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Long-chain polyunsaturated fatty acid supplementation increases levels in red blood cells and reduces the prevalence and severity of squamous gastric ulcers in exercised Thoroughbreds

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doi.org/10.2460/javma.22.06.0275

OBJECTIVE
To assess the relationship between plasma and RBC fatty acid composition and incidence and severity of squamous gastric ulcers when altered by short-chain (SC) or long-chain (LC) polyunsaturated fatty acid (PUFA) supplementation.

ANIMALS
13 fit Thoroughbred horses in training.

PROCEDURES
Horses were evaluated by gastroscopy for squamous ulcer score, gastric pH, and blood fatty acid composition prior to supplementation (UNSUPP) and after 3 months of supplementation with a corn-flax oil blend of alpha-linolenic acid and linoleic acid (SC-PUFA) or a gamma-linolenic acid (GLA)-fish oil blend of GLA, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA; LC-PUFA) in a crossover design. Prior to gastroscopy and blood collection, horses performed a 4,600-m standardized exercise test on the racetrack as a stressor.

RESULTS
Three months of supplementation with LC-PUFAs increased RBC levels of GLA, dihomo-gamma-linolenic acid (DGLA), arachidonic acid (AA), EPA, and DHA, and reduced severe ulcer prevalence (38% UNSUPP vs 8% LC-PUFA with a severe ulcer score of grade 3 to 4). Short-chain PUFA supplementation did not effectively elevate RBC GLA, DGLA, AA, EPA, or DHA and severe ulcer incidence was not different (38% UNSUPP vs 23% SC-PUFA with a severe ulcer score of grade 3 to 4). Lower levels of RBC GLA, DGLA, AA, and EPA correlated with severe squamous gastric ulceration (grade 3 to 4).

CLINICAL RELEVANCE
Equine gastric ulcer syndrome is prevalent in high-performance horses and is a concern to owners and trainers. Long-chain PUFA supplementation increased levels of GLA, DGLA, AA, EPA, and DHA, unlike SC-PUFA supplementation, and was associated positively with prevention or resolution of severe squamous gastric ulceration. Further studies are needed to evaluate different management styles and exercise intensities.

Omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) are essential nutrients for horses. Omega-3 PUFAs include alpha-linolenic acid (ALA; 18:3n3), eicosapentaenoic acid (EPA; 20:5n3), docosapentaenoic acid (DPA; 22:5n3), and docosahexaenoic acid (DHA; 22:6n3). Omega-6 PUFAs include linoleic acid (LA; 18:2n6), gamma-linolenic acid (GLA; 18:3n6), dihomo-gamma-linolenic acid (DGLA; 20:3n6), and arachidonic acid (AA; 20:4n6). PUFAs with ≤ 18 carbons are considered short chain (SC), whereas those with ≥ 20 carbons are long chain (LC). SC-PUFAs from the omega-3 and omega-6 pathways can desaturate and elongate to LC-PUFAs through competing enzymes. Long-chain–PUFAs serve as precursors for numerous lipid mediators, which are together referred to as eicosanoids and include prostaglandins (PGs), thromboxanes, and leukotrienes. Eicosanoids produced from AA tend to be proinflammatory, whereas DGLA, EPA, and DHA give rise to lipid mediators, which are anti-inflammatory and inflammation resolving. Some of these eicosanoids, such as prostaglandin E2 (PGE2),

are involved in the pathophysiology, prevention, and resolution of gastric ulcers.

Traditionally, PUFAs in equine diets have been provided as SC-PUFAs from vegetable oils and forage. The predominant form of the omega-6 SC-PUFA (LA) comes from vegetable oils, such as corn and soy oils, whereas the major SC-PUFA omega-3 (ALA) is supplied from forage or vegetable oils such as flaxseed oil. To produce desirable eicosanoids, these SC-PUFAs must go through a multitude of rate-limiting steps. Supplementation with LC-PUFAs, as more immediate precursors, may produce a more efficient response.

RBCs are longer lived than platelets and lipoproteins, so their fatty acid composition is more stable and reflective of chronic omega-3 status.[1] Data from mice demonstrated that the gastrointestinal tissues are highly responsive to dietary LC-PUFA supplementation and that the sum of EPA + DHA in RBCs, expressed as a percentage of total fatty acids (the omega-3 index[2]), can serve as a valid biomarker for assessing dietary EPA + DHA incorporation into gastrointestinal tissues.[3]

This study was conducted to evaluate how supplementation with SC- or LC-PUFAs affects plasma and RBC PUFA composition, and how these changes relate to the incidence and severity of squamous gastric ulcers.

