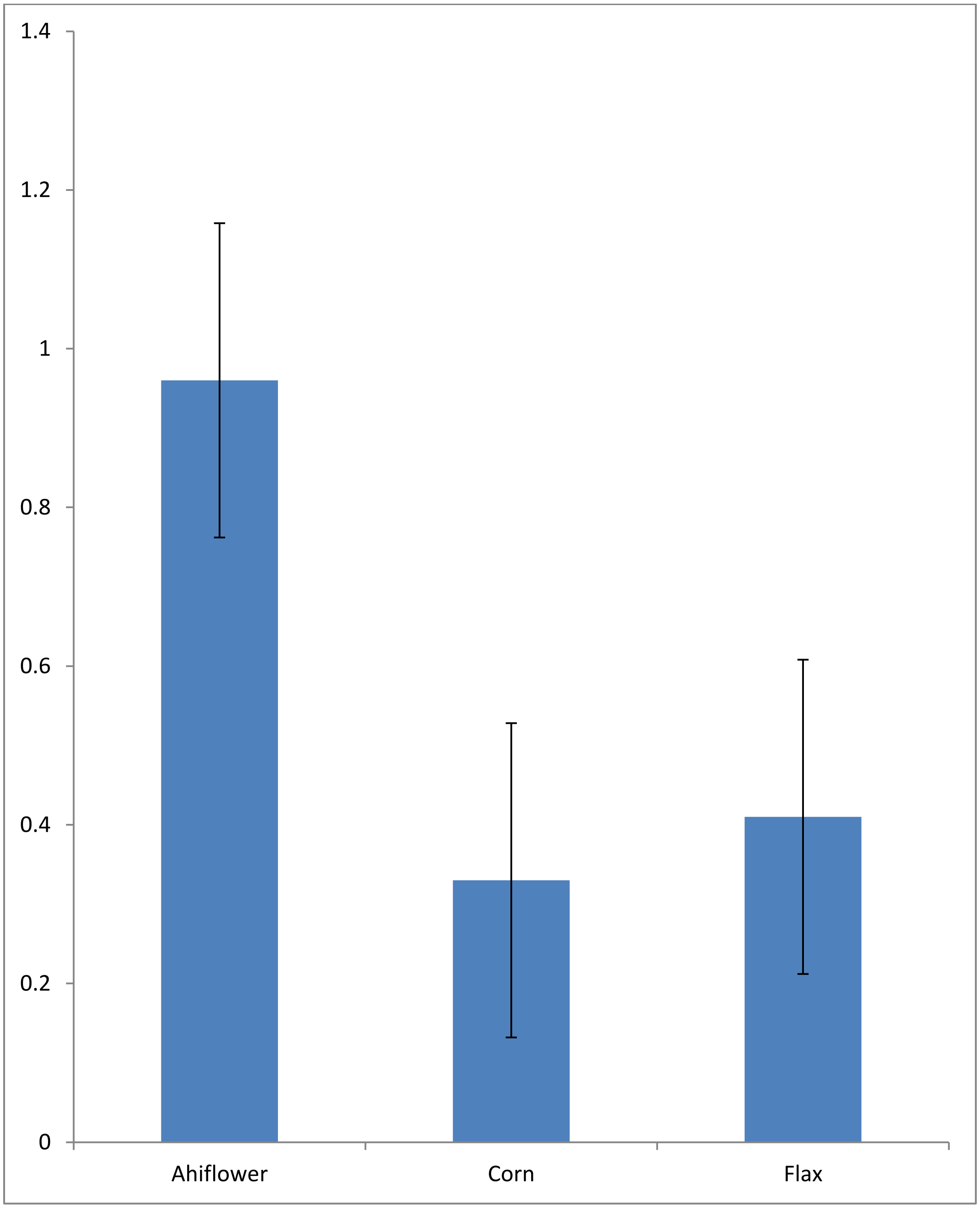

Figure 2. Total omega-3 fatty acid level in blood of horses fed one of three dietary oils for 70 days.