Materials and Methods

Horses and feeding management

Thirteen fit Thoroughbred horses (age 4.1 ± 1.9 years; mean body weight [BW] ± SD, 496.0 ± 36.5 kg) housed at the Kentucky Equine Research Performance Center in Ocala, Florida, were used in a 3-period study. During each 88-day period, the horses were fed 4.9 ± 1.4 kg/day (mean ± SD) of a textured horse feed fed in 3 meals/day, along with 1.0% to 1.5% BW/day of timothy hay and 60 g loose salt. The horses were stalled for 8 hours/day and were turned out in bahiagrass-pasture paddocks for 16 hours each night. The proximate analysis and PUFA composition of the feed, hay, and pasture is shown in **Table 1**, along with the PUFA supplements. The feed, hay, and pasture provided 2.3, 4.9, and 2.9 g/kg (as fed) of ALA and 24.9, 1.5, and 0.8 g/kg (as fed) of LA, respectively. These feedstuffs did not supply measurable quantities of other PUFAs. The study protocol was reviewed and approved by an internal company Institutional Animal Care and Use Committee.

PUFA supplementation

During period 1, the horses received no PUFA supplementation (UNSUPP) other than what was contained in the feed, hay, and pasture. After period 1, horses were split into 2 groups balanced for age, concentrate intake, BW, and baseline values for exercise speed, gastric pH, and ulcer score. During periods 2 and 3, the horses received either 35 mL/day of a corn oil-flax oil supplement (SC-PUFA) or 60 mL/day of a high GLA safflower oil-fish oil supplement (LC-PUFA) in a crossover design. Seven horses received LC-PUFAs first, whereas the other 6 horses received SC-PUFAs first before switching to the other treatment. During a 10-day washout period between periods 2 and 3, the horses did not receive PUFA supplementation. Each supplement was divided into 2 daily feedings. The SC-PUFA supplement provided 8.0 g/day LA and 10.2 g/day ALA. The LC-PUFA supplement

Table 1—Proximate analysis and fatty acid composition of feed, hay, pasture, and polyunsaturated fatty acid (PUFA) supplements.

Nutrient	Textured feed (as fed)	Timothy hay (as fed)	Pasture (100% DM)	SC-PUFA	LC-PUFA
DE (Mcal/kg)	2.97	1.90	1.98	—	—
CP (%)	12.7	10.3	17.3	—	—
CF (%)	4.9	2.3	2.8	100	100
ADF (%)	7.9	32.5	35.9	—	—
NDF (%)	14.6	53.9	62.2	—	—
Starch (%)	36.2	0.7	1.8	—	—
WSC (%)	8.4	11.3	3.9	—	—
NSC (%)	44.6	12.0	5.7	—	—
Fatty acid[a] (%)					
LA	48.4	16	14.6	28.9	5.6
GLA	0.1	.03	0.2	0.2	12.1
DGLA	—	—	—	—	0.2
AA	—	—	—	—	0.8
ALA	4.5	51.1	51.3	37	1.4
EPA	—	—	—	—	11.1
DPA	—	—	—	—	2
DHA	—	—	—	—	9.5

AA = Arachidonic acid. ADF = Acid detergent fiber. ALA = Alpha-linolenic acid. CF = Crude fat. CP = Crude protein. DE = Digestible energy. DGLA = Dihomo-gamma-linolenic acid. DHA = Docosahexaenoic acid. DM = Dry matter. DPA = Docosapentaenoic acid. EPA = Eicosapentaenoic acid. GLA = Gamma-linolenic acid. LA = Linoleic acid. LC = Long chain. NDF = Neutral detergent fiber. NSC = Nonstructural carbohydrate. SC = Short chain. WSC = Water-soluble carbohydrate. — = Not detected.

[a]Grams/100 g identified fatty acids.

provided 2.5 g/day LA, 5.4 g/day GLA, 0.3 g/day AA, 4.4 g/day EPA, 0.8 g/day DPA, and 3.8 g/day DHA. Each supplement provided a similar amount of total omega-6 (8.0 g SC-PUFAs vs 8.2 g LC-PUFAs) and omega-3 (10.2 g SC-PUFAs vs 9.6 g LC-PUFAs) per day.

Exercise schedule

During the first 77 days of each period, horses were exercised 3 times per week on a 1,200-m dirt racetrack. For each session, horses were outfitted with heart rate monitors (Polar H10; Polar Electro Inc) and GPS through a smartphone application (KER ClockIt Race) to evaluate heart rate, speed, and distance. Across all periods, each session averaged 3,640 ± 590 m and lasted 12.3 ± 0.5 minutes. During each session, horses walked 275 ± 67 m (1 to 3 m/s), trotted 1,115 ± 285 m (3 to 5 m/s), cantered 1,520 ± 470 m (5 to 10 m/s), and galloped 730 ± 325 m (> 10 m/s). The horses cantered or galloped 4.0 ± 1.1 minutes of each session with heart rates ≥ 154 bpm. The horses also walked 30 minutes/session 3 days/week on a 20-m-diameter mechanical walker.

On day 81 of each period, the horses performed a 4,600-m standardized exercise test (SET) on the racetrack consisting of a 1,800-m trot (4.5 ± 0.4 m/s), a 1,200-m canter (8.2 ± 1.0 m/s), a 600-m fast gallop (15.3 ± 0.6 m/s), and a 1,000-m canter (8.7 ± 1.7 m/s). Horses did not perform any forced exercise for 2 weeks after each SET.

Gastroscopy and gastric fluid collection

Five days after each SET (day 86), the gastric mucosa of the horses was examined using a flexible 3-m video-endoscope (Olympus OSF-V60) while sedated with 0.01 mg/kg detomidine (Dormosedan; Zoetis). Prior to gastroscopy, feed and hay were withheld for 15 to 18 hours and water was withheld 2 to 3 hours. The severity of squamous gastric ulceration was scored on a scale of 0 to 4 in accordance with an equine gastric lesion scoring system[4] by a researcher (BMW) who was unaware of the diet each horse was being fed. A score of 0 was considered free of ulceration, scores of 1 or 2 were considered mild to moderate ulceration, and scores of 3 or 4 were considered severe ulceration. Gastroscopic evaluations followed the collection of 200 to 300 mL gastric fluid through the biopsy port of the endoscope for pH analysis. Gastric fluid samples were evaluated immediately for pH after collection using a benchtop pH meter (model 35419-12; Oakton pH 700 Benchtop Meter).

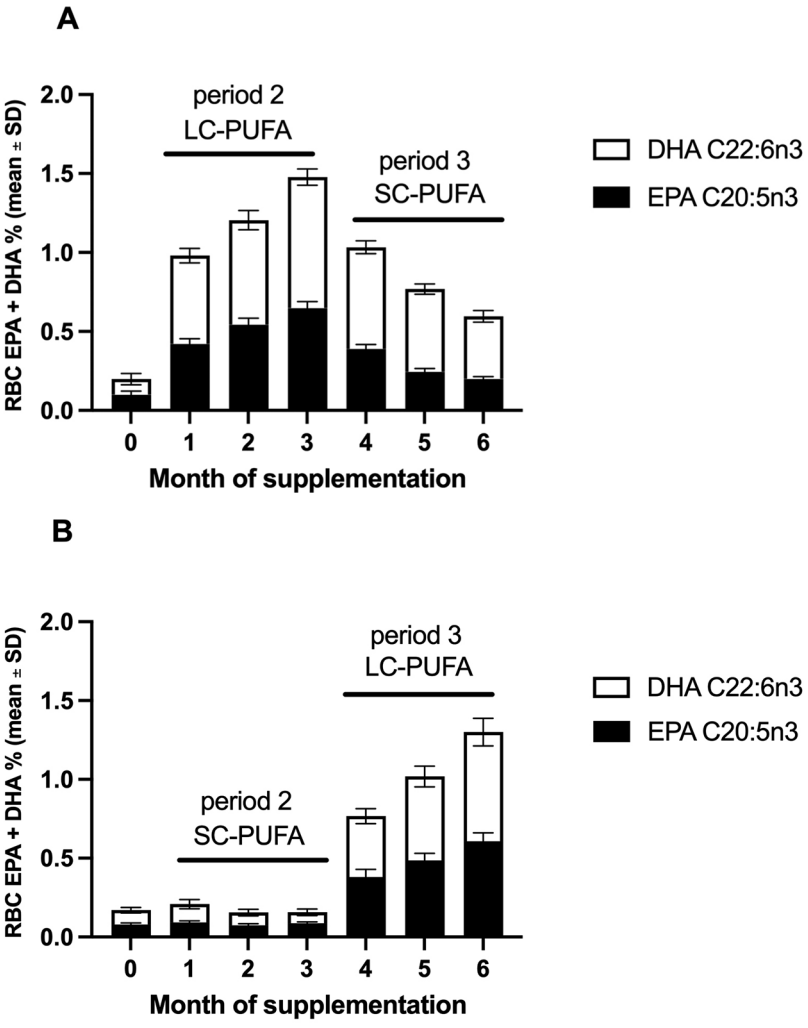

Figure 1—RBC eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in horses (n = 7) that received long-chain polyunsaturated fatty acids (LC-PUFAs) before short-chain polyunsaturated fatty acids (SC-PUFA; A) and in horses (n = 6) that received LC-PUFAs after SC-PUFAs (B) during periods 2 and 3.

Blood collection and fatty acid analysis

Blood samples were collected monthly during each period to evaluate changes in plasma and RBC fatty acid composition. The final sample from each period was collected 2 days after the gastroscopy (day 88). Prior to morning feeding (6 to 7 AM), samples were collected via venipuncture of the jugular vein directly into K_2EDTA vacutainers. Vacutainers were placed at 4 °C until they could be centrifuged at 1,500 X *g* for 10 minutes as soon as possible (≤ 10 minutes). After centrifugation, plasma was collected and placed into separate aliquots. The buffy coat was discarded carefully via aspiration to access RBCs. RBCs were then collected and placed into separate aliquots. Samples were either sent immediately overnight at ambient temperature (22 to 30 °C) to a commercial laboratory (OmegaQuant Analytics), where they were frozen at −80 °C until analysis, or they were

Table 2—RBC and plasma PUFA composition (day 88) when horses were unsupplemented or fed SC-PUFA or LC-PUFA supplements (g/100 g total identified fatty acids).

	Unsupplemented	SC-PUFA			LC-PUFA		
Fatty acid	**Period 1 (n = 13)**	**Period 2 (n = 6)**	**Period 3 (n = 7)**	**Total (n = 13)**	**Period 2 (n = 7)**	**period 3 (n-6)**	**Total (n = 13)**
RBC fatty acids							
LA C18:2n6	39.64 ± 0.93 a	39.93 ± 0.97	38.33 ± 0.82	39.07 ± 1.19[a] a	37.98 ± 1.13	37.12 ± 0.70	37.59 ± 1.02 b
ALA C18:3n3	1.27 ± 0.33 a	1.34 ± 0.26	1.09 ± 0.18	1.21 ± 0.25 a	1.04 ± 0.24	1.10 ± 0.24	1.07 ± 0.23 b
GLA C18:3n6	0.10 ± 0.04 a	0.12 ± 0.01	0.17 ± 0.03	0.15 ± 0.03 [a] b	0.38 ± 0.04	0.37 ± 0.09	0.37 ± 0.07 c
DGLA C20:3n6	0.27 ± 0.05 a	0.25 ± 0.02	0.38 ± 0.05	0.32 ± 0.07[a] a	0.57 ± 0.13	0.51 ± 0.08	0.54 ± 0.11 b
AA C20:4n6	1.48 ± 0.26 a	1.31 ± 0.21	1.39 ± 0.24	1.36 ± 0.22 b	1.68 ± 0.23	1.53 ± 0.22	1.61 ± 0.23 c
EPA C20:5n3	0.09 ± 0.05 a	0.09 ± 0.02	0.2 ± 0.04	0.15 ± 0.06 [a] b	0.65 ± 0.11	0.61 ± 0.13	0.63 ± 0.12 c
DHA C22:6n3	0.09 ± 0.07 a	0.12 ± 0.07	0.39 ± 0.10	0.27 ± 0.17 [a] b	0.83 ± 0.14	0.69 ± 0.21	0.77 ± 0.18 c
EPA + DHA	0.18 ± 0.11 a	0.16 ± 0.07	0.60 ± 0.11	0.39 ± 0.24 [a] b	1.47 ± 0.20	1.30 ± 0.34	1.39 ± 0.28 c
DGLA + AA	1.76 ± 0.30 a	1.57 ± 0.23	1.77 ± 0.26	1.67 ± 0.26 a	2.25 ± 0.32	2.04 ± 0.29	2.15 ± 0.31 b
DGLA + AA + EPA + DHA	1.94 ± 0.37 a	1.72 ± 0.27	2.36 ± 0.24	2.07 ± 0.41[a] a	3.72 ± 0.47	3.34 ± 0.59	3.55 ± 0.54 b
Plasma fatty acids							
LA C18:2n6	54.04 ± 1.4 6 a	55.12 ± 1.24	54.80 ± 0.45	54.94 ± 0.88 a	53.52 ± 1.19	52.34 ± 1.01	52.98 ± 1.23 b
ALA C18:3n3	2.87 ± 1.04 a	2.63 ± 0.38	2.10 ± 0.23	2.34 ± 0.40 a	1.81 ± 0.46	1.89 ± 0.48	1.85 ± 0.45 b
GLA C18:3n6	0.08 ± 0.04 a	0.13 ± 0.01	0.17 ± 0.06	0.15 ± 0.05 [a] b	0.83 ± 0.12	0.77 ± 0.22	0.80 ± 0.16 c
DGLA C20:3n6	0.33 ± 0.08 a	0.30 ± 0.03	0.51 ± 0.05	0.41 ± 0.12[a] a	0.69 ± 0.15	0.81 ± 0.09	0.75 ± 0.13 b
AA C20:4n6	1.39 ± 0.21 a	1.14 ± 0.14	1.25 ± 0.13	1.20 ± 0.14 b	1.45 ± 0.19	1.47 ± 0.14	1.46 ± 0.16 a
EPA C20:5n3	0.09 ± 0.04 a	0.07 ± 0.02	0.18 ± 0.06	0.13 ± 0.07 a	0.57 ± 0.09	0.65 ± 0.06	0.61 ± 0.08 b
DHA C22:6n3	0.07 ± 0.05 a	0.12 ± 0.06	0.35 ± 0.12	0.24 ± 0.15 [a] b	0.86 ± 0.12	0.85 ± 0.12	0.85 ± 0.12 c
EPA + DHA	0.16 ± 0.06 a	0.18 ± 0.08	0.53 ± 0.14	0.36 ± 0.21 [a] b	1.43 ± 0.17	1.5 ± 0.17	1.46 ± 0.17 c
DGLA + AA	1.73 ± 0.26 a	1.44 ± 0.15	1.76 ± 0.13	1.61 ± 0.21 a	2.14 ± 0.27	2.28 ± 0.12	2.20 ± 0.22 b
DGLA + AA + EPA + DHA	1.89 ± 0.29 a	1.62 ± 0.18	2.29 ± 0.10	1.98 ± 0.37[a] a	3.57 ± 0.36	3.77 ± 0.26	3.66 ± 0.32 b

[a] Period effect seen in SC-PUFA due to carryover effect of LC-PUFA supplementation in period 2.
Different lowercase letters in a row are significantly different.
See Table 1 for key.

frozen at −80 °C after collection and sent overnight on dry ice to the same laboratory. Plasma and RBC samples were processed and analyzed for fatty acid composition using gas chromatography with flame ionization as described elsewhere.[5] Grass, hay, and grain samples were treated similarly, except samples were first ground, homogenized, and extracted with a modified Folch extraction before the organic layer was dried in a speed vacuum. Oil samples were diluted with a chloroform/methanol mixture (2:1 volume/volume) and dried in a speed vacuum. The rest of the processing for dietary samples followed that used for plasma samples. A total of 24 fatty acids were identified for every sample.

Statistical analysis

Data were analyzed using Prism 9 for macOS version 9.4.1 (GraphPad Software, LLC). Values are presented as mean ± SD unless specified otherwise, and levels of significance were set at $\alpha \leq 0.05$. Significant differences in RBC and plasma fatty acid levels between treatments were determined using a 1-way ANOVA for repeated measures followed by a Tukey post hoc test. Significant differences in RBC fatty acid composition between ulcer groups were determined using a 2-tailed, unpaired *t* test. Odds ratios were used to quantify the relationship between PUFA supplementation and severity of squamous ulcer score (OR calculator version 20.115; MedCalc Software Ltd).

Results

Supplementation with LC-PUFAs resulted in a decrease in RBC and plasma LA and ALA ($P < .05$), and an increase in RBC and plasma GLA, DGLA, EPA, and DHA ($P < .05$) compared to UNSUPP or SC-PUFA–supplemented horses. RBC AA was also greater in LC-PUFA–supplemented horses ($P < .05$). RBC GLA, DGLA, EPA, and DHA were elevated in SC-PUFA horses compared to UNSUPP horses during period 3 as a result of a carryover effect after LC-PUFA supplementation in period 2 **(Figure 1; Table 2)**.

Gastric ulcers

The overall prevalence of gastric ulcers (grade 1 to 4) was 54%, 46%, and 31% in the UNSUPP, SC-PUFA, and LC-PUFA groups, respectively **(Figure 2)**. Five of the 13 horses (38%) had severe ulcers (grade 3 or 4) at the end of the UNSUPP period. None of the other 8 horses developed severe ulcers during the rest of the study. When supplemented with LC-PUFAs, significantly fewer horses (1/13 [8%]) had a severe ulcer (OR, 0.13; 95% CI, 0.013% to 1.36%; $P < .05$; *z* score, 1.70). Short-chain-PUFA–supplemented horses had a 23% (3/13) incidence of severe ulceration, which was not different from UNSUPP or LC-PUFAs ($P > .05$).

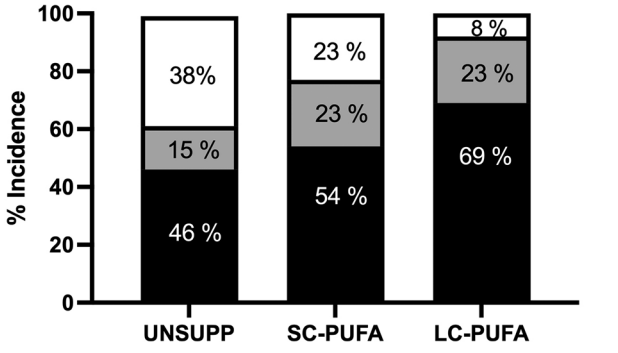

Figure 2—Percent incidence of squamous ulcer scores of 0, 1 to 2, and 3 to 4 per treatment. ***See*** Figure 1 for remainder of key.

All observations (n = 39) were grouped to compare RBC fatty acid composition to ulcer score. The PUFA composition of all horses with no or mild to moderate ulcers (grades 0 to 2; n = 30) is shown in **Table 3** compared to horses with severe (grade 3 to 4) ulcer scores (n = 9). RBC GLA, DGLA, EPA, and AA levels were all greater in horses with grade 0 to 2 ulcers compared to horses with severe ulcers (grade 3 or 4; $P < .05$). Gastric fluid pH was also greater (1.69 ± 0.20) in the horses with no or mild to moderate ulcers compared to the horses with severe ulcers (1.51 ± 0.22; $P < .05$).

The 5 horses that had severe ulceration initially (period 1, UNSUPP) were subgrouped to evaluate supplementation. In these horses, supplementation with LC-PUFAs resulted in greater RBC DGLA, EPA, and DHA levels ($P < .05$; **Figure 3**), and 4/5 had no ulcers (grade 0) after 3 months of LC-PUFA supplementation, regardless of period. Two of these horses redeveloped ulcers when placed subsequently on the SC-PUFA supplement, and 1 horse was resistant to any changes.

Table 3—RBC PUFA composition of horses with either grade 0 to 2 ulcers (n = 30) or grade 3 to 4 ulcers (n = 9).

	Ulcer score		
RBC fatty acid[a]	0–2 (n = 30)	3–4 (n = 9)	*P* value
ALA	1.23 ± 0.26	1.03 ± 0.32	.06
LA	38.58 ± 1.36	39.4 ± 1.18	.11
GLA	0.23 ± 0.14	0.13 ± 0.05	< .05
DGLA	0.40 ± 0.15	0.30 ± 0.09	< .05
EPA	0.33 ± 0.27	0.14 ± 0.13	< .05
DHA	0.42 ± 0.34	0.21 ± 0.21	.09
AA	1.54 ± 0.24	1.29 ± 0.22	< .01
DGLA + AA	1.94 ± 0.35	1.59 ± 0.24	< .01
EPA + DHA	0.75 ± 0.61	0.35 ± 0.32	.06
LC-PUFA	2.69 ± 0.88	1.94 ± 0.40	< .05

[a]Grams/100 g total fatty acids.
See Table 1 for key.

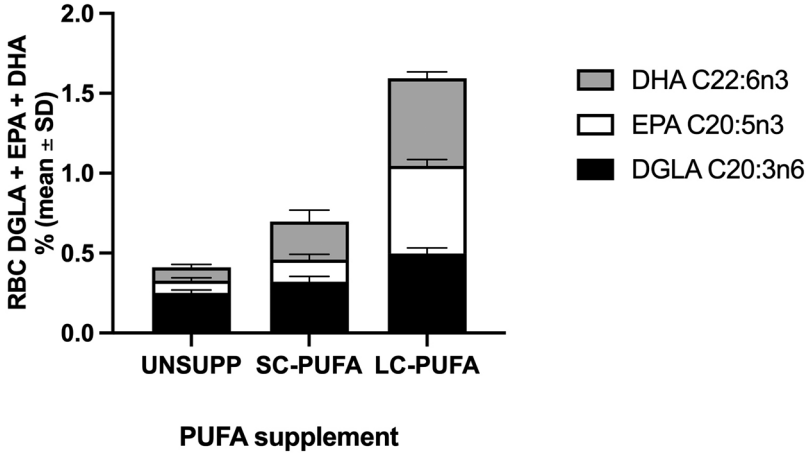

Figure 3—RBC dihomo-gamma-linolenic acid (DGLA) EPA and DHA (g/100 g of total fatty acids) in 5 horses with severe ulcers (grade 3 to 4) when UNSUPP and in these same horses after 88 days of either SCPUFA or LC-PUFA supplementation. ***See*** Figures 1 and 2 for remainder of key.

Discussion

In our study, horses fed supplemental LA and ALA from corn oil and flax oil did not increase LC-PUFAs in plasma or RBC. Horses fed LC-PUFAs had reduced RBC and plasma LA and ALA levels, and increased EPA, DHA, and AA levels. This agrees with previous studies[6–8] in which horses fed fish oil had greater ($P < .05$) proportions of EPA, DHA, and AA in plasma and RBC, and plasma LA and ALA levels were less ($P < .05$) compared with those supplemented with flax oil. In addition, horses not supplemented directly with EPA and DHA did not increase their plasma or RBC levels.[7,9–11] This reflects an inability to elongate adequately dietary SC-PUFAs such as ALA and LA to LC-PUFAs, and suggests the benefits of supplementing LC-PUFAs directly. This allows the horse to avoid minimally effective and competitive elongation steps. Interestingly, GLA is considered a SC-PUFA, but has been shown both here and in other research (JD Pagan, Kentucky Equine Research, unpublished data, 2020) to be efficiently elongated to the LC-PUFA DGLA by the horse. It is possible that concurrently supplying LC-PUFAs EPA and DHA allow for GLA to be converted efficiently to DGLA and AA and their respective eicosanoids. Some research[12] has shown a reduction in AA when fed fish oil, but our study has demonstrated an increase in DGLA and AA that is likely attributable to supplying GLA. The LC-PUFA treatment with GLA increased RBC levels of AA compared to UNSUPP; the SC-PUFA treatment showed a decrease in RBC AA. Although plasma levels of AA were not different between LC-PUFA and UNSUPP, SC-PUFAs had significantly lower AA levels than UNSUPP.

Prostaglandins produced from fatty acids can influence acid, mucus, and bicarbonate secretion in addition to accelerated healing of ulcers.[13] Arachidonic acid from the omega-6 pathway produces PGE2, whereas PGE1 and PGE3 are produced from DGLA and EPA through the omega-6 and omega-3 pathway, respectively. NSAIDs block the production of all 3 PGEs through disruption of the cyclooxygenase pathway and have been implicated in increased risk for gastric ulcers. When it comes to gastric protection, PGE2 from AA is thought to play an important role during stress. Studies have shown that a PGE2 deficiency can increase mucosa susceptibility to ulceration and that increased erosion has been seen with decreased levels of AA, largely as a result of a decrease in PGE2 production.[12,14] When supplied with LA as a precursor to AA, the horse has been able to increase gastric PGE2 production and lower gastric acid output.[15] However, GLA supplementation was preferred to LA in rats subjected to aspirin-induced gastric hemorrhage.[16] GLA supplementation produced greater levels of AA and no gastric hemorrhage compared to hemorrhage in 3/8 rats on the LA diet. They believed GLA was able to bypass depressed levels of delta-6-desaturation in the LA group and protect gastric mucosa through improved synthesis of AA and PGEs. Similarly, supplementation of LA in the SC-PUFA treatment in our study did not increase AA levels but reduced levels, and supplementation with GLA in the LC-PUFA treatment increased AA levels. Horses with severe ulceration (grade 3 or 4) had lower RBC AA levels as well as lower GLA, DGLA, and EPA levels compared to those with no or mild to moderate ulcers (grade 0 to 2). These results suggest the use of a fatty acid further downstream is ideal. Because PGE2 production is linked to ulceration, greater levels of AA—although potentially proinflammatory—may be preferred in horses in which ulceration from stress is a concern. Although gastric PGE2 was not measured in our study, it may be inferred that an increase in AA levels in the LC-PUFA treatment likely resulted in an increase in PGE2 and could be attributed to the reduced pH and ulcer prevalence and severity in the LC-PUFA group.

Previous studies[7,9,10,17,18] in horses demonstrated augmented levels of omega-3 PUFAs in plasma, RBCs, leukocytes, synovial fluid, and skeletal muscle after EPA and DHA supplementation. Although gastrointestinal tissue was not measured in our study, levels of fatty acids in RBCs, including EPA + DHA, were altered by dietary supplementation and may be reflective of changes to local gastric tissue. Other studies in mice have shown chronic or acute administration of EPA or DHA from fish oil to be effective in reducing or protecting gastric injury when subjected to ethanol-induced hemorrhagic gastritis[19,20] or mechanical, chemical, and thermic stress.[21] Docosahexaenoic acid was also found to be protective against indomethacin-induced gastric damage akin to omeprazole.[22] This gastroprotective effect was found not to be through mediation

of PGE2, but by a decrease in gastric B4 leukotriene levels. A collection of data indicates that fish oil is able to protect gastric mucosa by both offensive (inhibiting acid secretion) and defensive (enhancing mucus secretion, activity of antioxidant enzymes) factors.[23] Overall, the use of a LC-PUFA product with GLA, EPA, and DHA may allow for the use of multiple approaches to gastric protection and ulcer prevention. Long-chain PUFA supplementation used in our study was associated positively with the prevention or resolution of severe squamous gastric ulceration in exercising Thoroughbreds.

A limitation in study design did produce a carryover effect in period 3 data as a result of the length of the washout period. Greater levels of LC-PUFAs were seen in the SC-PUFA treatment in period 3 as a result of the treatment order. When supplementing fatty acids for any chronic period of time, a longer washout is generally necessary.

Importantly, most research has used an induced-ulceration model for study, and various models are used that can complicate the interpretation of results. However, this study was able to evaluate naturally occurring ulcers from performance horses subjected to typical stress exercise as recommended by the Equine Gastric Ulcer Council.[4] The difference between the generation of naturally occurring ulcers and those induced by mechanical, chemical, or other stress factors may be significant in characterizing the mechanisms behind preventing and improving ulceration of gastric mucosa. Mucosal damage depends on the nature of the injury and has been shown to be affected significantly by fatty acid composition.[12] Although ulcers were naturally occurring in our study, the prevalence (31% to 54%; grades 1 to 4) was less compared to surveys of other racehorses (52% to 92%,[24] 66%,[25] 72%,[26] 82%,[27] 93%[28]). Our study was considered less intense than race training (64% of training distance and 77% of time with a heart rate >154 bpm),[29] and horses were housed outside at night compared to stabled approximately 23 hours/day. This moderation in horse management may have also played a role in the ulcer prevalence and results observed in our study. Horses undergoing different exercise intensities and management styles require further study. In addition, this study only assessed squamous gastric ulcers. Any effect on glandular mucosa also requires further evaluation.

Although the ideal omega-6:omega-3 ratio has not been established for horses, it is clear that the current feeding practices of performance horses has skewed the ratio drastically from a natural forage-based diet. An all-forage diet would provide a ratio of 0.3:1 (omega-6:omega-3). In our study, the basal diet provided about 140 g/day LA and 62 g/day ALA, for a ratio of 2.2:1. When horses were supplemented in our study, treatments were matched to provide similar omega-6:omega-3 ratios (0.8:1) to evaluate only the type of fatty acid (SC vs LC) fed rather than an impact on the ratio. Studies in other species have ratios from 5.3 to 665:1, and studies in horses have ratios of 0.8 to 7.5:1, if they are listed at all.[9,10,12] Because of the competitive nature of these pathways, differences in the balance of a dietary omega-6:omega-3 ratio between studies could affect data easily, influence conclusions, and lead to confusion with interpretation. Amounts and ratios used in our study were associated with a beneficial squamous gastric ulcer response, but further research may be warranted for different diets.

In summary, this study has shown that horses lack the ability to elongate SC-PUFAs to LC-PUFAs adequately, which agrees with findings of other researchers. This subsequently limits their ability to produce valuable eicosanoids such as PGE2 that have been implicated in the resolution of gastric ulceration. Supplementation with GLA, EPA, and DHA increases equine plasma and RBC levels of DGLA, AA, EPA, and DHA effectively and is associated with prevention or resolution of severe squamous gastric ulcers. The presence of squamous gastric disease in horses, especially those involved in high-performance competition, ranks high among health concerns of owners and trainers, and specific nutritional strategies to prevent and alleviate this condition are highly sought. Our results suggest that supplementation with LC-PUFAs, specifically a high-GLA safflower oil-fish oil blend, is a viable way to prevent or resolve severe squamous gastric ulceration in equine athletes during training.

Acknowledgments

Kentucky Equine Research supplied all of the funding for this study.

The compositions and methods described in this study are patent pending based on a patent application filed by Kentucky Equine Research.

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